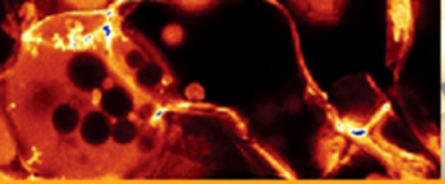
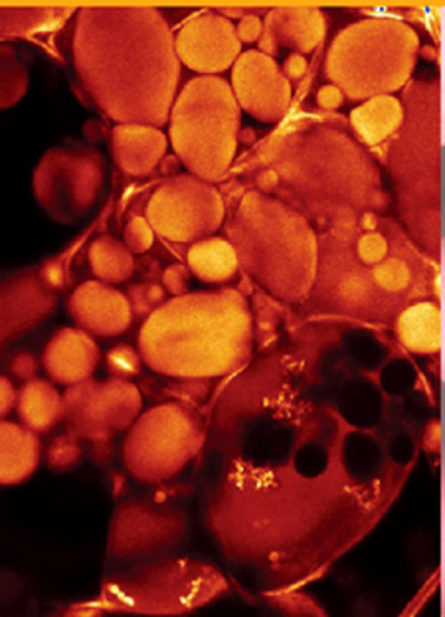



ADVANCES IN POTATO CHEMISTRY AND TECHNOLOGY

Jaspreet Singh • Lovedeep Kaur




Advances in Potato Chemistry and Technology

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Contents

Preface

The potato (*Solanum tuberosum* L.) which is grown in over 100 countries throughout the world is one of the staples of the human diet. Potato is an important raw material for the industry as well. Growing conditions, genetic origin and aging during post-harvest storage can affect potato quality. The chemical composition of potato is of utmost importance during processing at industrial scale. The potato components such as starch, non starch polysaccharides and minerals affect the quality of potatoes and their products to a significant extent. An understanding of the chemical structure of potato components is also important for the development of unique or novel potato products.

Potatoes are among the most efficient sources of energy and other nutrients including vitamins and minerals. With potatoes having such great importance, it is perhaps surprising that very few books about them have been written.

Considerable progress has been made by potato chemists and technologists over the years, which is available through a scattered scientific literature. The aim of this book is to provide a consistent set of our advanced knowledge on potato chemistry and technology in one volume. The first chapter provides a brief introduction about the potato tuber and forms the basis for the next chapters on the potato origin, breeding, potato composition including cell wall components, starch, proteins, lipids and minerals. Potatoes are either stored under controlled conditions or processed for consumption long after harvest. Storage conditions can promote extensive changes in the chemical composition of potato tubers, thereby altering the quality characteristics of the final product. A detailed chapter on the post-harvest storage of potatoes and its impact on potato quality cover the main components affected by post-harvest metabolism in potato tubers. The chapter on the analysis of glycoalkaloids, phenolic compounds and anthocyanins describes their analysis, toxicity and health benefits. Various modern and sophisticated analytical techniques such as differential scanning calorimetry, rapid visco-analysis, infrared spectroscopy, optical, electron and atomic force microscopy, nuclear magnetic resonance spectroscopy, X-ray diffraction, high performance size exclusion chromatography, and high performance anion exchange chromatography for the quality evaluation of potato and their products are discussed in the next chapter. The chapters on textural and rheological characteristics of raw and cooked potatoes, and fried and dehydrated potato products emphasize the technological and processing aspects; while the chapter on the potato starch modification deals with the various factors that may affect the extent of physical, chemical and enzymatic modification of potato

starch. The alteration in the physic-chemical, morphological and functional properties of potato starch which occur due to modification has been described in detail. The detailed nutritional aspects of potatoes have been presented in the form of two chapters. The chapters on the non-food uses of potatoes are a novel feature of this book and provide information on the industrial scale non-food use of potato and their products. The last chapter deals with the use of potatoes for human life support in space. We hope that this book will serve as a valuable reference for academics, potato scientists, chemists, industry professionals, and graduate students.

Jaspreet Singh
Lovedeep Kaur

Introduction

The potato (*Solanum tuberosum* L.) has an annual world production of 322 million metric tonnes, China being the major producer (FAOSTAT, 2007). Potato production has increased over the past years in both developed and developing countries much faster than other tuber or root crops because of its high yield per unit area and nutritive value (Karim et al., 1997). Potato plant is a perennial herb belonging to the family *Solanaceae*. The plant bears white to purple flowers with yellow stamens and some cultivars bear small green fruits each containing up to 300 seeds (Figure 1). Potato tuber develops as an underground stem (swollen part of a subterranean rhizome or stolon) bearing auxillary buds and scars of scale leaves and is rich in starch and storage proteins (Figure 2). Potatoes can be grown from the botanical seeds or propagated vegetatively by planting pieces of tubers. The eyes on the potato tuber surface, which are actually dormant buds, give rise to new shoots (sprouts) when grown under suitable conditions. A sprouted potato is not acceptable for consumption and processing. But optimum sprouting is a desired attribute when the tubers are used for propagation.

New cultivars of potatoes with better yield, disease resistance, and desirable end-use are being developed with the help of breeding techniques. In recent years, many potato cultivars with desired cooking texture (such as waxy, floury), flesh color, and disease resistance have been developed with the help of breeding. Following the rational development of genetic engineering, many genetically modified varieties of potatoes have been produced. However these transgenic varieties of potatoes are not permitted for food use in many countries because of the concerns related with the safety of the consumer and environment. Genetically modified potato varieties having very high amylose/amylopectin content of starch, antioxidants levels, and tuber yield are developed.

Morphologically, a potato tuber is usually oval to round in shape with white flesh and a pale brown skin, although variations in size, shape, and flesh color are also frequently encountered, depending on genetics of the cultivar. The color, size, and texture of potatoes are the main quality attributes assessed by the consumer for acceptability. Good-quality potatoes are considered to be relatively smooth, firm, and free from sprouts or any other disorders. In a potato tuber, about 20% is dry matter and the rest is water. The yield, dry matter, and composition of dry matter vary among different potato cultivars; soil type and temperature; location; cultural practices; maturity; post-harvest storage conditions and other factors (Burton, 1989). Starch is the major component of the dry matter accounting for approximately 70% of the total solids. Major part

Figure 1: A potato farmer.

Figure 2: Potato plants in the field.

of the fresh potato tuber is comprised of storage parenchyma in which the starch granules are stored as a reserve material. Potatoes are a rich source of high-value protein, essential vitamins, minerals, and trace elements. The average range of raw material composition of a potato tuber is as follows: starch (10–18%) having 22–30% amylose content, total sugars (1–7%), protein (1–2%), fiber (0.5%), lipids (0.1–0.5%), vitamin A (trace/100 g fresh weight, FW), vitamin C (30 mg/100 g FW), minerals (trace), and glycoalkaloids (1–3 mg/100 g FW). The average composition of a potato tuber is presented in Table 1. The less well-known constituents of potato tuber are carotenoids and phenolics, which are potent antioxidants. Carotenoid content of potatoes ranges from 50 to 100 μg per 100 g FW in white-fleshed cultivars to 2000 μg per 100 g FW

Table 1: An average composition of potato tuber, per 100 g, after boiling in skin and peeling before consumption (Source: USDA, National Nutrient Database)

Potato	
Water	77 g
Carbohydrates	20.13 g
Energy	87 kcal
Protein	1.87 g
Fat	0.1 g
Calcium	5 mg
Potassium	379 mg
Phosphorus	44 mg
Iron	0.31 mg
Niacin	1.44 mg
Thiamin	0.106 mg
Riboflavin	0.02 mg

Photographs by Jaswinder Gill.

in deeply yellow to orange-fleshed cultivars. Potatoes also contain phenolic compounds, predominantly chlorogenic acid, and up to 30 μg per 100 g FW of flavonoids in white-fleshed potatoes and roughly 60 μg per 100 g FW in red- and purple-fleshed potatoes. The total anthocyanin content of whole unpeeled red- and purple-fleshed potatoes may be around 40 mg per 100 g FW (Brown, 2005). The influence of potato chemical composition during industrial processing is of high significance to maintain the quality of the potato products. As an example, the texture of potato crisps is dependent mainly on starch content of the raw potato tubers. Apart from that, nonstarch polysaccharides also play a crucial part in determining the quality of the crisps. The cell wall materials are important determinants of potato texture and are important contributors towards the tuber fiber content. Potatoes with closely packed small and irregular parenchymatous cells have been observed to be relatively hard and cohesive. In contrast, potatoes with large, loosely packed cells are generally less hard.

Starch, the major component of the dry matter of potatoes, consists of amylose and amylopectin. The structural characteristics and amylose to amylopectin ratio of potato starch vary from cultivar to cultivar. The nutritional and processing quality of potatoes and potato products (frozen and dry) are greatly affected by their starch characteristics. Several chemical and enzymatic modifications are performed to improve the processing performance of potato starch. Most of these modifications of potato are generally recognized as safe (GRAS) by the safety authorities. The total sugars in potato tuber range from 1–7 g kg^{-1}. The reducing sugars (glucose, fructose) are at the highest levels in young tubers which decrease considerably towards the end of the growing season. The starch to sugars conversion during post-harvest storage also causes variation in the sugar content of tubers which is an important consideration in the potato crisp industry. In the past few years, advanced analytical and instrumentation techniques have been introduced to evaluate the quality of potato and its products. These techniques provide in-depth information about structure and functionality of potato components which help to tailor the potato products for desirable attributes. The nutritional characteristics such as glycemic index and relative glycemic impact are important in human health. The relationships between composition of potato tubers and its impact on the release of glucose in the blood have been studied by various researchers. The careful selection of suitable potato cultivars, cooking, processing, and storage conditions can prove helpful in getting better nutritional benefits from potatoes. Apart from food use, potato products are being use for non-food applications such as biodegradable packaging, fermentation, vaccines, and pharmaceuticals. Potatoes have been tested in various space studies at national aeronautics and space administration (NASA), USA, which reflects the importance of this source for future science.

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CHAPTER 1

Potato Origin and Production

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1.1 Introduction

The potato (*Solanum tuberosum*) is the world's fourth most important food crop after wheat, maize and rice with 314 million tonnes fresh-weight produced in 2006 (FAOSTAT). Over half of this production (159 million tonnes) was in Asia, Africa and Latin America where the potato is a major carbohydrate (starch) supplier in the diets of hundreds of million of people. It also provides significant amounts of protein, with a good amino acid balance, vitamins C, B6 and B1, folate, the minerals potassium, phosphorus, calcium, and magnesium and the micronutrients iron and zinc. The potato is high in dietary fiber, especially when eaten unpeeled with its skin, and is rich in antioxidants comprising polyphenols, vitamin C, carotenoids and tocopherols (Storey, 2007). Fresh potatoes are virtually free of fat and cholesterol. A guide to potato composition is shown in Table 1.1, but it must be appreciated that values are affected by both cultivar and growing conditions.

As a major food staple the potato is contributing to the United Nation's Millennium Development Goals of providing food security and eradicating poverty. In recognition of these important roles, the UN named 2008 as the International Year of the Potato. Food security and eradicating poverty are high on the agenda of the International Potato Center (CIP) in Lima, Peru. CIP was founded in 1970 as an international agricultural research center (IACR), and is now a Future Harvest Center. Since 1971, CIP has been supported by the Consultative Group on International Agricultural Research (CGIAR), whose aim is the eradication of human hunger and poverty through research. Eradicating poverty is helped where the potato provides not only food but also employment and income as a cash crop.

As a staple food and as a vegetable for table use, the potato needs to be cooked because of the indigestibility of its ungelatinized starch (Burton, 1989). Such cooking is frequently by baking, boiling, steaming, roasting, deep-fat frying or microwave cooking, although in the Andes a broad diversity of additional preparation methods are employed. Good appearance, texture and flavor are important to the consumer and the subject of much research (Taylor et al., 2007). When baked, boiled or mashed and eaten alone, potatoes generally have a high glycemic index

Table 1.1: Chemical composition of potatoes on a fresh-weight basis (from figures in Li et al., 2006; Storey, 2007)

Component	Content
Dry matter	15–28%
Starch	12.6–18.2%
Glucose	0.01–0.6%
Fructose	0.01–0.6%
Sucrose	0.13–0.68%
Dietary fiber	1–2%
Lipid (fat)	0.075–0.2%
Protein	0.6–2.1%
Asparagines (free)	110–529 mg/100 g
Glutamine (free)	23–409 mg/100 g
Proline (free)	2–209 mg/100 g
Other amino acids (free)	0.2–117 mg/100 g
Polyphenols	123–441 mg/100 g
Carotenoids	0.05–2 mg/100 g
Tocopherols	Up to 0.3 mg/100 g
Thiamin B1	0.02–0.2 mg/100 g
Riboflavin	0.01–0.07 mg/100 g
Vitamin B6	0.13–0.44 mg/100 g
Vitamin C	8–54 mg/100 g
Vitamin E	~0.1 mg/100 g
Folic acid	0.01–0.03 mg/100 g
Nitrogen (total)	0.2–0.4%
Potassium	280–564 mg/100 g
Phosphorus	30–60 mg/100 g
Calcium	5–18 mg/100 g
Magnesium	14–18 mg/100 g
Iron	0.4–1.6 mg/100 g
Zinc	~0.3 mg/100 g
Glycoalkaloids	< 20 mg/100 g

(GI), like other staple starchy foods such as some types of rice and white bread (Foster-Powell et al., 2002). However, boiled waxy or new potatoes, and potatoes prepared in different ways, have lower GI values and so carry reduced concern for diabetics (Henry et al., 2005). Eating potatoes in mixed meals will further alter GI levels, and the nutritional benefits of potato indicate that they are generally a useful and nutritionally beneficial component of the human diet (McGregor, 2007).

The potato is processed into French fries (chips in the UK) and chips (crisps in the UK), and is used for dried products and starch production. In North America and some European countries between 50 and 60% of the crop is processed (Li et al., 2006; Kirkman, 2007). Furthermore, processors are building factories in countries where the potato is primarily grown as a staple

Table 1.2: Potato production by region in 2006 (source FAOSTAT)

	Harvested area (hectares)	Quantity (tonnes)	Yield (tonnes/hectare)
Africa	1 499 687	16 420 729	10.95
Asia/Oceania	9 143 495	131 286 181	14.36
Europe	7 348 420	126 332 492	17.19
Latin America	951 974	15 627 530	16.42
North America	608 131	24 708 603	40.63
World	19 551 707	314 375 535	16.08
China	4 901 500	70 338 000	14.35
Russia	2 962 420	38 572 640	13.02
India	1 400 000	23 910 000	17.08
USA	451 430	19 712 630	43.67

food, and this is a trend that is likely to continue. Kirkman (2007) has estimated that global consumption in processed form will have increased from 13% of total food use in 2002 to nearly 18% by 2020. In some countries the potato is still fed to animals but this use is decreasing.

Potatoes were grown on 19.6 million hectares of land in 2006 (FAOSTAT), in 149 countries from latitudes 65°N to 50°S, and at altitudes from sea level to 4000 m (Hijmans, 2001). Potato production by region is shown in Table 1.2 and consumption by region in Table 1.3. The four largest potato producers are China (70 million tonnes), the Russian Federation (39 million tonnes), India (24 million tonnes) and the USA (20 million tonnes) with per capita consumption still much larger in Russia than in the other countries. Potatoes can be grown wherever it is neither too hot (ideally average daily temperatures below 21°C) nor too cold (above 5°C), and there is adequate water from rain or irrigation (Govindakrishan and Haverkort, 2006). In practice this means that they are grown as a summer crop in the tropical highlands of Bolivia, Peru and

Table 1.3: Potato consumption by region in 2005 (source FAOSTAT)

	Population	Consumption	
		Total (tonnes)	Per capita (kg)
Africa	905 937 000	12 850 000	14.18
Asia/Oceania	3 938 469 000	101 756 000	25.83
Europe	739 276 000	71 087 000	96.15
Latin America	561 344 000	13 280 000	23.65
North America	330 608 000	19 156 000	57.94
World	6 475 634 000	218 129 000	33.68
China	1 323 345 000	52 882 000	39.78
Russia	143 202 000	20 442 000	141.98
India	1 103 371 000	18 253 000	16.06
USA	298 213 000	16 399 000	54.39

Mexico, all the year round in parts of China and Brazil and in the equatorial highlands of South America (e.g., Ecuador and Colombia) and East Africa (e.g., Kenya and Uganda), as a winter crop in the lowland subtropics (e.g., northern India and southern China), as spring and autumn crops in the Mediterranean (e.g., North Africa), and in summer in the lowland temperate regions of the world (North America, western and eastern Europe, northern China and Australia and New Zealand).

The growing season can be as short as 75 days in the lowland subtropics, where 90–120 days is the norm, and as long as 180 days in the high Andes. In the lowland temperate regions where planting is done in spring and harvesting in autumn, crop duration is typically 120–150 days, and yields are potentially high. Average fresh-weight yields vary tremendously by country from 2 to 45 t/ha with a global average of 16.1 t/ha in 2006 (FAOSTAT). As potatoes cannot be grown year round in most parts of the world, it is normal to have to store both seed tubers for planting the next crop and ware tubers for consumption. Hence post-harvest infrastructure in terms of road transport and cold storage facilities is also an important aspect of potato production.

This opening chapter provides a brief introduction to the origin of the potato and its transformation into a crop that makes a major contribution to the feeding of humankind. The following books proved useful sources of information and references: The Potato (Burton, 1989), The Potato Evolution, Biodiversity and Genetic Resources (Hawkes, 1990), Handbook of Potato Production, Improvement, and Postharvest Management (Gopal and Khurana, 2006), Potato Biology and Biotechnology Advances and Perspectives (Vreugdenhil, 2007) and Propitious Esculent (Reader, 2008).

1.2 Potato Origin

1.2.1 Wild tuber-bearing Solanum species

Wild tuber-bearing *Solanum* species are distributed from the southwestern United States (38°N) to central Argentina and adjacent Chile (41°S) and cover a great ecogeographical range (Hawkes, 1990; Spooner and Hijmans, 2001). In the southwestern USA and in Central America wild species generally occur at medium to high altitudes. In South America they are found along the Andes from Venezuela to northwest Argentina and also in the lowlands of Chile, Argentina, Uruguay, Paraguay and southeastern Brazil. The adaptive range among the different species is very great and includes the high Andean regions from 3000 m to the vegetational limit at 4500 m where frosts are common, dry semi-desert conditions and scrub and cactus deserts, cool temperate pine and rain forests, woodlands and coastal plains. Wild species have also developed resistances to a wide range of pests and diseases. Hence they are a tremendous resource for potato breeding and research for which purposes it is important to appreciate their wide geographical distribution and great range of ecological adaptation (Hawkes, 1994). There have been numerous collecting expeditions, from those pioneered by the Russians in the 1920s (Hawkes, 1990) to

the more recent ones of the 1990s (Spooner and Hijmans, 2001). The germplasm is maintained in a number of genebanks around the world which together comprise the Association for Potato Intergenebank Collaboration (http://www.potgenebank.org).

The taxonomy of wild tuber-bearing *Solanum* species is complicated and under continuous revision. Hawkes (1990) recognized 219 wild tuber-bearing species and arranged them into 19 series of subsection *Potatoe* of section *Petota* of subgenus *Potatoe* of genus *Solanum* (Table 1.4). He grouped series I to IX in superseries *Stellata* and series X to XIX in superseries *Rotata.* He considered the sequence of subsections, superseries and series to reflect an approximate evolutionary one and suggested a possible scenario for the evolution of wild potato species, while acknowledging that modification may be required as a result of continuing experimental work particularly with molecular markers. He also recognized a further nine closely related non-tuber-bearing species that he grouped into two series of subsection *Estolonifera*, but these have been excluded from section *Petota* in more recent taxonomic reviews, leaving a section comprising all tuber-bearing species (Spooner and Salas, 2006).

Spooner and Hijmans (2001) reviewed accepted species based on a literature survey, including new species described and names placed in synonymy since Hawkes' treatment, and listed 196 wild tuber-bearing species. Further changes in the delimitation of species are being reported as molecular marker and DNA sequence data are used to clarify species relationships. The latest summary by Spooner and Salas (2006) recognizes 188 wild potato species for section *Petota* that are grouped into four clades, based on plastid DNA, rather than 19 series (Table 1.4). Clade 1 comprises the US, Mexican and Central American diploid species, exclusive of *S. bulbocastanum*, *S. cardiophyllum* and *S. verrucosum*; Clade 2 comprises *S. bulbocastanum* and *S. cardiophyllum*; Clade 3 comprises all examined members of the South American series *Piurana* and some South American species classified to other series; and Clade 4 comprises all remaining South American species and the US, Mexican and Central American polyploid species and *S. verrucosum*. However, this plastid-based classification splits similar species into different clades and so may not properly represent groupings made on the basis of nuclear DNA. Furthermore, it is not an appropriate means of classifying allopolyploid groups. The number of species may be further reduced in the future, and clade composition based on chloroplast DNA may change as extensive nuclear DNA sequence data become available. Of more interest to potato breeders is the origin and relatedness of the genomes in wild and cultivated potatoes, including hybrid taxa, and their accessibility for breeding via crossing.

The wild species form a polyploid series from diploid ($2n = 2x = 24$) to hexaploid ($2n = 6x = 72$) in which genomes were classified into five groups A, B, C, D and P by Matsubayashi (1991), with a sixth group E recognized in closely related non-tuber-bearing species. Spooner et al. (2004) summarized the putative genome compositions of the polyploid species, but it is clear

Table 1.4: Classification of wild tuber-bearing *Solanum* species (Section *Potatoe*) based on Hawkes (1990) and Spooner and Salas (2006) and species mentioned in text

Superseries	Series	Species Numbers	Ploidy	EBN	Area[1]	Plastid clade
Superseries *Stellata*						
I	*Morelliformia*	1	2*x*	1	Mex	1
II	*Bulbocastana*	2	2*x*	1	Mex	1
	S. bulbocastanum		2*x*	1	Mex	2
III	*Pinnatisecta*	11	2*x*	1	Mex	1
	S. cardiophyllum		2*x*	1	Mex	2
IV	*Polyadenia*	2	2*x*	1	Mex	1
V	*Commersoniana*	2	2*x*	1	SA	4
VI	*Circaeifolia*	3	2*x*	1	SA	4
VII	*Lignicaulia*	1	2*x*	1	SA	4
VIII	*Olmosiana*	1	2*x*	1	SA	4
IX	*Yungasensa*	9	2*x*	2	SA	4
	S. chacoense		2*x*	2	SA	4
Superseries *Rotata*						
X	*Megistacroloba*	11	2*x*	2	SA	4
	S. megistacrolobum		2*x*	2	SA	4
XI	*Cuneoalata*	3	2*x*	2	SA	4
XII	*Conicibaccata*	40	2*x*,4*x*,6*x*	2,2,4	SA,Mex	4
XIII	*Piurana*	15	2*x*,4*x*	2	SA	3
XIV	*Ingifolia*	2	2*x*	2	SA	4
XV	*Maglia*	1	2*x*	2	SA	4
XVI	*Tuberosa*	96	2*x*,4*x*	2	SA	4
	S. brevicaule		2*x*	2	SA	4
	S. bukasovii		2*x*	2	SA	4
	S. canasense		2*x*	2	SA	4
	S. leptophyes		2*x*	2	SA	4
	s. multidissectum		2*x*	2	SA	4
	S. sparsipilum		2*x*	2	SA	4
	S. spegazzinii		2*x*	2	SA	4
	S. vernei		2*x*	2	SA	4
	S. verrucosum		2*x*	2	Mex	4
XVII	*Acaulia*	4	4*x*,6*x*	2,4	SA	4
	S. acaule		4*x*	2	SA	4
XVIII	*Longipedicellata*	7	4*x*	2	Mex	4
	S. stoloniferum		4*x*	2	Mex	4
XIX	*Demissa*	8	6*x*	4	Mex	4
	S. demissum		6*x*	4	Mex	4

[1] *SA = South America, Mex = Southwestern USA, Mexico and Central America.*

that further research is required to resolve the origins of the genomes in polyploid potato species. Nearly all of the diploid species are outbreeders, with a single S-locus, multiallelic, gametophytic self-incompatibility system (Dodds, 1965), whereas the tetraploids and hexaploids are mostly self-compatible allopolyploids that display disomic inheritance (Hawkes, 1990). The crossability of species has been determined through artificial pollinations done across many years (Jansky, 2006). The results can be explained primarily but not exclusively in terms of Endosperm Balance Number (EBN) which can be regarded as the effective rather than the actual ploidy of the species (Johnston et al., 1980). Today breeders can usually achieve sexual hybridization between wild and cultivated potatoes by manipulation of ploidy with due regard to EBN (Ortiz, 1998, 2001; Jansky, 2006). They can also use somatic (protoplast) fusion to achieve difficult or impossible sexual hybridizations (Wenzel, 1994; Thieme and Thieme, 2005; Veilleux, 2005). But where, when and how did domestication occur, which species were involved in the process, and what changes did potatoes undergo as they adapted to human cultivation?

1.2.2 Domestication

The wild species progenitors of cultivated potatoes have been the subject of much discussion. Recently Spooner et al. (2005a) provided molecular taxonomic evidence for a single domestication in the highlands of southern Peru from the northern group of members of the *S. brevicaule* complex of diploid species. This group contains species such as *S. canasense, S. multidissectum* and *S. bukasovii*, some of which are not always clearly resolved and perhaps could be better reduced to a single species, *S. bukasovii* (Table 1.4). Sukhotu and Hosaka (2006) also concluded from chloroplast data that species such as these were first domesticated in Peru with a later spread to Bolivia. The result of domestication was a diploid cultigen *S. tuberosum* Group Stenotomum (Dodds, 1962) from which all other cultivated potatoes were derived.

Hawkes (1990) concluded that the potato is an ancient domesticate, with preserved food plant remains found at various excavated sites on the coast of Peru and one site in the high Chilca canyon, south of Lima (Engel, 1970). The Oxford Radiocarbon Accelerator dated the tuber remains found by Engel to about 7000 years before present. The journey from gathering wild tubers to cultivating them and finally domesticating them started early in the human colonization of the Americas as wild potato remains have been found in a late Pleistocene settlement in south central Chile dated around 12 500 years before present (Ugent et al., 1987; Moseley, 2001). Lack of more evidence of cultivation in highland sites where the progenitor species are found probably simply indicates poor preservation conditions in high regions with seasonally wet climates, compared with better conditions in arid environments. Indeed, subsequent agricultural development in the Peruvian highlands was impressive, with crops of maize, potatoes and other food plants grown on terraces built frequently of dressed stone, and a suite of locally domesticated plants and animals. Subsequent to its domestication, the potato was grown for at least four millennia prior to the development of ceramics and hence Hawkes (1990) concluded

that fresh potatoes were most likely baked in the embers of a fire or cooked in an earth oven on hot stones.

An ancient method of potato preservation was chuño production, where potatoes were in effect freeze-dried in the high, cold mountains, a process that has continued in use to the present day (Hawkes, 1990) yielding a product highly prized by the peoples of the Andean countries. Processing of chuño by soaking is a necessary prelude to the consumption of bitter potatoes as it removes toxic glycoalkaloids. Hence it is likely to have been more widespread when Andean people were using only wild potatoes. It also gives a dehydrated product that can be stored over several years and used when fresh potatoes are scarce.

Potatoes, like their ancestral wild species, reproduce by sexual means and also by setting tubers which propagate genotypes clonally. Potatoes flower and set true seed in berries following natural pollination by insects capable of buzz pollination, such as some bee species, which can release pollen from the poricidal anthers of potatoes (Scurrah et al., 2008). This sexual reproduction creates an abundance of diversity by recombining the variants of genes that arose through mutation and are carried by the population. The genetically unique seedlings that grow from true seeds produce tubers that can be replanted as seed tubers and hence distinct clones can be established and maintained by asexual (vegetative) reproduction. It is assumed that domestication involved selection for less bitter and hence less toxic tubers, but interestingly Johns and Alonso (1990) found that some genebank accessions of *S. bukasovii* had tuber glycoalkaloid levels which were consistently close to the levels found in many clones of *S. tuberosum* Group Stenotomum. They concluded that exploitation and domestication of this species would have required little or no selection for lower glycolkaloid level, unlike their samples of *S. canasense*, *S. leptophyes* and *S. sparsipilum*.

Andean farmers certainly retained a much wider variety of tuber shapes and skin and flesh colors than is seen in wild species (Simmonds, 1995), and this diversity can only have arisen by mutation under domestication. Furthermore, naturally occurring tetraploid types of potato came to be selected in preference to their diploid ancestors, presumably because farmers found them superior for yield and other traits. Although self-incompatibility does not operate in these tetraploids, sufficient natural cross-pollination occurs for sexual reproduction to continue to maintain high genetic diversity (Brown, 1993). Subsequent selection for appropriate maturity and dormancy, higher yields and harvest index, and resistance to abiotic and biotic stresses must have occurred in many environments. Indeed, a striking characteristic of our modern potato is its high harvest index of 0.81 (the proportion of the whole plant's dry-weight which is harvestable tuber) compared with values as low as 0.09 in some wild species (Hofius and Bornke, 2007).

Although the timescale of domestication is unknown, some of the processes prior to the spread of potato cultivation throughout South America can be envisaged. Early Andean cultures were likely to be 'vertical' ones, moving up and down the mountains with the changing seasons

(Moseley, 2001). It is likely that the herding of animals and the domestication of llama and alpaca preceded the settled cultivation of crops. Seasonal visits to favored sites for food plants would mean that domestication would proceed gradually, with favored types being re-planted to ensure harvests at subsequent re-visits. The stresses of plant life at high altitudes and possibly unstable weather patterns from the El Niño Southern Oscillation may have promoted the development of vegetative storage organs in a number of plant genera in the high Andes. The native peoples domesticating potato also brought into cultivation a range of other roots and tubers, including oca (*Oxalis tuberosa*), mashua (*Tropaeolum tuberosum*) and ulluco (*Ullucus tuberosus*). The duration of domestication is a current debate for crops in general as can be seen in a recent review 'Seeking Agriculture's Ancient Roots' (Balter, 2007). For the potato the rates of each of the stages in the domestication process are unknown, but the extensive abandoned cultivation terraces throughout parts of the Andes suggest that it was a very widespread crop in the Andes prior to the discovery of South America by the Spanish in 1533 (Hawkes, 1990). In contrast, although wild species are native to Central America and Mexico, the cultivated potato seems to have been introduced after the New World was discovered by Columbus in 1492 (Hawkes, 1990).

1.2.3 Cultivated potatoes in Latin America

Following its creation, CIP assembled a collection of more than 15 000 accessions of potato cultivars (landraces) native to nine countries in Latin America: Argentina, Bolivia, Chile, Colombia, Ecuador, Guatemala, Mexico, Peru and Venezuela. Subsequently duplicate accessions were identified and the number of individual cultivars was reduced to 3527 (Huaman et al., 1997). The taxonomy of these cultivated potatoes, like that of their wild relatives, has been reassessed several times in recent decades, and molecular methods are now providing some additional clarity. Dodds (1962) classified cultivated potatoes into five informal groups within one species *S. tuberosum* in which Groups Andigena, Chaucha, Phureja and Tuberosum were derived from Group Stenotomum (Figure 1.1). He also recognized two additional hybrid species involving wild species (*S.* x *curtilobum* and *S.* x *juzepczukii*) to which subsequent authors added *S.* x *ajanhuiri* (Figure 1.2), making eight groups in total. These eight groups are used in the rest of this chapter to avoid ambiguity. Hawkes (1990) gave Groups Andigena and Tuberosum subspecies status and the other six groups species status (Figures 1.1 and 1.2), making seven cultivated species in total. Huaman and Spooner (2002) returned to classifying all eight groups as Groups with a single species *S. tuberosum*. Finally, using molecular data, Spooner et al. (2007) have argued for four species. They regard frost-tolerant *S.* x *ajanhuiri* (diploid), frost-resistant *S.* x *juzepczukii* (triploid) and frost-resistant *S.* x *curtilobum* (pentaploid) as separate hybrid species derived from crosses between domesticates and wild relatives (Figure 1.2). These 'bitter potatoes' are grown at high altitudes (up to 4500 m for *S.* x *juzepczukii*) in the central Andes of Peru and Bolivia (Hawkes, 1990). *S*. x *ajanhuiri* is the hybrid of *S. tuberosum* Group Stenotomum with the wild frost-resistant diploid species *S. megistacrolobum*, *S.* x *juzepczukii* is the hybrid

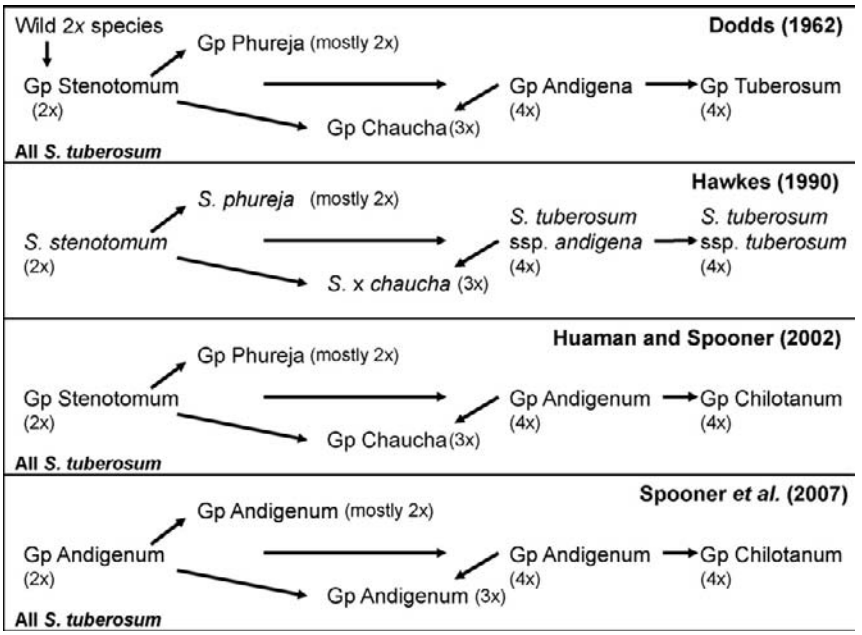

Figure 1.1: Cultivated groups excluding wild species hybrids, named according to Dodds (1962), Hawkes (1990), Huaman and Spooner (2002) and Spooner et al. (2007).

of *S. tuberosum* Group Stenotomum with the wild frost-resistant tetraploid species *S. acaule* and *S.* x *curtilobum* is the hybrid between an unreduced gamete of triploid *S.* x *juzepczukii* and a normal gamete of *S. tuberosum* Group Andigena (Figure 1.2). More controversially, Spooner et al. (2007) regard Groups Andigena, Chaucha, Phureja, Stenotomum and Tuberosum (called by them Chilotanum) as a single species *S. tuberosum*, but now divided into two Cultivar Groups. These are the Andigenum Group of upland Andean genotypes containing diploids, triploids and tetraploids, and the Chilotanum Group of lowland tetraploid Chilean landraces (Figure 1.1). This is controversial because the dendrogram in their paper indicates that Groups Andigena, Phureja,

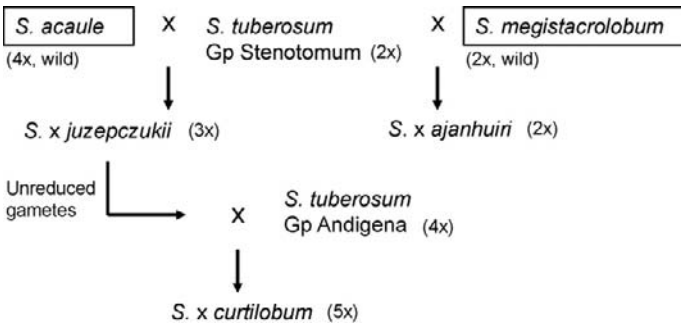

Figure 1.2: Origin of *S.* x *ajanhuiri*, *S.* x *juzepczukii* and *S.* x *curtilobum*; cultivated species with bitter taste and frost tolerance.

Stenotomum and Tuberosum of Dodds (1962) and Huaman and Spooner (2002) can be discriminated, and the rejection of Phureja as a valid group due to polyploid forms within the group does not take into account the specific inclusion of tetraploids in *S. phureja* as described by Hawkes (1990). It is now useful to return to consider how these landraces arose.

First it is necessary to consider how *S. tuberosum* Group Andigena arose from Group Stenotomum. Sukhotu and Hosaka (2006) concluded from chloroplast and nuclear DNA markers that Group Andigena arose from Group Stenotomum through sexual polyploidization from unreduced gametes many times at many places in the fields of Group Stenotomum. These tetraploids were subsequently modified by occasional and unintentional selection of natural hybrids with neighboring wild species to give present-day Group Andigena. Scurrah et al. (2008) have shown that closely related species growing around farmers' fields can hybridize with Group Andigena and that some hybrid progeny would be selected by present-day Andean farmers. These results explain the chromosome behavior and tetrasomic inheritance of tetraploid *S. tuberosum* and why it can be regarded as the autotetraploid of diploid Group Stenotomum for practical purposes. Earlier hypotheses about the origin of tetraploid *S. tuberosum* are referred to by Bradshaw (2007a, b). Hosaka (2004) suggested that Chilean Tuberosum cytoplasm is derived from the southern wild species *S. tarijense* so that Group Tuberosum is not simply Group Andigena that has been selected to tuber in long days. Spooner et al. (2007) show that the T cytoplasm is also found at low frequency in Andean landraces including some diploids, indicating that the T cytoplasm moved northwards as well as becoming predominant in Chilean germplasm. However this does not contradict the view that the Chilean landraces are secondarily derived from the Andean ones and that the long-day adapted landraces of coastal Chile are genetically distinct from the short-day adapted ones of the Andes (Raker and Spooner, 2002).

Returning to the other landraces, *S.* x *chaucha* is the triploid hybrid between diploid *S. tuberosum* Group Stenotomum and tetraploid *S. tuberosum* Group Andigena and, like Group Stenotomum, is confined to the central Andes of Peru and Bolivia. In contrast, *S. tuberosum* Group Phureja (diploid) was selected from Group Stenotomum by Andean farmers for lack of tuber dormancy and faster tuber development so that they could grow up to three crops per year in the lower, warmer, eastern valleys of the Andes. Phureja potatoes were therefore able to spread into northern Ecuador, Colombia and Venezuela and are the second most widely cultivated type in South America, after Andigena, which is grown throughout the upland Andes of South America. Interestingly Ghislain et al. (2006) found that 32 out of 102 accessions of Phureja in the CIP collection of landraces were triploid or tetraploid, not diploid, in agreement with Hawkes (1990) that not all Phureja are diploid. This provides justification for not using ploidy as a species criterion.

1.2.4 Introduction to Europe

It is assumed that Pizarro and his men were the first Europeans to see potatoes being cultivated, in the Andes of Peru in 1533 during the Spanish conquest of the Incas, but there is no written

record (Hawkes, 1990). In fact potatoes were first recorded in 1537 in what is now Colombia (Hawkes, 1990). The first record of cultivated potatoes outside South America is their export in 1567 from Gran Canaria in the Canary Islands to Antwerp in Belgium (Hawkes and Francisco-Ortega, 1993). This was 6 years before they were first recorded in Spain in 1573 in the market archives of the Hospital de La Sangre in Seville, the archives having been checked by Hawkes and Francisco-Ortega (1992). These authors therefore concluded that the potato was first introduced from South America into the Canary Islands around 1562, and from there to mainland Europe (Hawkes and Francisco-Ortega, 1993).

The first water-color painting of a potato is dated 1588 and was sent by Philippe de Sivry, Prefect of Mons in Belgium, to the herbalist Clusius in Vienna in 1589. Clusius had already in 1588 received two tubers and a fruit from de Sivry who in turn had received them from a friend of the Papal Legate in Belgium in 1587 (Hawkes, 1990). Clusius' potato was very late maturing and had red tubers of irregular shape and deep eyes (Salaman, 1926). Potatoes in Chile were first mentioned by Sir Francis Drake in 1578 (Drake, 1628) but as he was on a round-the-world voyage he could not have brought them back to Britain in a living state (Salaman, 1937). The first printed illustration of a potato was by the Englishman, John Gerard, in his Herball of 1597, and it has been suggested, but not proved, that Sir Francis Drake brought this potato to England from Cartegena on the coast of what is now Colombia in either 1586 or 1590 (Glendinning, 1983). Gerard's potato had white-skinned tubers of irregular shape with deep eyes but was not as late in maturity as Clusius' potato (Salaman, 1926). The cooking methods mentioned by Gerard were baking in embers and boiling in water which subsequently became the main method because it is quicker, as little as 15 minutes in boiling water at 100°C compared with 75 minutes baking at 180°C. Interestingly, hot baked potatoes became popular again on the streets of London in Victorian times in the 1850s (Reader, 2008), and are still popular today.

All of the authors mentioned so far concluded that the early introductions of potatoes to Europe most likely came as ships' stores from Colombia and were of Columbian, or possibly Peruvian, origin and hence were primarily tetraploid Group Andigena potatoes. They further concluded that as the growing of potatoes spread north-eastwards across Europe, the potato became adapted to the long summer days of northern Europe and in this respect resembled Chilean potatoes. However, extant Canary Island potatoes comprise both Andean- and Chilean-type landraces and Rios et al. (2007) have suggested that there were multiple early introductions of both types. Furthermore they suggest that the early European potato was selected from the Chilean introductions because they were better adapted to European conditions. Potato introductions from South America were reviewed by Glendinning (1983), but one can not say with certainty how many there were and what their contribution was to the subsequent spread of the potato in and from Europe, as reviewed by Hawkes (1990). It now seems safest to assume that the early introductions of cultivated potatoes to Europe came from both the Andes and coastal Chile (Hosaka et al., 1994; Spooner et al., 2005b; Rios et al., 2007). Analysis of DNA from 49

herbarium specimens has confirmed the presence in Europe of Andean potatoes from around 1700 and Chilean potatoes from 1811 (Ames and Spooner, 2008). Hence the genetic base of modern potato breeding (see below) most likely traces back to potatoes of Andean and Chilean origin. Furthermore, North America's most popular cultivar, Russet Burbank, was derived from cultivar Rough Purple Chili by three generations of open pollination with selection and was released in 1914 (Ortiz, 2001). Rough Purple Chili was a Chilean landrace introduced into the USA in 1851 (Goodrich, 1863) whose descendants were widely employed as female parents in crosses with European and North American cultivars at the end of the 19th century. Chilean Tuberosum cytoplasm predominates in modern cultivars.

1.2.5 Transition to major food crop

After its introduction to Europe, the potato initially remained a botanic curiosity, being grown and studied in physic gardens for interest and medicinal properties. Its potential as a food crop was first seen in Ireland at the end of the 17th century and throughout the 18th century (Burton, 1989). The climate and soil of Ireland proved suitable for the potato but there were also societal and economic reasons for its increase in importance as a food crop. As a consequence, the population of Ireland increased in size from 2 million in 1700 to 8.5 million in 1845 (Reader, 2008). But over-reliance on the potato meant that the late blight epidemics of 1845 and 1846 resulted in famine in Ireland with profound societal consequences. In Britain potatoes were first grown as field crops in the west where there was demand from Irish immigrants. Potatoes were plentiful in these regions by the end of the 17th century and 100 years later were providing the food required by the cheap labor that underpinned the Industrial Revolution (Reader, 2008). Indeed, it was food shortages that proved to be the stimulus to potato cultivation throughout Europe because military and economic strength in the 18th century depended upon adequately fed manpower (Burton, 1989). Frederick the Great of Prussia, for example, in 1744 enforced by decree the cultivation of potatoes in what is now Poland, and in France, Parmentier did much to encourage potato cultivation after famine in 1770. Although the potato was brought to Russia from Holland by Peter the Great at the end of the 17th century, again it was a royal decree by Catherine the Great in 1765 that led to its spread from around St Petersburg to all parts of the country (Hawkes, 1992). In summary, the 18th century saw the potato accepted as a foodstuff throughout Europe and the 19th century saw its ascendancy as a major food crop with boiling the main method of cooking (Burton, 1989), before a decline in potato production and consumption set in during the 20th century. Globally, however, this decline was offset by what happened in the rest of the world.

1.2.6 Spread of potato from Europe to the rest of the world

The 16th century onwards saw the countries of Europe develop widespread political and commercial interests in the rest of the world and the European colonists and missionaries took with them their common crops which came to include potatoes (Burton, 1989). By the late

17th century potatoes had been introduced to the Philippines by the Spanish and by the late 18th century to the Dutch East Indies by the Dutch, to India by the British, to Africa by the Portuguese and to Oceania by the French and British. Earlier dates are possible, for example to India in the 17th century by the Portuguese (Pandey and Kaushik, 2003; Spooner et al., 2005b). Furthermore, the potato had been introduced from England into Bermuda by 1613 and from there to Virginia, in what is now the USA, in 1621. It had also been introduced into Taiwan and coastal China by European missionaries in the 17th century (Pandey and Kaushik, 2003), and likewise into central China by Russian traders also during the 17th century.

The spread of the potato around the world was no doubt followed by farmer selection for better adaptation to the environments in which it was being grown, selection that made use of naturally occurring variation as previously discussed. Potato production expanded worldwide during the 19th century, but it was expansion in China and India during the second half of the 20th century that led to these countries becoming the first and third most important producers in the world.

1.3 Potato Production

1.3.1 Potato breeding

Modern potato breeding began in 1807 in England when Knight made the first recorded hybridizations between varieties by artificial pollination (Knight, 1807), although named cultivars can be traced to the 1730s (Reader, 2008). It flourished in Europe and North America during the second half of the 19th century when exchanges of germplasm started to occur and many new cultivars were produced by farmers, hobby breeders and seedsmen. Even then, the raising of seedlings from seed of self-set berries remained a common practice which continued into the 20th century. Modern potato breeding started later in the 1930s in China and India, but as we have seen, these countries are now two of the leading potato producers in the world with rapid expansion in China since 1978 (Gaur and Pandey, 2000; Jin et al., 2004). During the 20th century potato breeding was helped by incorporating an understanding of the principles of the new science of genetics and by the use of a wider range of germplasm (Bradshaw, 2007a, b). The introgression of genes from wild species into successful cultivars started in 1909 and had been significant but fairly limited in number by the end of the 1980s. Resistance to late blight had been introgressed from *S. demissum* and *S. stoloniferum*, to viruses from these species together with *S. chacoense* and *S. acaule* and to cyst nematodes from *S. vernei* and *S. spegazzinii* (Figure 1.3) (Bradshaw, 2007a, b). Further improvements in the resistance of cultivated potatoes to abiotic and biotic stresses are coming from a greater use of wild species in potato breeding, and molecular-marker-assisted introgression is starting to speed progress. Genes from Andean potatoes have also been introgressed into long-day-adapted cultivars and starting in 1959, a number of programs worldwide have selected populations of Andean potatoes to tuber in long days (Bradshaw, 2007a, b). These biodiverse populations are providing parents suitable for direct incorporation into European and North American potato-breeding

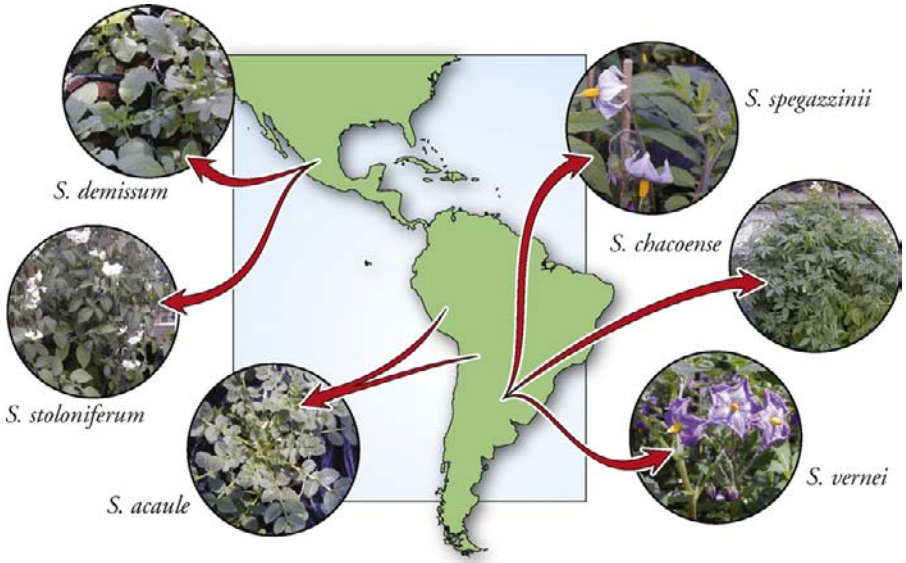

Figure 1.3: Past utilization of wild species in breeding programs in Europe, North America, China and India.

programs, but they require further improvement to achieve greater use in the breeding of successful cultivars.

The extent of progress since 1807 can be judged by the latest World Catalogue of Potato Varieties (Hils and Pieterse, 2007) which lists over 4200 cultivars from more than 100 countries covering all potato-growing regions in the world. Although a much smaller number has been widely grown, this is nevertheless a remarkable achievement for a crop which was unknown outside of Latin America until almost 500 years ago, and which was derived from a narrow genetic base. It represents adaptation of the potato to a wide range of environments and to the end uses which are considered later in this chapter.

Finally, there is a new route to potato improvement, namely the genetic modification of successful cultivars. This has been possible since potato transformation using *Agrobacterium*-mediated systems was developed during the 1980s, first with *A. rhizogenes* (Ooms et al., 1986) and then more successfully with *A. tumefaciens* (Stiekema et al., 1988). The technique is now relatively straightforward, but regeneration of plants can still be a problem with some genotypes (Dale and Hampson, 1995). Targeted improvements can be brought about in three ways. When desirable genes are found in cultivated potatoes and their cross-compatible wild relatives, they can be cloned and introduced by genetic transformation. Where the control of biochemical pathways of interest is understood, down-regulation of gene expression using antisense technology as well as up-regulation using different promoters can achieve desired modifications. Finally, and more controversially, the use of exotic sources of genes in transformation has the clear value

of permitting the introduction of traits not found in cross-compatible relatives, and this can produce novel function. Examples have been summarized by Bradshaw (2007a, b).

1.3.2 Seed production and certification

Asexual reproduction through seed (daughter) tubers allows a genetically unique seedling to be multiplied, maintained and distributed to growers as a new cultivar. However, once potatoes became widely grown in the 19th century, tuber-transmitted disease problems appeared which were 'cured' by producing and growing new stocks from the true seed harvested from naturally occurring berries. This practice accelerated the proliferation of new varieties but it is not clear how much selection was practiced. The most serious disease problems, which were recognized by Salaman (1921) to be the result of virus infections, were less troublesome in some areas and these became seed-growing areas. For example, in Britain, Scotland and Yorkshire became seed-growing regions and seed potatoes were produced by specialist growers and a trade in seed tubers was established. As a result varieties can attain wide and lasting popularity.

Potato yields and quality are certainly best when crops are planted with high-quality disease-free seed tubers. Experience around the world during the 20th century showed that this is most likely to be achieved through statutory seed certification schemes operating in areas where potatoes are grown only for seed. Such areas will usually be geographically and climatically less favorable to the aphid vectors of viruses which cause systemic infection (Jeffries et al., 2006). Meristem culture and micropropagation have now become routine in seed certification schemes and have been reviewed by Wenzel (1994) and Veilleux (2005). Meristem-tip culture can be used in combination with thermotherapy and chemotherapy to eliminate viruses. Rooted plantlets are indexed for virus testing and the virus-free plants are increased by clonal multiplication, starting with *in vitro* axillary node and shoot production. The resulting axillary plants can be used to produce nodal cuttings for *in vitro* production of microtubers, greenhouse production of mini-tubers, and for rooting in soil and transplanting to the field. Field generations of multiplication need to be kept to a minimum to maintain high seed health. It is common practice for the Certifying Authority to hold *in vitro* pathogen-free nuclear stocks of cultivars under multiplication. Finally it is important to point out that strict quarantine procedures are required when potatoes are transferred from one country to another to prevent the introduction of diseases, particularly non-indigenous ones. Again advances with *in vitro* techniques are proving useful.

1.3.3 True potato seed

Most potato cultivars are clonally (vegetatively) propagated through seed (daughter) tubers and are genetically uniform. There are, however, circumstances where cultivars based on current methods of true potato seed (TPS) propagation are an attractive proposition. In the torrid zones of the lowland tropics and subtropics the difficulty of establishing a TPS crop, later maturation and less uniformity can be outweighed by three advantages (Golmirzaie et al., 1994). Seed costs

are reduced due to the much smaller amounts of planting material required. Planting time is flexible because the farmer does not have to consider the physiological age and condition of seed tubers. Finally, tubers are free from tuber-borne diseases with the possible exception of the few caused by true seed-borne viruses. TPS potatoes are established in Bangladesh, China, Egypt, India, Indonesia, Nicaragua, Peru, Philippines, southern Italy and Vietnam (Almekinders et al., 1996; Ortiz, 1997; Simmonds, 1997). Chilver et al. (2005) have recently reviewed on-farm profitability and prospects for TPS and concluded that widespread geographic adoption is unlikely in the immediate future, but that investment in a small but sustained TPS breeding effort can be justified in both China and India. Breeding cultivars for TPS was in fact started back in 1972 by CIP with the aim of high yields and acceptable uniformity. This latter trait is important because none of the current breeding methods can deliver genetic uniformity (Golmirzaie et al., 1994; Ortiz, 1997; Bradshaw, 2007a, b), and TPS cultivars are genetically variable and inferior to the best genotype that exists within the TPS progeny. While TPS can be propagated by direct drilling and transplanting, preference is now given to rapid multiplication by cuttings or shoot-tip culture to give first-generation tubers or mini-tubers that can be chitted before planting (Simmonds, 1997). Methods involving multiplication allow some selection to be practiced within a TPS progeny, and hence are a compromise between vegetative and true seed multiplication.

Synthetic seed is an alternative to TPS that would avoid the need to develop parents that generate uniform hybrids. Hence there is currently renewed interest in somatic embryogenesis in potato, and sufficient progress has been made for serious consideration to be given to exploiting synthetic potato seed, as seen in the review by Veilleux (2005).

1.3.4 Potato growing and storage

The literature is vast on modern methods of planting, growing and harvesting potatoes, and the scientific basis of these methods. The same is true for post-harvest management and controlling the many pests and diseases of potato. The interested reader is referred to the books edited by Gopal and Khurana (2006) and Vreugdenhil (2007). It is, however, worth recalling that the modern fertilizer industry began in the middle of the 19th century and was well established by the end of that century. Likewise, the 19th century saw the start of progress in the chemical control of pests and diseases, but it was the middle of the 20th century onwards that saw the systematic search for new compounds and the establishment of the agrochemical manufacturing of herbicides, insecticides and fungicides (Spedding, 1983).

Average fresh-weight yields vary tremendously by country from 2 to 45 t/ha with a global average of 16.1 t/ha in 2006 (FAOSTAT). In contrast, yields of 120 t/ha have been achieved experimentally in the absence of pests and pathogens and with adequate inputs of water and fertilizers (Mackay, 1996). Many countries now achieving over 40 t/ha have in fact more than doubled their yields during the 20th century through planting better-quality seed tubers, better

cultural practices including irrigation and applications of mineral fertilizers, better control of pests and diseases and cultivars better adapted to the available growing seasons (see Crop Management chapters in Vreugdenhil, 2007). Further increases in stable potato production are certainly going to be needed to meet increased demand for food from population growth, particularly in Asia, Africa and Latin America, during a period of climate change.

In the early 19th century potatoes were stored in unventilated clamps, and still are in some countries for short-term storage of ware potatoes (Gottschalk and Ezekiel, 2006). Potato stores with outside-air cooling systems were first constructed in the 1930s for long-term storage of large quantities of tubers. Stores with electronically controlled environments have been developed since the 1960s, with refrigerated storage the best option for long-term storage. The purpose is to maintain quality as long as possible with minimum losses, particularly weight losses from respiration and transpiration. Cold storage (2–4°C) helps in controlling sprout growth and in maintaining a low respiration rate, and is recommended for seed potatoes. However, low-temperature storage is not suitable for potatoes meant for chips (crisps) or French fries (chips) because low temperatures cause sweetening in most varieties as a result of excessive reducing sugar accumulation. This results in discoloration during frying and acrylamide formation from the Maillard reaction between asparagine and reducing sugars. As there is evidence that acrylamide may be directly or indirectly carcinogenic, processors are under pressure to lower acrylamide concentrations in their finished products, and to this end varieties with lower reducing sugar concentrations could help (Kirkman, 2007; Storey, 2007). This should be feasible either by conventional breeding or through transgenic approaches. Currently processors commonly use warm storage temperatures above 7°C, but the tubers need to be treated with sprout inhibitors. Potatoes for other uses are stored at intermediate temperatures.

1.3.5 Potato processing

Although potatoes are traditionally used as food after baking, boiling or roasting, their commercial value is increased considerably when they are processed into edible products that appeal to consumers on flavor, texture, appearance and most of all convenience (Kirkman, 2007). During the last 100 years potato processing has grown into a global industry which expanded rapidly after the Second World War (1939–1945) and is still expanding.

Ancient chuño production produced the first dehydrated potatoes, but for a modern context we need to move forward to the end of the 18th century. The first European reference (Burton, 1989) to dried potato was by Parmentier in France in 1781 in the form of biscuits made from boiled potatoes for use by sailors. He also discussed methods of drying cooked potatoes in forms to be reconstituted by the addition of boiling water. Although the first US patent for dehydrating mashed potatoes was granted to Edwards in 1845 (Eskew, 1959), it was in wartime in industrialized countries in the 20th century that dehydration was widely practiced. Dehydrated potatoes provided combatant troops with a food that was less bulky than fresh potatoes and easier

to store and prepare as food. In succeeding periods of peace, dehydration declined, except for products which found acceptance as 'convenience foods' in the second half of the 20th century. The reader is referred to Burton (1989) for accounts of traditional Andean *Papa seca*, potato strips and dice, riced potato, potato powder or granules (e.g. mashed potato powder produced in the UK during the second half of the 1939–45 war), potato flakes and potato flour, which is not rehydrated like the other products mentioned. Dehydrated products do deteriorate on storage, but dehydrated potatoes dried to about 6% moisture, with the addition of sulfite, and where necessary antioxidants, can be stored in a fully acceptable condition for at least 6 months in air at room temperature (Burton, 1989).

Today the major processed products are potato chips (crisps) and French fries (chips) and other frozen products, followed by dehydrated products, chilled-peeled potatoes and canned potatoes. Potato processors require potato varieties with specific characteristics to meet demand for quality products, and hence are drivers of modern potato breeding. Appropriate tuber morphology, adequate solids and low glucose content are important as well as freedom from mechanical damage, bruising and internal defects. Processing usually first involves washing, peeling, slicing or cutting the tubers and defect removal. Then further washing for chips and blanching for French fries and other frozen products, drying, and finally frying which is carried to completion in chip manufacture whereas it is partial par-frying in the manufacture of frozen fries and other products. The following brief account is taken from the reviews by Burton (1989), Li et al. (2006) and Kirkman (2007).

Potato chips (crisps) are very thin slices of potatoes (1–1.5 mm) which have been deep-fried at around 180°C until they were dry and brittle, with a finished moisture concentration of 1.3–1.5% to ensure stability of crispness in the product. They are fried in different types of vegetable oil with a range of added flavors. Because of their large surface to volume ratio the uptake and surface retention of fat is considerable at around 40–45% in domestic frying and 36% in commercial frying. This does raise health issues because the World Health Organization (2005) has identified increased consumption of energy-dense foods high in saturated fats as a primary factor in an obesity pandemic that is already affecting 5% of the world's adult population. Global manufacturers of chips and other processed products have mostly eliminated the use of trans-fats (saturated oils) and are launching a range of low-fat and fat composition changes to meet the challenge (Kirkman, 2007).

Potato chips have a US origin dating back to 1853 in a hotel kitchen at Saratoga Springs, New York, but commercial production did not get underway until 1895. Since the 1920s there have been a number of technical innovations which have contributed to current state-of-the-art chip manufacture. These include the mechanical potato peeler (1920s), packaging in sealed bags (1920s), seasoning technology (1950s), microprocessor-controlled weighing heads (1985), optical sorting to remove defective product (1990) and nitrogen-fill to preserve freshness (1995).

The term 'potato chip' also includes Baked Lays® and Pringles® which are formed from a dough made with dehydrated potato, and either baked or flash-fried. Today the international snacks market is dominated by Frito-Lay which has been part of Pepsico since 1965. The company operates approximately 67 plants in 27 countries worldwide. Interestingly, for potato chip manufacture, raw potatoes may be transported long distances whereas finished products have a more limited distribution, reflecting a product with a low weight per unit volume and hence high transport costs.

French fries are made from potatoes that have been cut into thin strips (around 1 cm square in cross-section), washed briefly in cold water, partly dried to remove surface moisture and deep fried in vegetable oil at around 180°C to a light golden color. The final product comprises around 10% fat, most of which is retained on the surface. Frozen fry manufacturers ship their products raw, par-fried, or partially cooked and drizzled with oil for baking, to suit the end user. The product is frozen at −40°C and stored at −20°C. Moisture content needs to be less than 70% in par-fried fries to prevent limpness and separation of the interior and the crust. Heterogeneity in moisture content between strips can result in variability in texture. Frying is finished by immersing the frozen product in deep fat at about 200°C until the desired color and texture are achieved.

Pommes frites became a culinary item in France around 1800 and were introduced into England in the 1860s where they were soon sold with fish, thus establishing a 'fish and chips' tradition which for many years was the most sustaining meal of many poor families. The industrial production of French fries came much later in 1945 and was then developed during the early 1950s. The invention of the industrial process is generally attributed to Jack Simplot of the J. R. Simplot Company of Idaho, USA. Today the global players are McCain, Simplot and Lamb Weston with McCain having 55 plants in 13 countries and markets in 110 countries. In 2004 some 7 million tonnes of frozen product was produced with McDonald's reputed to utilize 27% of the world's production of frozen fries. French fry factories are usually located close to their raw material sources but the finished products may travel long distances to reach the markets, reflecting their relative weights per unit volume. Other frozen potato products include waffles, wedges, hashed brown potatoes, rosti, pre-formed mashed potatoes, patties, potato rounds, diced potatoes, baby roasts and a variety of shaped potato products with child-appeal.

Canned potato production constitutes just a small part of the overall market for processed potatoes. Production of chilled-peeled potatoes became established in Europe during 1995–2005 to supply a growing demand by restaurants, takeaways and the catering business.

1.3.6 Potato starch

Some 65–75% of the dry matter of the potato tuber consists of starch that is readily extractable in water (Burton, 1989). In early 19th century households in potato-growing areas it was

commonly prepared for domestic use, before being displaced by factory-starch. The first potato starch plant was established in the USA in 1831 at Antrim, New Hampshire (Treadway, 1959). Since then the industry has developed in North America and Europe, particularly in the Netherlands, Poland, France and Germany (Burton, 1989), and high dry matter starch varieties have been bred. The main extraction process is wet-milling, including tuber washing, peeling, soaking, disintegration, centrifuging and drying (Li et al., 2006). Potato starch has numerous useful functional properties such as thickening, coating, gelling, adhesion, and encapsulation. Some of these functionalities are unique to the polymer as a result of the structure of linear amylose (17–25% of starch) and highly branched amylopectin and their organization. Today, through effective chemical and physical modifications, potato starch is used in a wide variety of industrial products, including food ingredients, sizing agents for paper and textiles, and starch-based biodegradable plastics (Li et al., 2006). Furthermore, high amylopectin starch has been produced by the down-regulation of the granule-bound starch synthase gene that controls amylose synthesis (Visser et al., 1991) and high amylose starch by concurrently down-regulating two starch-branching enzymes, A and B (Schwall et al., 2000). However, commercialization of these genetically modified potatoes is still to take place. Potato starch can also be used for alcohol production.

1.3.7 Molecular farming

Molecular farming is the use of plant cells to express recombinant genes and produce value-added products, usually proteins. Since the late 1990s, the potato has proved useful for this purpose because of its easy genetic transformation, high productivity, suitability for storage and vegetative propagation. Potato has been used successfully for developing vaccines or medicines against cholera, other bacteria, cancers, hepatitis B, rabbit hemorrhagic virus, enteric virus, Norwalk virus, foot and mouth disease virus, and diabetes (Li et al., 2006).

1.4 Future Trends

In Asia (particularly China and India), Africa and Latin America there is a need for increased potato production to meet increasing demands for food from human population growth during a period of environmental (including climate) change. Hence the overriding need is for increased and stable yields through new cultivars and better agronomy in the widest sense. Although the potato is a wholesome food, further improvements in its nutritional and health properties are worth considering. Thus there is current interest in improving the health of poor people by breeding staple foods that are rich in micronutrients (biofortification), particularly iron, selenium, zinc and beta-carotene (vitamin A). Higher beta-carotene content is now a realistic target for potato given recent success in modifying carotenoid biosynthesis through genetic transformation (Ducreux et al., 2005). Increased protein content and better amino acid balance has also been achieved through transformation with a non-allergenic seed albumin gene (*AmA1*) from *Amaranthus hypochondriacus* (Chakraborty et al., 2000).

In Europe, North America and Oceania, food security has been achieved and their potato industries are trying to increase potato usage in an economically and environmentally sustainable way. The issues facing potato production are the need for economic benefits through more yield of saleable product at less cost of production, whether the potatoes are for processing or table use. Next, there is a need for environmental benefits through a reduced use of pesticides and fungicides and a better use of water and fertilizers, both nitrogen and phosphate. Finally, there is a need to meet consumer demands for convenience foods, nutritional and health benefits, improved flavor and novel products. New cultivars will have an important role to play in achieving these objectives and their production should be greatly helped by the genetic knowledge which is expected from sequencing the potato genome, a task that will be completed by around 2010 (www.potatogenome.net). Targets will be improvements in built-in resistances to pests and diseases, better water and mineral use efficiency, and improved quality and health-promoting attributes. Two examples of the latter are as follows. Varieties with a low GI could be of value in lowering the glycemic load of the Western diet, thus decreasing the risk of type-2 diabetes, cardiovascular disease and obesity (Storey, 2007). The introduction of inulin to potato is another way to improve its nutritional value because compounds such as inulin reduce the energy density of food and are used to enrich food with dietary fiber or to replace sugar and fat. Hellwege et al. (2000) have developed transgenic potato tubers that synthesize the full range of inulin molecules naturally occurring in globe artichoke (*Cynara scolymus*).

In summary, understanding the factors that affect the chemical composition of the potato is going to be of increasing importance in ensuring that it continues to make a major contribution to providing humans with adequate supplies of wholesome food.

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CHAPTER 2

Breeding, Genetics, and Cultivar Development

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2.1 Introduction

The potato is widely grown worldwide and ranks first in production among vegetable crops. Its popularity is due to its high yield, nutritional quality, and broad array of culinary uses. Until the early 1900s, potato breeders grew seed from open-pollinated fruits, which likely resulted from a mixture of self- and cross-pollination. They inevitably selected for day-length adaptation, disease resistance, and high yield. In addition, they probably also selected for compact plants, large tubers, and smooth tuber shape. These breeding goals are similar to those of modern potato breeders. However, breeders today must also consider processing traits and, increasingly, nutritional quality. During the past 150 years, potato breeders have developed cultivars with earlier maturity, smoother tubers, and improved processing quality (Douches et al., 1996; Love et al., 1998). However, it is a surprise to note that total yield has not improved over time.

2.2 The Germplasm Resource

Wild *Solanum* species are found in 16 countries, from the southwestern United States to central Chile (Figure 2.1) (Spooner and Salas, 2006). The greatest diversity of species is found in central Mexico at 20°N, and in the southern hemisphere, especially in the highlands of the Andes between 8° and 20°S (Hijmans and Spooner, 2001). Wild potatoes grow from sea level to 4300 m, but are most commonly found at altitudes of 2000–4000 m. Collectively, these species represent a more diverse and accessible germplasm resource than in any other crop (Ross, 1986; Hanneman, 1989; Peloquin et al., 1989; Hawkes, 1990). Wild species are adapted to a much wider range of habitats than the cultivated potato. They are found in a tremendously diverse array of environments, including the cold high grasslands of the Andes, hot semi-desert and seasonally dry habitats, humid subtropical to temperate mountain rainforests, cultivated fields, and even as epiphytes in trees (Figure 2.2) (Hawkes, 1990; Hijmans et al., 2002). These wild species contain genes encoding numerous traits not found in cultivars and represent an especially rich source of disease-resistance and tuber-quality genes (Hanneman, 1989; Spooner and Bamberg,

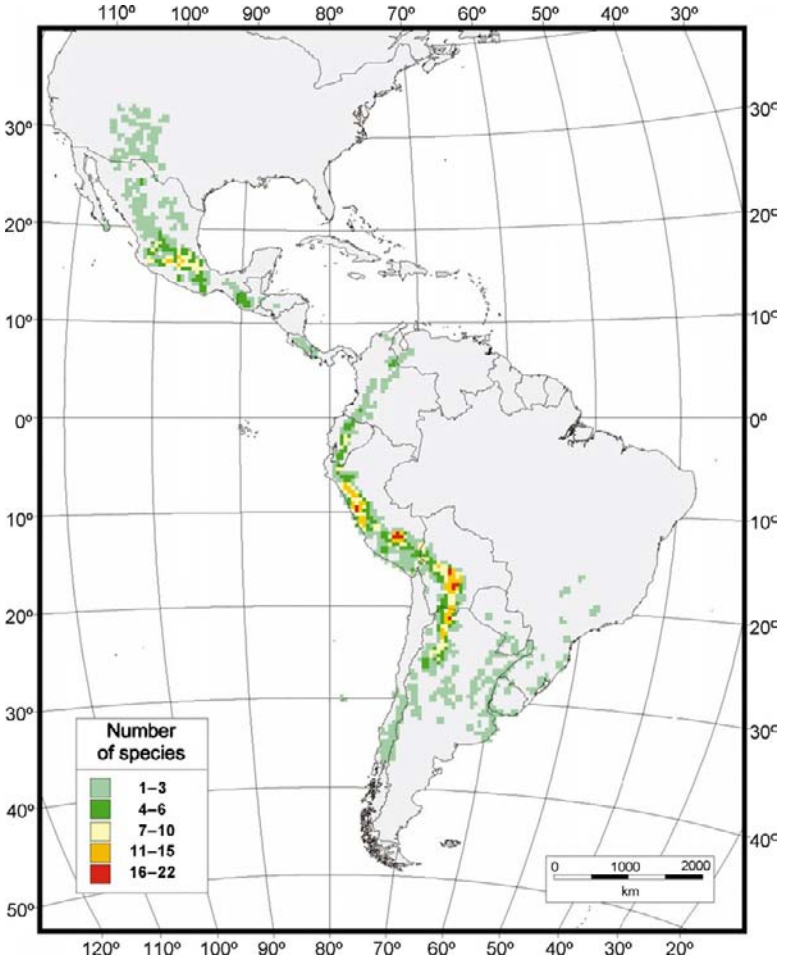

Figure 2.1: The distribution of wild *Solanum* species in the Americas.

1994; Jansky, 2000). About 64% of wild *Solanum* species are diploid ($2x = 24$), with most of the remaining species tetraploid ($4x = 48$) or hexaploid ($6x = 72$) (Hijmans et al., 2007).

The wild and cultivated relatives of potato are extensively represented in gene banks throughout the world. Potato gene banks include the International Potato Center (CIP, Lima, Perú), United States Potato Introduction Project (NRSP-6, Sturgeon Bay, Wisconsin,USA), Dutch German Potato Collection (CGN, Wageningen, The Netherlands and BGRC, Braunschweig, Germany), Institute of Plant Genetics and Crop Plant Research (GLKS, Gross Luessewitz, Germany), Commonwealth Potato Collection (CPC, Dundee, Scotland), N.I. Vavilov Institute (VIR, St. Petersburg, Russia), and the Instituto Nacional de Tecnología Agropecuaria (INTA, Balcarce, Argentina).

Figure 2.2: A wild *Solanum* species (*S. bukasovii*) in its native habitat in the Andean highlands.

In disease and tuber-quality screening studies, significant variation exists among accessions within a species and even among plants within an accession. Therefore, while a species may be characterized as resistant to cold sweetening, for example, it is important to realize that not every plant in that species carries that trait. A fine screening evaluation must be used, in which individuals within resistant accessions are screened and then the most resistant individuals are maintained clonally for use by breeders (Bamberg et al., 1996; Douches et al., 2001; Jansky et al., 2006; Zlesak and Thill, 2004; Jansky et al., 2008).

The cultivated potato contains low levels of glycoalkaloids, typically solanine and chaconine, which are bitter and can be toxic at high levels. High levels of glycoalkaloids were selected against during the domestication of potato (Johns and Alonso, 1990). However, wild potato species may contain high levels of glycoalkaloids, some of which are not found in the cultivated potato (Friedman, 2006). It is important, therefore, for breeders to continue to monitor glycoalkaloid levels in tubers when wild species have been introgressed into the cultivated potato. Because a number of glycoalkaloids are produced by complex biochemical pathways in potato, QTL analysis has been employed to elucidate the genetic control of this trait. Major QTLs have been identified on several chromosomes (Yencho et al., 1998). Chromosome I seems to be an especially important source of genes involved in controlling glycoalkaloid levels (Sørensen

et al., 2008). It is interesting to note that QTLs for α-solanine and α-chaconine content were mapped to the same location as those for total glycoalkaloid content.

2.3 Reproductive Biology

2.3.1 Self incompatibility

Almost all diploid *Solanum* species are self-incompatible due to a gametophytically controlled system (Swaminathan and Howard, 1953; Pandey, 1962; Cipar et al., 1964). A highly polymorphic locus, called the *S* gene, expressed in pollen tubes as they grow through the style, prevents the fertilization of an egg cell by a sperm cell of the same genotype. The style produces an S-RNase that prevents the normal growth of genetically matching pollen tubes (Luu et al., 2000). Therefore, attempts to self-pollinate most wild diploid *Solanum* species fail. On the other hand, polyploids, including potato cultivars, are capable of self-fertilization. The pistil is capable of inhibiting pollen, but pollen tubes are incapable of eliciting an incompatibility reaction (Levin, 1983).

Genetic systems that overcome self-incompatibility in diploid potatoes have been reported in two germplasm sources. Hybrids between cultivated diploid potatoes and an *S. tuberosum* haploid (US-W4) were reported to be self-compatible due to the action of a dominant self-incompatibility inhibitor in US-W4 (De Jong and Rowe, 1971). Three generations of selfed individuals were generated. A dominant self-incompatibility inhibitor (*Sli*) has been reported in the wild diploid species *S. chacoense* (chc) (Hosaka and Hanneman, 1998a, c). This gene has been mapped to the distal end of chromosome 12 (Hosaka and Hanneman, 1998b). The *Sli* gene is independent of the *S* locus, which maps to chromosome 1 (Tanksley and Loaiza-Figueroa, 1985). The *Sli* gene has been transferred to a number of diploid genotypes, allowing them to be self-pollinated (Phumichai et al., 2006).

2.3.2 Unilateral incompatibility

Unilateral incompatibility is a phenomenon in which self-compatible species can be crossed as a female, but not as a male, to self-incompatible species (Abdalla and Hermsen, 1972). Pollen tubes fail to penetrate stylar tissue in self-incompatible (female) X self-compatible (male) crosses. Although most diploid *Solanum* species are self-incompatible, the Mexican species *S. verrucosum* is self-compatible. Dinu et al. (2005) found that *S. verrucosum* could be crossed as a female, but not as a male, to self-incompatible species. It is sometimes possible to find exceptional plants that do not exhibit unilateral incompatibility in self-incompatible X self-compatible interspecific crosses (Pandey, 1962). The identification of such plants allows a breeder to overcome the unilateral incompatibility crossing barrier. For example, exceptional plants (‘acceptors’) that accept *S. verrucosum* pollen and produce fertile hybrids have been reported (Eijlander et al., 2000). It is interesting that some ‘acceptor’ plants will accept pollen

of any *S. verrucosum* plant, while others only accept pollen from certain *S. verrucosum* plants (Hermsen 1978).

2.3.3 Male sterility

Male sterility due to deleterious nuclear genes is common in the cultivated potato (Howard, 1970). Because the marketable product is not seed, there is no selection pressure for high fertility in breeding programs. In fact, fruit development may partition resources away from tuber yield, so breeders may inadvertently select against high fertility (Jansky and Thompson, 1990). In addition, deleterious recessive alleles can accumulate in tetraploid potato cultivars because they are more easily masked than in diploids.

Potato also exhibits male sterility due to interactions between cytoplasmic genes and nuclear genes. Cytoplasmic-genetic male sterility has been reported in a number of interspecific hybrids (Dionne, 1961a, b; Grun et al., 1962; Grun and Aubertin, 1966; Grun, 1970; Abdalla and Hermsen, 1973; Hermundstad and Peloquin, 1985b; Tucci et al., 1996; Santini et al., 2000; Phumichai and Hosaka, 2006). For example, crosses between Chilotanum Group haploids and cultivated Andigenum Group clones produce male fertile hybrids when the haploids are the male parent, but male sterile hybrids when the haploids are the female parent (Grun et al., 1962; Ross et al., 1964; Carroll, 1975).

Levels of cytoplasmic genetic male sterility are frequently variable, presumably due to genetic and environmental influences (Hanneman and Peloquin, 1981). Breeders can, therefore, overcome this type of sterility by either carrying out reciprocal crosses or selecting parents that do not contain sensitive cytoplasm or dominant nuclear sterility genes (Iwanaga et al., 1991; Tucci et al., 1996). In addition, fertility restorer genes may be identified. For example, there is a dominant gene (*Rf*) that restores fertility to plants that contain the dominant male sterility gene (*Ms*) in the presence of sensitive cytoplasm (Iwanaga et al., 1991). Selection of Chilotanum Group haploids carrying the *Rf* gene allows for the production of male fertile offspring even when the male parent contains the dominant male sterility gene *Ms*. Tucci et al. (1996) also identified a male fertility restorer gene in a haploid X *S. chacoense* hybrid.

2.3.4 2n gametes

Most polyploid crop plants originated from the union of numerically unreduced (2n) gametes. These gametes are produced in plants carrying meiotic mutations. These mutations interrupt meiosis so that gametes contain the parental (sporophytic) chromosome number rather than half that number. These meiotic mutations occur naturally and frequently in cultivated and wild potatoes (Peloquin et al., 1999; Carputo et al., 2000a). Some meiotic mutations result in the production of 2n eggs (Stelly and Peloquin, 1986b; Werner and Peloquin, 1991a), while others produce 2n pollen (Quinn et al., 1974; Mok and Peloquin, 1975b). As discussed later, a cross

between a tetraploid and a 2n gamete-producing diploid will produce tetraploid offspring. 2n pollen is easily detected microscopically because diploid pollen grains are larger than monoploid pollen grains (Quinn et al., 1974). 2n eggs can also be detected microscopically via a stain clearing technique (Stelly et al., 1984), but this is a laborious procedure and not practical for large-scale screening. Diploid clones that produce 2n eggs can be identified by simply crossing them as females to tetraploids (Erazzú and Camadro, 2007). If seeds are produced, then 2n eggs were present in the diploid parent.

The cytological change that results from a meiotic mutation produces dramatic genetic consequences. Normally, in anthers, the four products of meiosis are separated so that their poles define a tetrahedron. Cytokinesis then produces four haploid microspores. In contrast, one type of meiotic mutant called parallel spindles produces two microspores, each with an unreduced (sporophytic) chromosome number. The first division is normal, but in the second division, the spindles are parallel. When cytokinesis follows, two diploid microspores are produced. Even though the first meiotic division occurs in this mutant, the genetic result of parallel spindles is equivalent to first division restitution (FDR) because gametes contain non-sister chromatids from the centromere to the first crossover. The parallel spindles genotype exhibits variable expressivity and incomplete penetrance (Mok and Peloquin, 1975a). Consequently, not all gametes of a mutant plant are 2n, and not all plants carrying the mutation produce 2n pollen. While the genetic consequence of 2n pollen formation in potato is typically FDR, that of 2n egg formation is second division restitution (Stelly and Peloquin, 1986a; Werner and Peloquin, 1990). Second division restitution gametes contain sister chromatids from the centromere to the first crossover.

The genetic consequences of FDR 2n gametes are very different from those of SDR 2n gametes. In an FDR 2n gamete, all loci from the centromere to the first crossover on each chromosome have the same genetic constitution as the parent of that gamete. That is, all dominance (intralocus) interactions up to the first crossover are maintained in the gametes (Figure 2.3). Even in the chromosomal region beyond the first crossover, half of the loci that were heterozygous in the parent will remain so in 2n gametes. Since potato chromosomes are small, there is typically only one crossover per chromosome (Yeh et al., 1964). Consequently, FDR 2n gametes provide a unique and powerful method of transmitting blocks of advantageous dominance (intralocus) and epistatic (interlocus) interactions to polyploid offspring even following meiosis, which usually breaks up such interactions. In contrast, SDR 2n gametes contain non-sister chromatids from the centromere to the first crossover. While FDR 2n gametes transmit 80% of the diploid parent heterozygosity to tetraploid offspring, SDR 2n gametes transmit less than 40% (Peloquin, 1983).

2.3.5 Endosperm Balance Number

In angiosperms, double fertilization results in the production of an embryo and endosperm, both of which are critical for the development of viable seed. Diploid plants produce diploid embryos and triploid endosperm tissue. The endosperm contains two genomes of the maternal parent and

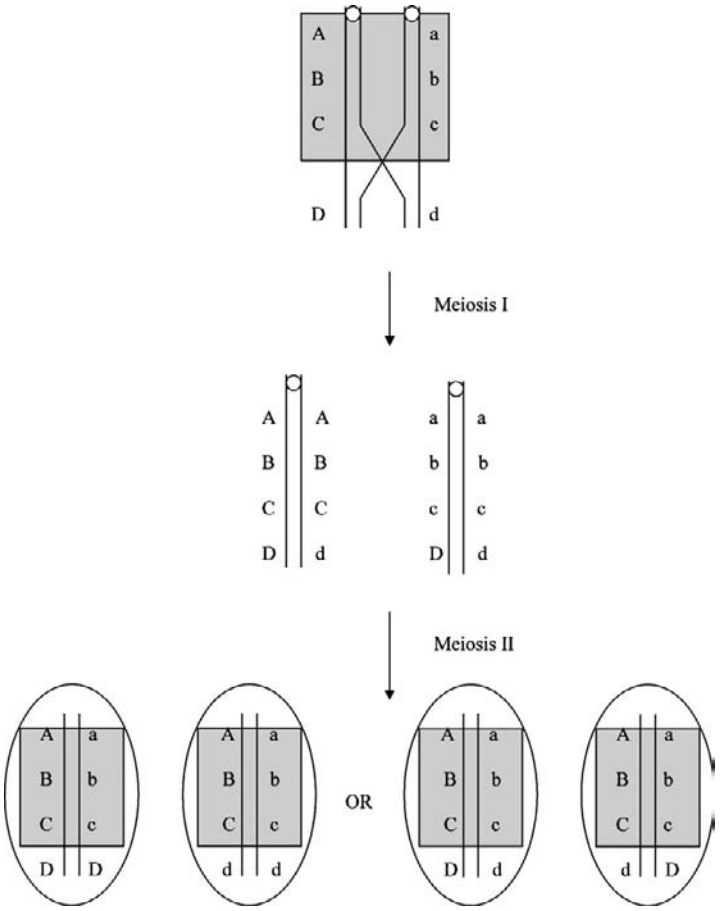

Figure 2.3: All genetic interactions from the centromere to the first crossover are transmitted to offspring in gametes produced by a first division restitution mechanism such as parallel spindles.

one genome of the paternal parent. Intraspecific intraploidy crosses in potato typically produce viable seeds containing well-developed endosperm. Conversely, in most interploidy crosses, inviable seeds are produced due to endosperm failure (Brink and Cooper, 1947). However, endosperm may also fail to develop adequately in some intraploidy, interspecific crosses, while some interploidy crosses succeed. A 2:1 maternal:paternal ratio of endosperm balance factors, rather than genomes, is necessary for normal endosperm development in potato (Johnston et al., 1980). The nature of these endosperm balance factors has yet to be elucidated, although genetic models have been proposed (Ehlenfeldt and Hanneman, 1988; Camadro and Masuelli, 1995). *Solanum* species have been assigned endosperm balance numbers (EBN) based on their ability to hybridize with each other (Hanneman, 1994). Barring other crossing barriers, viable seeds will be produced from crosses between plants with matching EBN values. This will produce a

2:1 maternal:paternal ratio of endosperm balance factors after fertilization of the central cell to produce endosperm. The most common ploidy, EBN combinations in potato are 6x (4EBN), 4x (4EBN), 4x (2EBN), 2x (2EBN) and 2x (1EBN).

Breeders use EBN values to determine whether interspecific crosses will succeed. The EBN concept also allows them to design strategies to access wild germplasm by manipulating EBN (Johnston et al., 1980). Endosperm balance number can be increased through somatic doubling (Ross et al., 1967; Sonnino et al., 1988) or the production of 2n gametes. Endosperm balance number can be reduced through anther culture or parthenogenesis (discussed below).

An example will serve to illustrate the value of the EBN concept to access wild *Solanum* germplasm. Triploid hybrids were formed between the Mexican 4x (2EBN) species *S. stoloniferum* and 2x (2EBN) cultivated diploids, both of which were selected for 2n gamete production (Brown, 1988). These triploid plants produced fertile 2n (3x, 2 EBN) pollen, allowing them to be crossed onto 4x (4EBN; gametes 2 EBN) cultivated potato clones (Brown and Adiwilaga, 1990). The majority of offspring were pentaploid, as expected if the functional pollen grains from the triploid parent were numerically unreduced (2n). The advantage of producing triploids rather than hexaploids in this cross is that homoeologous chromosome pairing (between the two species) is more likely to occur in triploids, increasing opportunities for introgression of chromosomal segments of the wild species into the cultivated genome.

It is important to note that, while knowledge of EBN and 2n gamete production allows for successful cross prediction most of the time, there are exceptions. Sometimes intra-EBN crosses fail and, at other times, inter-EBN crosses succeed even without the presence of 2n gametes. EBN is only one component of a complex system of pre- and post-zygotic interspecific crossing barriers (Masuelli and Camadro, 1997; Chen et al., 2004).

2.3.6 Haploids

Haploids are sporophytes with the gametophytic chromosome number. Haploids (2x) of tetraploid potato cultivars provide a mechanism for direct gene transfer from wild 2x relatives and allow breeders to work at the diploid level. As discussed below, selection progress is more rapid with diploids than tetraploids. Haploids can also be used to measure the genetic load in the tetraploids from which they are derived, since deleterious alleles hidden in tetraploids are often expressed in haploids.

Haploids can be produced from tetraploid cultivars and breeding clones via parthenogenesis (Hougas and Peloquin, 1957). When a tetraploid is crossed with any of several selected diploid clones, some of the offspring are diploid. In these crosses, both sperm cells from the pollinator enter the central cell, allowing normal endosperm to develop. This stimulates the division of the egg cell in the absence of fertilization, resulting in the production of a haploid (2x) embryo

(von Wangenheim et al., 1960; Montelongo-Escobedo and Rowe, 1969; Peloquin et al., 1996). Sometimes, functional 2n pollen in the pollinator produces tetraploid offspring. It is important to distinguish between seeds that resulted from fertilization of the egg cell by 2n pollen (which would be tetraploid and undesirable) and those that were not (and are therefore haploids). The pollinators are homozygous for a dominant seed spot marker, so seeds expressing the marker are discarded and those lacking the marker are retained with the expectation that they are haploids (Peloquin and Hougas, 1959; Hermsen and Verdenius, 1973).

Populations of haploids provide unique opportunities for the genetic analysis of polygenic traits. A population of haploids from a single highly heterozygous tetraploid clone represents a random pool of female gametes. Genetic analyses can be carried out on this population without the confounding effects of fertilization. In addition, genetic variability hidden in polyploids can be revealed in populations of haploids (Peloquin et al., 1991). As a result of segregation, haploids may express traits that were not found in their tetraploid parents. Haploid populations have been used to characterize the genetic basis of total tuber yield, average tuber weight, tuber number, dry matter content, tuber dormancy, vine maturity, and tuber glucose levels (Kotch et al., 1992).

Breeders usually select haploid parents based on traits useful for the generation of hybrids (fertility, vigor, profuse flowering) and for the ability to produce offspring with high-quality tubers (Yerk and Peloquin, 1990b; Werner and Peloquin, 1991b). Genetic variation among haploids for plant and tuber traits is common and has been widely reported (Peloquin and Hougas, 1960; DeMaine, 1984; Matsubayashi, 1979; Rousselle-Bourgeois and Rousselle, 1992; Hutten et al., 1995b). Disease resistance traits are also variable among haploids, with some haploid clones exhibiting better resistance than their parents. Haploids with resistance to Verticillium wilt, soft rot, common scab, blackleg, potato virus X, and potato cyst nematode have been reported (Hutten et al., 1995b; Carputo et al., 1996; Jansky et al., 2003; Ercolano et al., 2004).

Tetraploid potatoes are typically more vigorous and higher yielding than their haploid offspring (Peloquin and Hougas, 1960; DeMaine, 1984; Kotch 1987). The loss of vigor and yield in haploids is due to ploidy reduction and inbreeding depression. The magnitude of this loss at the diploid level differs depending on the tetraploid clone from which the haploids were derived (Kotch, 1987).

Potato monoploids (1x) can be produced from diploids via anther culture (Veilleux et al., 1985) or pollination (Uijtewaal et al., 1987). While the production of monoploids through anther culture is possible, it can be difficult because it requires the presence of genes for androgenic competence ('tissue culturability'), which is not found in all potato cultivars (Sonnino et al., 1989). A 'monoploid sieve' selects against deleterious recessive alleles, allowing only the genotypes with high fitness values to develop into monoploid plants. These monoploids can be somatically

doubled to produce homozygous diploids for heterosis breeding as described later (Lightbourn and Veilleux, 2007).

2.4 Germplasm Enhancement

2.4.1 Haploid-species hybrids

Haploids (2x) of tetraploid potato cultivars are commonly used to access the germplasm of wild diploid *Solanum* species (Jansky et al., 1990). These haploids readily cross with most diploid *Solanum* species, often producing fertile interspecific hybrids (Hermundstad and Peloquin, 1985a; Yerk and Peloquin, 1989). Wild *Solanum* species require a short critical photoperiod for tuberization, so they produce stolons rather than tubers under the long day conditions of summer in northern temperate regions (Rudorf, 1958). However, when these wild species are crossed to haploids of the cultivated potato, the majority of the resulting hybrids tuberize under long days (Figure 2.4) (Hermundstad and Peloquin, 1986; Yerk and Peloquin, 1989). Haploid–wild species hybrids therefore allow breeders to capture valuable genes from wild species in an adapted form that can be maintained clonally as tubers. Both the haploid parent (Hermundstad and Peloquin, 1986; Yerk and Peloquin, 1989) and the wild species parent (Yerk and Peloquin, 1989) influence the photoperiod adaptation in hybrid families.

It is difficult to evaluate the contribution of wild *Solanum* species for tuber traits such as nutritional quality because they do not tuberize in the field in temperate zone production areas. However, tremendous heterosis for yield is often observed in haploid–wild species hybrids (Leue, 1983; Hermundstad and Peloquin, 1986; Santini et al., 2000). The high yield and large tuber size in hybrids allows breeders to determine the contributions of wild species to tuber traits such as dry matter content, dormancy, starch composition, nutritional components, and processing quality (Yerk and Peloquin, 1989; Janskv et al., 1990; Yerk and Peloquin, 1990a; Serquén and Peloquin, 1996; Rousselle-Bourgeois and Rousselle, 1992; Santini et al., 2000; Oltmans and Novy, 2002; Ortega and Carraso, 2005). Surprisingly, even though half of the genes in these hybrids are from wild species, they exhibit acceptable tuber color, shape and size. In contrast, hybrids between cultivated diploid relatives produce tubers with irregular shape, deep eyes, and short dormancy (De Jong and Tai, 1977). In addition to variation for tuber traits, haploid–wild species hybrids exhibit useful variation for disease resistance and stress tolerance (Watanabe et al., 1995; Carputo et al., 1996; Tucci et al., 1996; Carputo, 2000; Jansky and Rouse, 2000, 2002; Ortega and Carraso, 2005).

Haploid–wild species hybrids are generally male fertile, although fertility varies by wild species parent (Leue, 1983; Hermundstad and Peloquin, 1985b; Yerk and Peloquin, 1988; Tucci et al., 1996). In contrast, when the cultivated diploids from Phureja and Stenotomum Groups are used as male parents in crosses to haploids, their offspring exhibit cytoplasmic-genetic male sterility (Ross et al., 1964; Grun and Aubertin, 1966). Reciprocal crosses, using pollen from haploids,

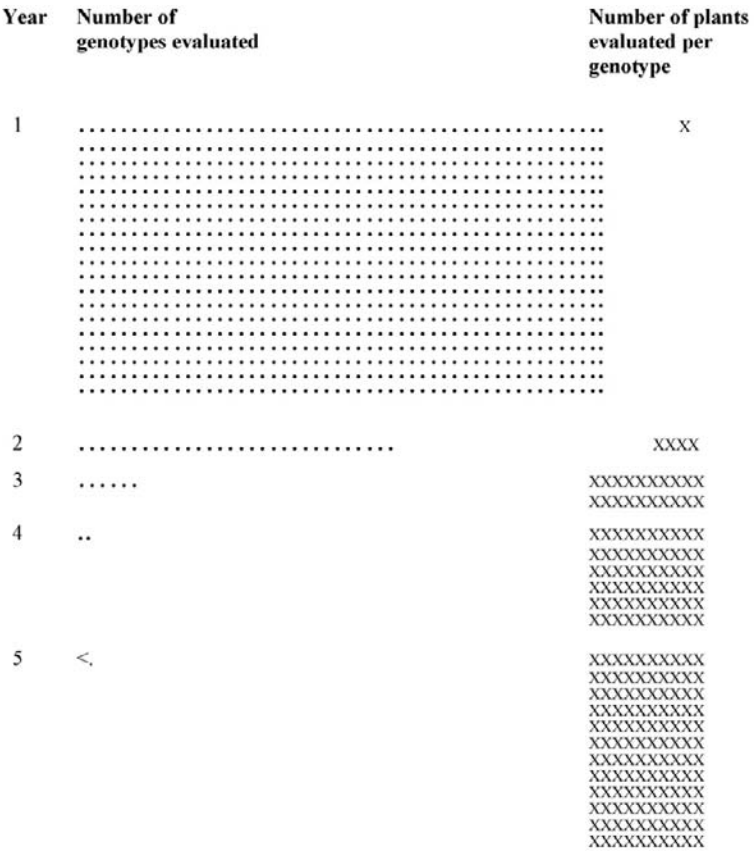

Figure 2.4: Selection intensity in a typical potato breeding program. Each dot represents 100 genotypes. Each x represents a single plant.

produce male fertile hybrids. However, many haploids are male sterile and cannot be used as pollen parents (Peloquin and Hougas, 1960).

2.4.2 Sexual polyploidization

While the development of potato cultivars at the diploid level sounds appealing, it is not likely to be successful. In potato, intralocus (dominance) and interlocus (epistasis) interactions are necessary to maximize yield (Mendoza and Haynes, 1974b). Tetraploidy offers many more opportunities to create such interactions. Two options are available to bring diploid hybrids to the tetraploid level. First, they can be somatically doubled through chemical means such as colchicine (Ross et al., 1967) or through tissue culture (Sonnino et al., 1988). However, tetraploids produced by this method do not exhibit a yield increase, because new interlocus and intralocus interactions are not created (Rowe, 1967; Maris, 1990; Tai, 1997). Somatic doubling

can produce only one type of heterozygote (duplex-AAaa) and a maximum of two alleles per locus. An alternative method to double chromosome number is through sexual polyploidization (Chase, 1963) using 2n gametes. Unilateral sexual polyploidization results from polyploidization of one parent, while the other parent is already at the polyploid level (4x female X 2x male or 2x female X 4x male to produce 4x offspring). Bilateral sexual polyploidization results from polyploidization of both parents (2x X 2x to produce 4x offspring). Triploid offspring are not produced from these crosses due to endosperm failure. Sexual polyploidization can produce three types of heterozygotes (simplex-Aaaa, duplex-AAaa, and triplex-AAAa) and up to four alleles per locus. Complex combinations of triallelic ($A_1A_2A_3A_3$) and tetraallelic ($A_1A_2A_3A_4$) loci can also be produced. In addition, sexual polyploidization produces a wide array of complex epistatic (interlocus) interactions.

Initial studies of sexual polyploidization in potato focused on tuber yield and quality. A high degree of heterosis for yield has been demonstrated following unilateral sexual polyploidization in which the tetraploid female parent is typically a potato cultivar or advanced breeding selection and the diploid male parent is a haploid X wild species hybrid or a cultivated diploid X haploid hybrid (De Jong et al., 1981; Bani-Aameur et al., 1991; Tai and De Jong, 1991; Buso et al., 1999a, 2000, 2003; Alberino et al., 2004). Yield heterosis is realized in 4x X 2x crosses because the diploid parent contributes allelic diversity and 2n gametes transmit a large proportion of heterozygous loci and epistatic interactions to the tetraploid offspring. Another advantage of the USP breeding scheme is that it provides the opportunity to select high-yielding clones with good quality from relatively small segregating populations (Concílio and Peloquin, 1991; Buso et al., 1999b). This is because a much larger proportion of clones from 4x X 2x crosses exhibit high tuber yield and acceptable tuber appearance than from 4x X 4x crosses. Products of unilateral sexual polyploidization also typically exhibit high levels of yield stability across environments, presumably due to the buffering provided by allelic diversity (Darmo and Peloquin, 1990; Ortiz et al., 1991). This is likely to result in more effective selection in early generations of a breeding program.

Recent studies have focused on the use of sexual polyploidization to transfer additional traits, including stress tolerance, processing quality, and disease resistance, to tetraploid offspring. Stable resistance to internal heat necrosis and high specific gravity have been reported in progeny of 4x X 2x crosses (Sterrett et al., 2003). Tetraploids with good chip color and resistance to cold sweetening have been produced via 4x X 2x crosses (De Jong and Tai, 1991; Hutten et al., 1996; Hayes and Thill, 2002a). Unilateral sexual polyploidization has successfully created hybrids with resistance to bacterial wilt (Watanabe et al., 1992), early blight (Herriott et al., 1990), common scab (Murphy et al., 1995), potato cyst nematode (De Maine et al., 1986; Ortiz et al., 1997), Verticillium wilt (Frost et al., 2005), and soft rot (Carputo et al., 2000; Capo et al., 2002). Tetraploids with Colorado potato beetle resistance have been created by crossing tetraploids with diploids containing the cry3Aa transgene (Johnson and Veilleux, 2003; Johnson

et al., 2003). Iwanaga et al. (1989) crossed root knot nematode-resistant diploids to tetraploid 'Atzimba' and to a haploid (2x) of 'Atzimba,' both of which are susceptible to the nematode. A significantly higher proportion of resistant offspring (25%) was obtained in the 4x X 2x crosses than in the 2x X 2x crosses (11%). Presumably, nematode resistance alleles at loci between the centromere and the first crossover on each chromosome were transferred to offspring intact in FDR 2n pollen in the 4x X 2x crosses, but those alleles segregated randomly in the formation of normal pollen in 2x X 2x crosses. This may also explain why 2n gametes transmit resistance to bacterial wilt, root-knot nematodes, late blight, and glandular trichomes to a high proportion of 4x X 2x offspring (Watanabe et al., 1999).

It is interesting that dramatic differences are often observed between 4x (female) X 2x and 2x (female) X 4x crosses. This is presumably because the diploid parent in a 4x X 2x cross produces 2n pollen via a first division restitution mechanism, while that in a 2x X 4x cross produces 2n eggs via a second division restitution mechanism. As discussed above, the genetic consequences of first division restitution mechanism are very different than those of second division restitution. Offspring from 4x X 2x crosses produce higher yields and tuber dry matter content than those from 2x X 4x crosses (Kidane-Mariam and Peloquin, 1974; Hutten et al., 1994). However, there is no difference between the cross types for vine maturity and chip color.

Bilateral sexual polyploidization provides an alternative sexual polyploidization strategy. In this scheme, both parents are diploid and produce 2n gametes. The potential advantage of bilateral sexual polyploidization is that highly heterotic offspring can be produced by crossing diverse diploid parents. The disadvantage is that both parents must produce 2n gametes. In addition, since the meiotic mutations that produce 2n gametes exhibit variable expressivity (n and 2n gametes are produced by the same plant), both diploid and tetraploid offspring will be produced. Tetraploid progeny from bilateral sexual polyploidization are highly heterotic and typically outyield their diploid full-sibs (Mendiburu and Peloquin, 1977; Hutten et al., 1995a) and even tetraploid commercial cultivars (Werner and Peloquin, 1991c). The yield gains from bilateral sexual polyploidization are typically higher than those from unilateral sexual polyploidization, presumably due to the contributions of heterozygosity from both parents (Werner and Peloquin, 1991c).

2.4.3 Polyploidization by somatic fusion

The cultivated potato and many of its relatives are amenable to cell and tissue culture procedures, including protoplast isolation and fusion. Somatic cell fusions have frequently been used to combine the genomes of *Solanum* species that are sexually incompatible because of pollen–stylar interactions or mismatched EBN numbers. Somatic fusion circumvents sexual reproduction and results in novel combinations of not only nuclear genomes, but also cytoplasmic genomes (Trabelsi et al., 2005; Bidani et al., 2007; Lovene et al., 2007). However, recalcitrant genotypes

are common, so some combinations can not be made. In addition, genetic recombination may not occur in some somatic hybrids, limiting introgression of foreign genes.

A common somatic fusion strategy fuses protoplasts of tetraploid cultivars with those of sexually incompatible diploid wild species. The resulting hexaploid hybrids are often fertile and crossable with the tetraploid cultivars (Austin et al., 1987; Fish et al., 1988; Helgeson et al., 1988; Carputo, 1997; Helgeson et al., 1998). The pentaploid offspring are also fertile and tetraploid clones are recovered after a few backcrosses. Alternatively, diploid cultivated potato clones can be fused with diploid wild species to produce tetraploid hybrids (Rokka et al., 1994; Carputo, 1997; Przetakiewicz et al., 2007). However, hexaploid somatic hybrids from 4x + 2x fusions are typically more successful in crosses to cultivars than are tetraploid hybrids from 2x + 2x fusions (Helgeson et al., 1988). Most somatic fusions have been carried out to capture disease resistance genes, but somatic fusion hybrids with improved salt tolerance have also been developed (Bidani et al., 2007). While chromosomes from both parents are typically found in somatic fusion hybrids, the genetic contributions of some wild species are lost more rapidly than others in backcross generations (Naess, 2001).

Another application of somatic fusion is to combine disease resistance genes from two sexually compatible parents. When protoplasts of diploids carrying a major gene for PVX resistance (Hines and Marx, 2001) were fused with those of diploids carrying a major gene for PVY resistance (*Ry*), most of the hybrids expressed both genes (Thach, 1993). Similarly, when clones carrying genes for resistance to different pathotypes of potato cyst nematode were fused, some of the hybrids were resistant to both pathotypes (Rasmussen, 1996). Foliar and tuber late blight resistance have also been combined in somatic fusion hybrids (Rasmussen et al., 1998). The fusion of diploid *S. verrucosum* protoplasts with those of cultivated potato clones combined PLRV resistance from *S. verrucosum* with adaptation and tuber yield from the cultivated potato donor (Carrasco, 2000).

Sometimes levels of resistance are not as high in somatic fusion hybrids as in the donor clones, presumably due to a dilution effect in the polyploid hybrids (Cooper-Bland, 1994; Rasmussen et al., 1998; Carputo et al., 2000b; McGrath, 2002; Gavrilenko, 2003). On the other hand, somatic hybrids produced from fusions of cultivated potato with the wild species *S. nigrum* were often more resistant than the resistant wild parent, perhaps due to complementation of resistance genes (Zimnoch-Guzowska, 2003).

2.5 Genetics

2.5.1 Polyploid versus diploid genetics

The cultivated potato is considered to be an autopolyploid because its subgenomes are similar. All four sets of chromosomes are capable of pairing with each other, so trivalents and

quadrivalents are observed in early stages of meiosis. A trivalent is an association of three homologous chromosomes in early stages of meiosis, while a quadrivalent is an association of four homologous chromosomes. Consequently, the cultivated tetraploid potato exhibits tetrasomic inheritance (Howard, 1970; Ross, 1986; Hawkes, 1990). Tetraploid potato cultivars are described as 2n = 4x = 48, meaning that the sporophyte generation (2n) contains four sets of chromosomes (4x) and there are 48 chromosomes in each somatic cell. Gametes from a cultivar are n = 2x = 24. In this case, 'n' refers to the gametophyte generation.

Three types of gene segregation occur in autotetraploids (Little, 1945; Little, 1952; Burnham, 1962). The type of segregation depends on the location of the gene relative to the centromere. If a gene is close to the centromere, then a crossover between that gene and the centromere is unlikely to occur and that gene will experience chromosome segregation. That is, the gene segregates with the chromosome on which it resides. Consequently, a triplex (AAAa) genotype will produce 50% AA and 50% Aa gametes. Notice that no aa gametes are produced. In the other two types of segregation, called random chromatid segregation and maximum equational segregation, the gene is far enough from the centromere that a crossover can occur during meiosis. Consequently, it is possible for the sister chromatids carrying the recessive allele to end up in the same gamete (aa) through a process called double reduction. Four requirements must be met to achieve double reduction: (1) A quadrivalent must form. That is, all four homologous chromosomes must associate with each other through crossing-over at meiosis. (2) Crossing-over must occur between the gene of interest and the centromere. (3) The two pairs of chromosomes that were involved in the crossover must end up at the same pole after the first meiotic division. (4) Chromatids must separate randomly during the second meiotic division. If these criteria are always met, then maximal equational separation occurs and the frequency of double reduction is 1/6. A less extreme type of segregation occurs when chromatids segregate randomly, resulting in 4/28 or 1/7 gametes carrying sister chromatids. Consequently, random chromatid segregation results in a frequency of double reduction of 1/7. It does not require a crossover between the gene and the centromere in every meiotic cell. With random chromatid segregation, all combinations of chromatids must be considered when determining gametic ratios. The gametes produced by a triplex (AAAa) individual would be all pairwise combinations of AAAAAAaa, which would be 15 AA, 12 Aa, and 1 aa, or 27 with the dominant genotype and one with the recessive genotype.

Two points should be emphasized regarding segregation ratios in polyploids with tetrasomic inheritance. First, the segregation ratio of a particular gene depends on its location on the chromosome. This is in contrast to diploid segregation ratios, which do not depend on a gene's chromosomal position. Second, large samples of segregating populations must be evaluated in order to characterize genetic ratios and to identify clones carrying genes for improved quality and disease resistance (Little, 1945). For example, it is necessary to evaluate at least 1700 plants to distinguish between chromosome segregation and random chromatid segregation when selfing a duplex clone. Consequently, genetic analysis at the tetraploid level is difficult. Segregation

ratios are not clearly delineated because they depend on the crossover frequency between the gene and the centromere, which may be environmentally variable. Consequently, statistical analyses are difficult to perform. Fixed ratios can be predicted from chromosome and random chromatid segregation models. However, they represent extremes. In reality, these extremes are rarely attained and ratios fall between them. Exact ratios can not be predicted because they are determined by crossover events, which differ in every meiotic cell. In addition, epistatic (interlocus) interactions are magnified in tetrasomic tetraploids, gene dosage effects are often important, and interactions with the environment can be complex. All of these complications result in a loss of resolution at the tetraploid level, so that qualitative traits are difficult to identify.

Historically, genetic studies in potato have been based on tetraploid families. As mentioned above, even major genes are difficult to identify at the tetraploid level due to the complexities of tetrasomic segregation. For example, Brown et al. (1997) intercrossed tetraploid cultivated potato clones and obtained high heritability estimates for potato leaf roll virus resistance. A few major genes are likely to be mainly responsible for resistance, but it was not possible to identify individual genes and their effects. In contrast, when Brown and Thomas (1994) carried out inheritance studies at the diploid level, with the wild species *S. chacoense*, a single dominant resistance gene was identified and parental genotypes were determined based on standard diploid segregation ratios.

Even highly selected tetraploid potato clones contain undesirable alleles along with desirable ones. The proportion of deleterious alleles in a plant is called the genetic load. Most deleterious alleles are recessive, so they are only expressed when homozygous. Consequently, the genetic load is high in tetraploids where homozygous recessive genotypes are less common than in diploids. These deleterious alleles are hidden by dominant alleles in tetraploid clones and do not typically have a negative effect. However, when tetraploid clones are self-pollinated or crossed to related clones, some of their offspring will be homozygous for deleterious recessive alleles and will exhibit reduced vigor and/or fertility. These clones are discarded as seedlings in breeding programs. Therefore, one method to measure the genetic load in parents used in breeding programs is to self them and measure the proportion of non-vigorous offspring. It may be beneficial to select parents, in part, based on low genetic load.

Gene expression was studied in a 1x, 2x, 4x polyploid series created by somatic doubling (Stupar et al., 2007). It is interesting that a linear correlation between gene expression and ploidy was rarely found. That is, the diploid and tetraploid clones exhibited similar gene expression patterns. The diploid plants in this study were actually more vigorous than the tetraploid ones. The cost to maintain more DNA and larger cells was apparently not compensated by higher gene expression. The tetraploids in this study were produced by somatic doubling, so they were not able to exploit the heterozygosity necessary for enhanced fitness in polyploids.

2.5.2 Major genes for economically important traits

Many genetic studies in potato have focused on disease resistance. A summary of disease resistance genes and quantitative trait loci in potato has recently been published (Celebi-Toprak et al., 2005b). In addition, major quantitative trait loci have been identified for tuber dormancy (chromosome II), tuber eye depth (chromosome X), flesh color (chromosome IV), tuber shape (chromosome II) and uniformity of tuber shape (chromosome III) (Sliwka et al., 2008).

Another important trait is pigment production, because it determines skin and flesh color. In 1911, Salaman indicated that, in tetraploid potato, the dominant allele of gene *D* is needed for coloration in the skin. Later, the dominant allele of gene *I* was reported to be necessary for skin coloration in diploid potatoes (Dodds and Long, 1956). Genes *D* and *I* are the same, so gene *D* will be used for the remainder of this discussion. Therefore, all *dd* genotypes lack pigmentation and produced white-skinned tubers. Genotypes with the *D* allele are red if they carry the dominant *R* allele and are homozygous recessive for the *P* gene (D-R-pp) (Dodds and Long, 1955). They are purple if they carry the dominant *P* allele, regardless of the genotype at the *R* gene (D—P-). The production of pelargonidin-based (red) anthocyanin pigments in plants requires the activity of dihydroflavonol 4-reductase (DFR), which is encoded by gene *R* (De Jong et al., 2003). The red allele of gene *R* is predicted to encode a 382-amino-acid protein that differs at ten amino acid positions from the gene products of alternative alleles. It is interesting to note that DNA sequence data from this study support the hypothesis that *R* was selected once during the domestication of the potato (Dodds and Long, 1955). The *P* locus codes for flavonoid 3',5'-hydroxylase, resulting in purple pigmentation (Jung et al., 2005). The production of anthocyanins in tuber flesh requires the dominant *Pf* gene, along with the *R* and *P* genes described above (De Jong, 1987) Genes *D* and *Pf* are closely linked (De Jong, 1987). The production of carotenoid pigments (yellow) in tuber flesh requires the dominant *Y* gene (Fruwirth, 1912). Russet skin is apparently controlled by three dominant genes, but the genetic system has not been clearly elucidated (De Jong, 1981).

An understanding of the genetic basis of the photoperiod response for tuberization would help geneticists and breeders to maximize their use of wild species germplasm. A two-gene model duplicate dominant epistasis model has been proposed for the control of tuberization under long-day conditions (Jansky et al., 2004). Clones with at least one dominant allele of one or two genes tuberize in temperate regions. In this model, wild species are homozygous recessive for both genes. Modifier genes are presumed to influence minor variability in the tuberization response.

Major genes also appear to control resistance to cold sweetening, an important processing trait. A three-gene model has been proposed for good potato chip quality when tubers are used directly out of cold storage (Thill and Peloquin, 1994). Another three-gene model explains chip quality when tubers are reconditioned prior to processing. There may be some overlap in these genes.

Acid invertase and UDP-glucose phosphorylase appear to be especially important components of the carbohydrate metabolism pathway as it relates to the accumulation of reducing sugars. Alleles associated with both enzymes have been found to be associated with resistance to cold sweetening (Sowokinos et al., 1997; Sowokinos, 2001; Li et al., 2005; McKenzie et al., 2005).

2.6 Cultivar Development

2.6.1 Choice of parents

A potato breeder's most important decision is which parents to use in crosses. Based on experience, breeders choose parents and parental combinations that result in a relatively high proportion of desirable offspring. Predictions of parental value can be obtained by visually scoring tubers of several families involving the same parent (Brown and Dale, 1998). In addition to specific parental combinations, the proportion of desirable phenotypes in a hybrid family depends on the cross type being evaluated. For example, a breeder is likely to select a higher proportion of clones from a cross between two white parents than from a cross between two red clones because the red family will contain a high proportion of clones with unacceptable skin color. That breeder will select an even lower proportion of clones from crosses between long, russet clones because both round and lightly russeted clones will be discarded. Typically, breeders will maintain separate sets of parents for each market type (red, white, russet). Crosses within market types are more common than across market types. When breeders find that they need to move toward a new market use, they typically make progress toward that goal by choosing appropriate genetically diverse parents. For example, when the U.S. processing industry began to expand dramatically in the mid-20th century, breeders responded by selecting for high dry matter content and chip color. Chip cultivars released in recent decades are far superior to previously released cultivars (Douches et al., 1996).

Genetic variance for yield in potato is mainly non-additive, involving intralocus and interlocus interactions (Mendoza and Haynes, 1974b; Tai, 1976). The genotypic value of a parental clone can be partitioned into additive, dominant, and epistatic contributions to its offspring. The additive component is due to alleles that contribute a fixed value to the genotype. It is the portion of an individual's breeding value that is passed on to offspring. The dominance component is composed of interactions between dominant and recessive alleles at a locus. In diploid populations, the dominance component of variance can not be passed on to offspring because it is lost when meiosis generates haploid gametes. Similarly, interactions among genes (epistatic interactions) are not transmitted intact to the next generation in diploid crosses. Of course, since tetraploids produce diploid gametes, they can pass complex combinations of genes and alleles on to their offspring. Quantitative genetic analyses in tetraploid breeding populations have shown that non-additive genetic variance is important for most economically important traits, including yield, specific gravity (dry matter content), tuber size, and tuber number (Killick, 1977). Consequently, selection can not be carried out on parental clones alone because parental

performance can not be used to predict progeny means, even if parental performance is known from other crosses. Therefore, one option is to make trial crosses of all parental combinations, grow out small families for evaluation, and then grow out larger samples of the best families. Another option is to measure the mean performance of progenies developed by selfing, which can provide some measure of prediction of progeny means of crosses (Gopal, 1998). Evaluations of families developed by selfing will also provide an approximation of the genetic load carried by each parent, which may impact parental value.

In potato, because non-additive (dominance and epistatic) genetic effects are so important for economically important traits, strategies have been developed to allow superior parents to transmit more than their additive genetic component to their offspring. In fact, 2n gametes are able to pass along intact groups of alleles retaining epistatic and dominance interactions. Major loci for tuber yield in potato are located in the chromosome regions between centromeres and proximal crossovers (Buso et al., 1999c). Transmission of heterozygosity associated with yield, then, is especially effective using FDR 2n gametes of potato.

2.6.2 Breeding strategies

Conventional potato breeding involves the intercrossing of heterozygous tetraploid clones (Bradshaw and MacKay, 1994). Segregation results in a very low percentage of desirable genotypes. Often, highly selected but genetically related parents are intercrossed, limiting breeding progress for both yield and yield stability (Mendoza and Haynes, 1974a) because potato exhibits severe inbreeding depression (De Jong and Rowe, 1971; Birhman and Hosaka, 2000). In a typical breeding program, up to 100 000 (and sometimes more) seedlings are grown from crosses between dozens of parents (Figure 2.4). A single tuber is collected from each plant in each family to create a family bag. These tubers are planted in the field as single 'hills' (plants). Selection is then carried out on single hills, mainly for tuber appearance and, to some extent yield and size. Selection pressure is very high at this stage, typically with less than 1% of the clones retained. Selection pressure is higher in some families than others, depending on breeding goals and phenotypic variability. In successive years, the number of genotypes planted decreases while the number of hills evaluated per genotype increases. It is important to evaluate advanced selections across multiple years and production environments to measure performance stability. Consequently, 10 or more years of evaluation are required before a clone is considered for cultivar status.

The phenotype of a potato plant is very plastic (environmentally variable) with regard to many traits of interest for potato breeders. The effect of production environment on traits such as yield, tuber number, tuber size, specific gravity, and processing quality presents a challenge that is difficult to overcome. It requires the testing of clones in multiple years and locations. For example, quantitative trait loci have been detected for tuber starch content, but few were stable across environments (Schäfer-Pregl et al., 1998). Similarly, environment has a large impact on

chip color and its components (Tai and Coleman, 1999; Blenkinsop et al., 2002; Hayes and Thill, 2003). In addition, levels of nutritional compounds, such as antioxidants and vitamins, vary with production environment (Hamouz et al., 1999; El-Morsi et al., 2000; Reyes et al., 2004; Rosenthal and Jansky, 2008). In a study on flavor components in baked potatoes, production and storage environments were found to be major sources of variation (Jansky, 2008).

Potato exhibits strong hybrid vigor due to genetic interactions within and among loci (Tai, 1976). Because high potato yield and vigor depend on interlocus and intralocus interactions, breeding strategies typically aim to maximize heterosis. The most extreme heterosis breeding strategy is to develop highly inbred clones at the diploid level and then combine genetically diverse diploids to produce heterotic hybrids. One heterosis breeding strategy creates a genetically diverse set of doubled monoploids and then somatically hybridizes them to create highly heterozygous diploids. This method has been attempted using diploid cultivated clones (Lightbourn and Veilleux, 2007). A number of roadblocks have limited the success of this first attempt to create heterotic diploids. These included genotypes that did not respond to anther culture, weak monoploids, the fixation of undesirable tuber quality traits, and polyploid somatic fusion hybrids. The monoploid sieve in this case may have been too dramatic, eliminating epistatic and intra-allelic interactions that were present in the heterozygous diploid donors. It is possible that some alleles that were valuable in a heterozygous condition were eliminated during the monoploid sieve.

Another option is to create homozygous diploids using self-incompatibility inhibitors to allow self-pollination (Birhman and Hosaka, 2000). This allows for the development of clones with fixed (homozygous) genes for traits of interest. Highly homozygous inbreds could also be used for heterosis breeding. Each generation of self-fertilization reduces heterozygosity by 50%. This rate of approach to homozygosity may be too high in potato, where inbreeding depression results in dramatic reductions in fertility and plant vigor (Phumichai et al., 2005; Phumichai and Hosaka, 2006). Sib-mating is an attractive alternative that provides a smoother transition to homozygosity.

Unilateral sexual polyploidization offers a modified form of conventional breeding that can maximize the effects of heterosis. Exceptionally high tuber yields have been observed in tetraploid ($2n = 4x = 48$) progenies obtained from 4x X 2x matings in potatoes (Hanneman 1968; Hanneman and Peloquin 1969; Kidane-Mariam and Peloquin 1972; Mok and Peloquin 1975a; Jansky and Peloquin, 2006). The progeny of 4x X 2x crosses are typically vigorous and relatively uniform for high tuber yield, which may at first seem surprising, considering the heterozygosity of the parents. The heterotic response is most commonly observed when the tetraploid is used as the female parent and the diploid parent produces 2n pollen by a first division restitution mechanism. In addition, families from 4x X 2x (first division restitution 2n pollen) crosses outyield 4x X 2x (second division restitution 2n pollen) and 4x X 4x families by about 50% (Mok and Peloquin, 1975b). Because intralocus and interlocus interactions contribute to high

yield in potato, this significant increase in yield by 4x X 2x (first division restitution pollen) hybridization is most likely due to the increase in transmission of heterozygosity and epistasis by 2n first division restitution gametes (Mendiburu and Peloquin 1977). However, high tuber yield must be accompanied by acceptable tuber quality. Schroeder (1983) reported that a large number of haploid (2x) X diploid *S. chacoense* hybrids used in USP schemes produced tetraploid offspring with good tuber appearance and size, along with high yield. Similarly, Buso et al. (1999) identified families from crosses between tetraploids and 2x haploid X *S. chacoense* hybrids with general tuber appearance scores similar to commercial cultivars.

2.6.3 Early generation selection

Most potato breeding programs evaluate tens of thousands of seedling genotypes every year. It is not unusual to discard over 99% of those plants based on visual appearance of tubers. In later generations, after the number of genotypes has been reduced considerably, breeders select for disease resistance and tuber quality in replicated plots with multiple hills (plants) of each clone (Louwes and Neele, 1987). However, early generation selection would allow breeders to retain superior genotypes before the population size has been dramatically reduced and while genetic variability is high (Caligari et al., 1986). Selection for tuber appearance in early generations enhances genetic gains for yield (da Silva et al., 2006). Significant genetic gains in chip potato populations were also reported when seedlings were selected for low glucose levels based on greenhouse-grown minitubers or in vitro grown microtubers (Xiong et al., 2002). In addition, specific gravity of field-grown tubers was found to be associated with that of minitubers and microtubers. It is often difficult to carry out severe selection for tuber quality traits and yield components in early generations, though, because they are strongly influenced by environment (Anderson and Howard, 1981; Brown et al., 1987; Love et al., 1997; da Silva et al., 2006). For example, early generation selection for resistance to cold sweetening is somewhat effective, but its efficiency is reduced by environmental effects on chip color (Thill and Peloquin, 1995; Hayes and Thill, 2002b; Hayes and Thill, 2003). Instead, a more moderate selection intensity or negative selection (eliminating clones with poor performance) is likely to be more effective (Tai and Young, 1984; Maris, 1988; Gopal et al., 1992; Love et al., 1997).

2.6.4 Marker-assisted selection

Some molecular markers associated with major genes are available for use by potato breeders, mainly for disease resistance traits. They include markers for resistance to late blight, potato virus X, potato virus Y, potato virus A, potato virus S, potato cyst nematode, potato root knot nematode, and soft rot (reviewed by Celebi-Toprak et al., 2005a). Recently, molecular markers for resistance to Verticillium wilt have also been identified (Simko, 2004; Simko et al., 2004a, b; Bae et al., 2008). Specific alleles for genes that control the carbohydrate pathway have been found to be associated with resistance to cold sweetening (Sowokinos et al., 1997; Sowokinos, 2001; Li et al., 2005). A marker associated with the gene for sucrose synthase has been found

to be effective in selecting for clones with light chip color (Kawchuk et al., 2008). Historically, markers have been developed using a genetic mapping approach, in which the trait of interest is genetically linked to the marker. This approach requires the development of a fine map in order to prevent recombination between the marker and the gene of interest. Markers based on candidate genes for the traits of interest are not influenced by recombination and are becoming more common as studies elucidate the molecular basis of disease resistance and quality traits. A number of quantitative trait loci have also been identified in potato for both disease resistance and agronomic traits such as starch content, protein storage, and glycoalkaloid production (reviewed by Celebi-Toprak et al., 2005a).

2.6.5 Traits of interest

Historically, breeders have focused on yield, tuber quality, and disease resistance during cultivar development. Progress toward the development of disease-resistant cultivars has recently been reviewed so this topic will not be considered here (Jansky, 2000). A complex set of external and internal quality traits is required for fresh market and processed potatoes. External quality traits include tuber size and shape, eye depth, skin color, and lack of blemishes due to bruising and disease. These traits are especially important for fresh market potatoes, but they may also impact processing quality. Internal quality includes dry matter content, nutritional quality, flavor, starch quantity and quality, and lack of defects such as hollow heart and internal necrosis. In recent years, breeders have been increasingly interested in improved nutritional quality and flavor. Since consumers in many countries eat potatoes more frequently and in larger quantities than other vegetables, any improvement in nutritional composition is likely to result in significant health benefits. Consequently, there is considerable potential to develop the potato as a functional food with health-promoting or disease-preventing properties beyond the basic function of supplying nutrients. These traits require complex chemical analyses and sometimes sensory evaluations, which currently limit the ability of breeders to identify superior phenotypes. A sample of quality traits is discussed below.

1. **Specific gravity**. Tuber specific gravity, which is a measure of dry matter content, is a critical processing quality trait. Cultivars with high dry matter are required for the production of fries, chips, and starch. The genotype–environment interaction for specific gravity is generally low, so rankings of cultivars do not change across years and production environments (Killick and Simmonds, 1974). Heritabilities are moderate to high, allowing for genetic gains to be made by selecting clones with high specific gravity (Killick, 1977; Tai, 1976; Haynes et al., 1989). High specific gravity is often noted in tetraploid clones derived from sexual polyploidization in which the diploid parent contains wild or cultivated potato relatives (Tai and De Jong, 1991; Ortiz et al., 1997; Buso et al., 2000).

2. **Ascorbic acid**. The potato is a significant dietary source of ascorbic acid (vitamin C), which is necessary for normal collagen formation and which acts as an antioxidant. In

addition, ascorbic acid inhibits enzymatic browning of tuber flesh, preventing a significant problem for the potato-processing industry (Finlay et al., 2003). Concentrations in cultivars range from 11 to 36 mg per 100 g tuber tissue (Dale et al., 2003; Love et al., 2003). The U.S.-recommended daily allowance is 60 mg. Ascorbic acid levels decrease over time in storage, but genotypic differences in the ability to maintain ascorbic acid levels during storage have been reported (Davies et al., 2002; Finlay et al., 2003). This quality is important because many potatoes are consumed after a storage period.

3. **Antioxidant capacity**. There is currently considerable interest in the nutritional value of antioxidants in vegetable crops. Carotenoid and phenolic compounds in potato are potent antioxidants (Brown, 2005). Major genes for carotenoid and flavonoid production have been identified in potato (De Jong, 1991; Van Eck et al., 1994). Carotenoid content increases exponentially with intensity of yellow flesh color (Lu et al., 2001), so it is easy to visually select for improved nutritional quality due to carotenoids. Anthocyanins are responsible for purple and red colors, and can be found at high enough levels to consider potato tubers as sources of natural dyes (Jansen and Flamme, 2006). The antioxidants in colored-flesh potatoes also contribute to potato disease resistance (Wegener and Jansen, 2007). High antioxidant capacity has been reported in white-fleshed potatoes, indicating that colorless flavonoids or phenolics may also be important (Hale, 2003). Antioxidant levels are strongly influenced by production environment and storage conditions (Reyes et al., 2004; Rosenthal and Jansky, 2008). Consequently, it is important to distinguish between genotypic and environmental sources of variation when breeding for enhance antioxidant capacity.

4. **Enhanced flavor**. Flavor is due to the combination of taste, aroma, and texture. Raw potatoes are bland, but become more flavorful when heated, as a result of chemical changes (Maga, 1994). Although potatoes are not considered to have a strong flavor, the components of flavor are complex (Coleman et al., 1981). Pyrazines are considered to be among the most important and characteristic components of baked potato flavor (Buttery et al., 1973). They are produced by the non-enzymatic Maillard reaction, in which sugars interact with amino acids at high temperatures. There is a strong positive relationship between pyrazines and organoleptic quality in both baked potatoes (Maga and Holm, 1992) and potato chips (Maga and Sizer, 1973). The breakdown products of RNA, 5' ribonucleotides, act as precursors for flavor enhancers called umami compounds. Steamed or boiled tubers of landrace cultivars with higher levels of glutamate and guanosine 5'-monophosphate (GMP) than *S. tuberosum* cultivars had higher acceptability scores in taste tests (Morris et al., 2007). The most important ribonucleotides for flavor enhancement are inosine 5'-monophosphate (Marcel et al., 2003) and GMP. They are present in low quantities in raw potatoes, but levels increase during cooking as RNA is degraded. Both levels and types of ribonucleotides vary among potato cultivars (Maga and

McNeill, 1986). This is probably due to differences in the activities and types of enzymes that break down RNA. A synergistic effect is detected when 5' ribonucleotides interact with amino acids, especially glutamate.

5. **Resistance to cold sweetening**. Most potato tubers are stored for a period of time before they are processed. When they are stored at low temperatures (<10°C) to prevent losses due to shrinkage and disease and to prevent sprouting, they undergo a phenomenon called low-temperature sweetening. This results primarily from the accumulation of reducing sugars (glucose and fructose) as starch breaks down. These sugars interact with amino acids in the Maillard reaction, causing unacceptably dark fried products (Coffin et al., 1987). It is often possible to reduce the effects of low temperature storage by reconditioning the cold-stored tubers at warmer temperatures prior to processing. This causes some of the sugars to be converted back into starch. However, reconditioning is not always effective (Coffin et al., 1987). Using wild *Solanum* relatives, however, breeders have been able to improve levels of resistance to cold sweetening in breeding clones and cultivars (Thill and Peloquin, 1994; Hayes and Thill, 2002a, c; Domanski et al., 2004; Hamernik et al., 2009). Quantitative trait loci have been identified which are linked to genes encoding enzymes in carbohydrate metabolic pathways (invertase, sucrose synthase 3, sucrose phosphate synthase, ADP-glucose pyrophosphorylase, sucrose transporter 1, and a putative sucrose sensor) (Menendez et al., 2002). It is interesting to note that most QTLs for glucose content mapped to the same positions as those for fructose content. Recent consumer concern about acrylamide levels in fried potato products may be alleviated by breeding for low levels of reducing sugars. Acrylamides result from the interactions of asparagine and reducing sugars during the Maillard reaction. The reducing sugar concentration is the limiting factor in this reaction, so minimizing reducing sugars in tubers is expected to reduce levels of acrylamides in finished products (Silva and Simon, 2005). Selection of cultivars with low levels of acrylamide precursors can alleviate concern about potatoes as significant sources of acrylamides (Vivanti et al., 2006).

6. **Starch content and quality**. Dry matter content in potato tubers is determined in large part by starch content. Starch content is especially important for the processing industry, but it is also of interest in fresh market potatoes because it influences texture. Early maturing cultivars typically do not produce as much dry matter as late maturing clones, which have more time to accumulate photosynthate. As expected, tuber starch content appears to be a polygenic trait. Quantitative trait loci influencing starch content have been identified on each of the 12 chromosomes of potato (Chen et al., 2001; Gebhardt et al., 2005). There is currently interest in breeding for increased levels of amylose in potato starch because high amylose starch has superior nutritional qualities. Following cooking, a portion of high amylose starch recrystallizes to form so-called resistant starch, which acts as a form of dietary fiber (Karlsson et al., 2007). In a survey of wild *Solanum* species,

a large amount of variability was found for dry matter content, starch content, protein content, percent amylose in starch, and the diameter of starch granules (Jansen et al., 2001). These wild species can be introgressed into the cultivated potato as described above, providing the means to improve important tuber quality traits.

2.7 Conclusions

Potato breeders are challenged by the tetraploid nature of the potato, limited variability for economically important traits in adapted breeding clones, and a complex set of requirements necessary for the successful adoption of new cultivars. However, they are fortunate to have access to rich germplasm resources. These resources are readily available in gene banks and the genetic diversity in *Solanum* relatives is typically easily transferred to cultivated potatoes via interspecific crosses. Since the majority of species are 2x (2EBN), the production of haploid–wild species hybrids offers an easy and effective method for capturing much of the diversity in wild *Solanum* species, while allowing breeding and genetic studies to be carried out at the diploid level. Unilateral or bilateral sexual polyploidization can then be used to transfer valuable wild species traits and genetic diversity back to the tetraploid level, where hybrids to cultivars are readily produced. To date, breeders have tapped into only a small fraction of the available genetic resources of potato using these methods. There is good reason for optimism about the prospects for continued genetic improvements in potato. The germplasm resources are abundant in genetic diversity, and breeders have access to a well-stocked toolbox for the utilization of those resources.

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CHAPTER 3

Cell-wall Polysaccharides of Potatoes

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3.1 Introduction

The bulk of potato tubers is made up of parenchyma cells that have thin, non-lignified, primary cell walls (Reeve et al., 1971; Bush et al., 1999, 2001; Parker et al., 2001). Unless stated to the contrary, 'potato cell walls' refers to parenchyma cell walls. These walls and their component polysaccharides are important for a number of reasons: they form part of the total intake of dietary fiber, influence the texture of cooked potato tubers and form much of the waste pulp that is produced in large amounts by the potato starch industry when starch is isolated. The pulp is usually used as cattle feed, but potentially could be processed in a variety of ways to increase its value (Mayer, 1998). For example, the whole cell-wall residues could be used as a food ingredient to alter food texture and to increase its dietary-fiber content, or cell-wall polysaccharides could be extracted and used in a similar way or for various industrial applications (Turquois et al., 1999; Dufresne et al., 2000; Harris and Smith, 2006; Kaack et al., 2006).

Because the parenchyma cells of potato tubers contain large proportions of starch granules (~20% fresh weight), but only small proportions of cell walls (~1% fresh weight), it is difficult to obtain cell-wall preparations that are entirely free from starch (Jarvis et al., 1981a; Harris, 1983; Jardine et al., 2002). Starch can be removed by treatment with a 90% (v/v) aqueous DMSO solution or with α-amylases (Ring and Selvendran, 1978; Harris, 1983). However, native starch granules are degraded only slowly by α-amylases and extensive treatment times are required. This problem can be overcome by first gelatinizing the starch using a short heat treatment, for example for 5 min at 100°C (Harris, 1983; Harris et al., 1991, 1997).

In addition to the walls of the parenchyma cells, the walls of the periderm (skin) cork cells form part of the total intake of dietary fiber and a waste product of potato processing for food as well as for starch. Although much is known about the suberin present in these cell walls (Bernards, 2002; Franke and Schreiber, 2007; Graços and Santos, 2007), little is known about their polysaccharides (Harris et al., 1991). Nonetheless, because of the presence of suberin, these cell walls are able to adsorb hydrophobic dietary carcinogens and their intake may be important in the prevention of colorectal cancer (Harris et al., 1991; Ferguson and Harris, 1998, 2001).

3.2 Overall Polysaccharide Composition of Potato Cell Walls

The overall polysaccharide composition of potato cell walls was established by carrying out analyses after sequential chemical fractionation, first with a chelating agent and then with alkali solutions of increasing concentrations, and by using specific polysaccharide-degrading enzymes (Ring & Selvendran, 1978, 1990; Jarvis et al., 1981a, b; Ryden & Selvendran, 1990). As in the primary cell walls of other eudicotyledons (Harris, 2005), in addition to cellulose, the cell walls contain large proportions of pectic polysaccharides (pectins), smaller proportions of xyloglucans and only minor proportions of heteromannans and heteroxylans. The proportions of the different polysaccharides have been estimated as follows: cellulose ~30%, pectic polysaccharides ~56%, xyloglucans ~11%, and heteromannans ~3%; heteroxylans are probably <3% (Oomen et al., 2003). The non-cellulosic polysaccharides form a gel-like matrix phase in which the cellulose occurs as a microfibrillar phase (Harris and Stone, 2008).

3.3 Individual Polysaccharides

3.3.1 Cellulose

Cellulose molecules are composed of β-D-glucopyranose (β-D-Glc*p*) residues linked (1 → 4)- in long, unbranched chains (Figure 3.1), which have an extended, ribbon-like conformation. This conformation allows the molecules to pack laterally to form microfibrils stabilized by intra- and inter-molecular hydrogen bonds and van der Waals interactions (Harris and Stone, 2008).

Examination of moist, isolated potato cell walls using atomic force microscopy (AFM) showed the cellulose microfibrils as an interwoven network (Kirby et al., 1996, 2006). Although accurate height measurements of cellulose microfibrils have not been obtained using AFM on potato cell walls, they have on similar parenchyma cell-wall preparations from onion (*Allium cepa*) and *Arabidopsis thaliana* (Davies and Harris, 2003). These studies showed that the microfibrils were 4–6 nm in diameter, and reduced to 3.2 nm (*A. thaliana*) when extracted to remove some of the non-cellulosic polysaccharides.

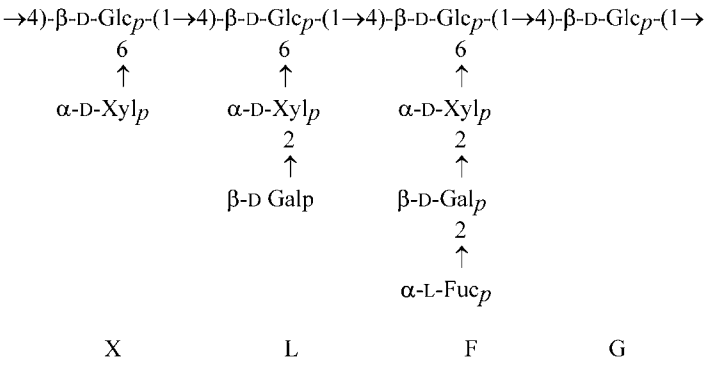
Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy of potato cell walls (Tang et al., 1999, 2000) and similar primary cell walls from a range of other flowering plants (angiosperms) showed the cellulose was crystalline (cellulose I) (Newman et al., 1994, 1996; Koh et al., 1997; Smith et al., 1998; Thimm et al., 2002), although the molecular conformation and hydrogen bonding arrangement was different on the crystallite surfaces (Vietor et al., 2002). Calculations from the studies on the cell walls of the other flowering plants showed the cellulose crystallite cross-sectional dimensions were ~2–3 nm and each crystallite contained ~20–25 molecules. Comparing these dimensions with those of the cellulose microfibrils, measured by AFM after extraction, indicated that each microfibril contained only one cellulose crystallite (Davies and Harris, 2003).

Cellulose [(1→4)-ß-D-Glucan]

→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→

Xyloglucans

Part of a fucogalactoxyloglucan that occurs in the primary walls of most eudicotyledons



Part of an arabinoxyloglucan that occurs in the primary walls of potatoes:

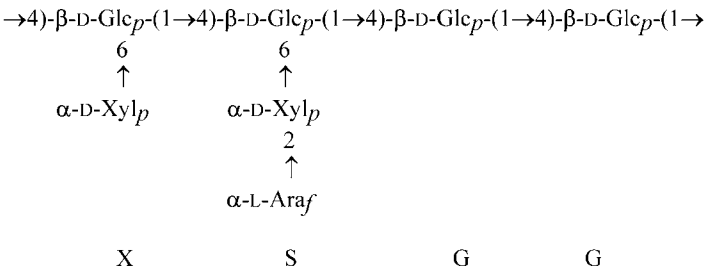


Figure 3.1: Structures of cellulose and two types of xyloglucans, a fucogalactoxyloglucan and an arabinoxyloglucan.

An experimental process has been described for extracting cellulose microfibrils from waste potato pulp and using these to manufacture starch–cellulose composites (Dufresne et al., 2000).

3.3.2 Pectic polysaccharides (pectins)

Pectic polysaccharides have very complex structures and are usually composed of four commonly occurring domains or blocks: homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II) and xylogalacturonan (XGA) (Ridley et al., 2001; Willats et al., 2001a; Willats et al., 2006; Mohnen et al., 2008). The backbones of the different domains are

likely to be glycosidically linked to each other in a linear fashion (Coenen et al., 2007). The HG domains are often referred to as 'smooth' regions, whereas the other domains, which are branched, are referred to as ramified 'hairy' regions. Small differences in the compositions of the pectic polysaccharides of potato cell walls were found between two different cultivars (van Marle et al., 1997a) and after different periods of tuber storage (van Dijk et al., 2002a).

3.3.2.1 Homogalacturonan (HG)

In potato cell walls, the HG domain comprises only ~20% of the total pectic polysaccharides, a much lower proportion than that in the pectic polysaccharides of other species (average ~65%) (Mohnen et al., 2008). HG is composed of linear chains of α-D-galacturonic acid residues linked (1 → 4)- (Figure 3.2). It is synthesized with a high proportion of the carboxyl groups methyl-esterified and is then partially de-esterified in the cell wall by a range of pectin methyl esterases giving different distribution patterns of methyl-esterified galacturonic acid residues (Willats et al., 2001b). Stretches of galacturonic acid residues that are not esterified can associate by calcium cross links to form junction zones ('egg-box' structures), resulting in gel formation (Jarvis, 1984).

Depending on the plant species, and stage of development of the tissues, the galacturonic acid residues may also be acetylated to different extents. The HGs of some species, including potato and sugar beet, are particularly highly acetylated (Turquois et al., 1999). The positions of the acetyl groups vary with species. In potato and spinach (*Spinacia oleracea*) HG, they are attached predominately to C(O)3 (Ishii, 1997; Perrone et al., 2002), but in sugar beet (*Beta vulgaris*) HG, they attached to C(O)2 and C(O)3 in about similar proportions; doubly substituted galacturonic acid residues were not found (Ralet et al., 2005).

In preparations of commercial pectins, the presence of 'hairy' regions, and methyl-ester and acetyl groups on the galacturonic acid residues in the HGs all affect pectin functionality, particularly its ability to form gels. The presence of 'hairy' regions and acetyl groups reduces the gelling ability of pectins (Willats et al., 2006). Pectins with a DE of 50% or more are known as high-ester pectins, and those with a DE less than 50%, low-ester pectins. The most commonly used pectins, from lemons (*Citrus limon*), limes (*Citrus aurantiifolia*) and apples (*Malus domestica*), are high-ester pectins, which gel at a low pH (<3.5) in the presence of high concentrations of soluble solids such as sucrose (>55%) (Harris and Smith, 2006). However, small amounts of low-ester pectins are produced, mostly from sunflower residues, for particular applications. These pectins gel without sucrose, but in the presence of calcium ions over the pH range 2–6 (Harris and Smith, 2006). Waste potato pulp is potentially another source of low-ester pectin. The DE of the HG in batches of pulp analyzed by Turquois et al. (1999) varied from 17.4 to 29.5%. These authors have developed an extraction procedure, involving treatment with alkali, which yielded a preparation with a low degree of acetylation and with high gelling ability in the presence of calcium.

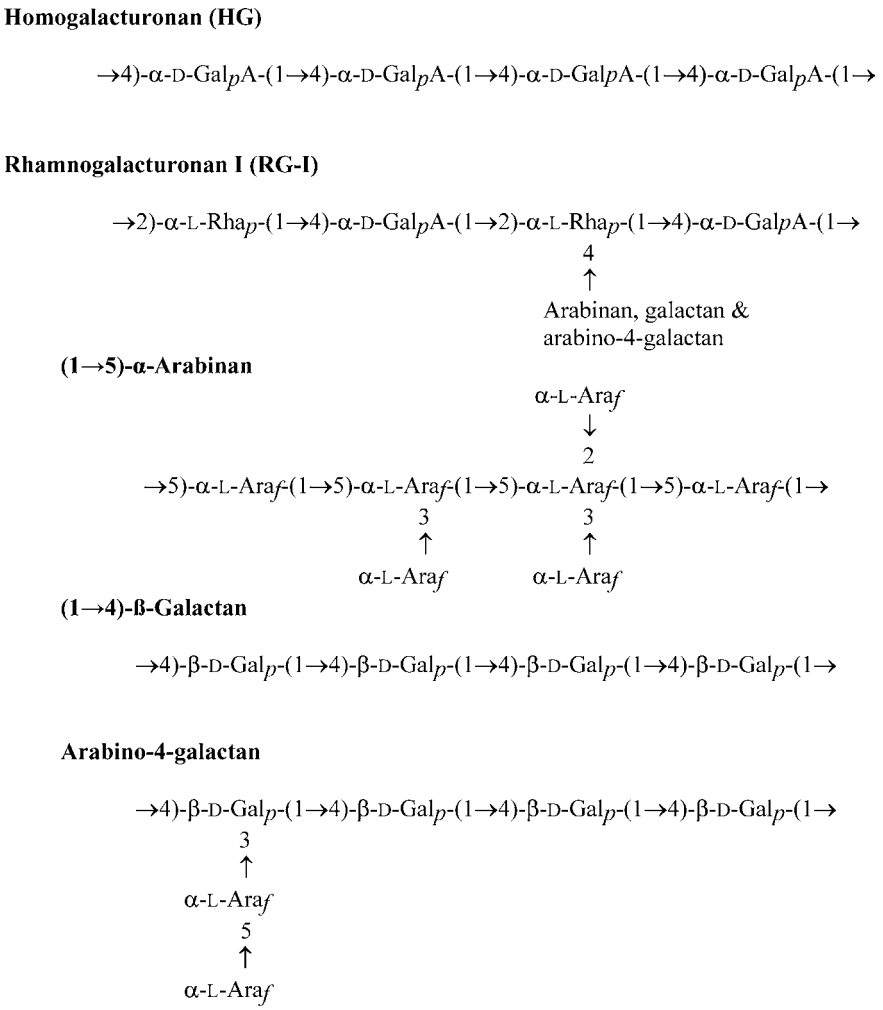


Figure 3.2: Structures of the pectic polysaccharides homogalacturonan (HG) and rhamnogalacturonan I (RG-I). (1 → 5)-α-Arabinan, (1 → 4)-β-galactan and arabino-4-galactan are side chains of RG-I.

3.3.2.2 Rhamnogalacturonan I (RG-I)

In potato pectic polysaccharides, the RG-I domain accounts for ∼75% of the total, a much higher proportion than in pectic polysaccharides of other species (∼20–35%) (Oomen et al., 2003; Mohnen et al., 2008). RG-1 consists of a backbone of alternating α-D-galacturonic acid and α-L-rhamnosyl residues, linked as indicated in Figure 3.3, which in potato accounts for ∼14% of the total RG-1 (Oomen et al., 2003). Although the galacturonic acid residues are probably not methyl-esterified, they may be acetylated. This is so in potato, where a preparation

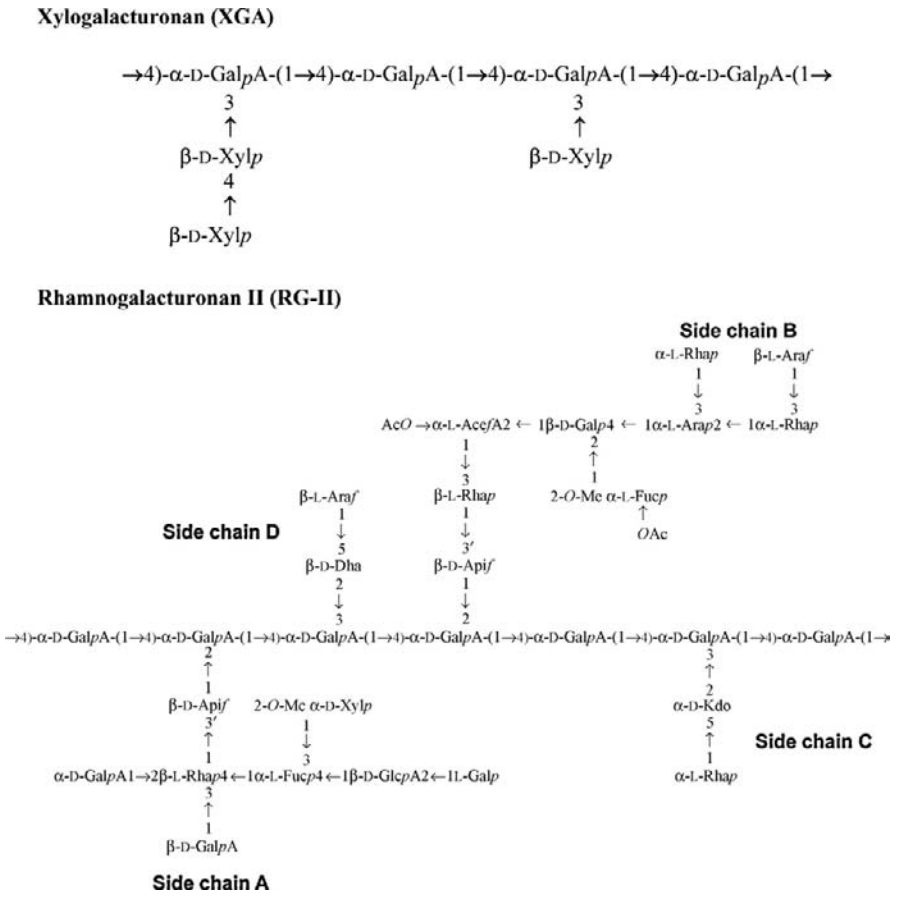


Figure 3.3: Structures of the pectic polysaccharides xylogalacturonan (XGA) and rhamnogalacturonan II (RG-II). In potato XGA, it is not certain if the terminal β-D-Xyl*p* residue is linked (1 → 4)-. The structure of side chain B of RG-II varies with species; the structure shown occurs in grape (wine) (*Vitis vinifera*).

rich in RG-1 was found to have a high acetyl content (Schols and Voragen, 1994). As in HG, the positions of the acetyl groups vary with species. In contrast to potato HG, the acetyl groups are attached predominately to C(O)2 of the galacturonic acid residues in potato RG-1 (Ishii, 1997). However, in spinach RG-1, they are attached to C(O)3, as in spinach HG (Perrone et al., 2002).

Polysaccharide or oligosaccharide side chains containing predominantly β-D-Gal*p* residues linked (1 → 4)- and/or α-L-Ara*f* residues linked (1 → 5)- are attached to up to 80% of the Rha*p* residues. The composition and lengths of the side chains vary with species and cell type (Harris, 2005). In potato cell walls, the predominant side chains are (1 → 4)-β-galactans (Figure 3.2) (Jarvis et al., 1981a, b; Ring and Selvendran, 1978; Ryden and Selvendran, 1990; Øbro et al.,

2004). Recently, it has been shown that small proportions of the β-D-Gal*p* residues in potato galactans are linked (1 → 3)- rather than (1 → 4)-, the ratio being 1:163 (Hinz et al., 2005). Small proportions of 3,4-linked Gal*p* residues, detected by linkage analysis (Ring and Selvendran, 1978), indicated that some arabino-4-galactans (Figure 3.2) are probably present. These have α-L-Ara*f* residues (1 → 5)-linked in short chains to C(O)3 of some of the Gal*p* residues. The likely presence of arabino-4-galactans was also indicated by experiments involving treatment with (1 → 4)-β-D-galactanase (Jarvis et al., 1981b; Øbro et al., 2004). Nevertheless, most of the α-L-Ara*f* residues linked (1 → 5)- are likely to be present as (1 → 5)-α-arabinans (Figure 3.2), with some branching. In the linkage analyses conducted by Jarvis et al. (1981a), small proportions of 3,6-linked Gal*p* residues were detected, indicating that traces of arabino-3,6-galactans may also be present as RG-I side chains. Such side chains have been found in RG-Is of other species (O'Neill and York, 2003; Harris and Stone, 2008). If the side chains of potato RG-I are regarded simply as (1 → 4)-β-galactans and (1 → 5)-α-arabinans, they would account for ∼67% and ∼19%, respectively, of the RG-1 (Oomen et al., 2003). Other residues, including 4-O-Me-β-D-Glc*p*A, β-D-Glc*p*A, and α-L-Fuc*p*, may be present in RG-1 side chains, but have not been reported in potato (Mohnen et al., 2008).

3.3.2.3 Rhamnogalacturonan II (RG-II)

Small proportions of the RG-II domain occur in the pectic polysaccharides of all vascular plants and bryophytes (embryophytes) (O'Neill et al., 2004). The proportion of RG-II in potato pectic polysaccharides has not been calculated, but in pectic polysaccharides from other eudicotyledon species it accounts for ∼10% of the total pectic polysaccharides (Mohnen et al., 2008). RG-II has a low molecular mass (∼5–10 kDa), yet it has an extraordinarily complex structure that varies only slightly among different species. Flowering plant RG-II contains five monosaccharides that may, in cell-wall polysaccharides, be confined to RG-II: L-aceric acid (L-Ace*f*A), 2-*O*-Me-L-Fuc*p*, 2-*O*-Me-D-Xyl*p*, 2-keto-3-deoxy-D-*lyxo*-heptulosaric acid (Dha), and 2-keto-3-deoxy-D-*manno*-octulosonic acid (Kdo); it also contains D- and L-Gal*p*, D-Glc*p*A, D-Gal*p*A, L-Ara*f*, L-Ara*p*, D-Api*f*, L-Fuc*p* and L-Rha*p*. It is a substituted HG, with at least seven galacturonic acid residues forming a backbone (HG) to which are attached four structurally different side chains: A-D (Figure 3.3). Side chains C and D are disaccharides; C contains Kdo, and D contains Dha. Side chain A is an octasaccharide and contains 2-*O*-Me-D-Xyl*p*; side chain B is, depending on species, a heptasaccharide, octasaccharides or nonasaccharide, and contains 2-O-Me-L-Fuc*p*. The structural variation in side chain B results from the substitution pattern of the L-Ara*p* residue (O'Neill et al., 2004; Rao et al., 2008). Except for the variability in side chain B, RG-II has a highly conserved structure.

RG-II has been isolated from potato cell walls (Ishii et al., 1999), but the detailed structure of its B side chain has not been determined. However, the monosaccharides that may be confined to RG-II have all been detected in potato cell walls (Stevenson et al., 1988; O'Neill et al., 2004). In cell walls, including those of potatoes, RG-II occurs mostly as a dimer cross-linked by 1:2

borate:diol esters. The ester cross-links the D-Api*f* residue of the A side chains in each of the dimer subunits; the D-Api*f* residue in each of the two B side chains is not esterified (O'Neill et al., 1999).

3.3.2.4 Xylogalacturonan (XGA)

The XGA domain is another substituted HG present in pectic polysaccharides in only small proportions. The proportion in potato pectic polysaccharides has not been calculated, but in *Arabidopsis thaliana* the proportion varies from 2.5 to 7% depending on organ (Zandleven et al., 2007). In potato pectic polysaccharides, this domain has single β-D-Xyl*p* residues and some disaccharide residues composed of two β-D-Xyl*p* residues probably linked (1 → 4)-, attached to C(O)3 of the α-D-GalA*p* residues of an HG backbone (Figure 3.3) (Zandleven et al., 2006). The disaccharide residues have been more extensively characterized in apple XGA, where they have been shown to be two β-D-Xyl*p* residues linked (1 → 4)- (Zandleven et al., 2006).

3.3.3 Xyloglucans

Xyloglucans have a linear backbone of (1 → 4)-linked β-D-Glc*p* residues, some of which are substituted at C(O)6 with α-D-Xyl*p* residues; these xylose residues may have other substituents attached to them (Figure 3.1). Because of this structural complexity, an unambiguous nomenclature was devised to describe xyloglucan structure (Fry et al., 1993). In this nomenclature, G, X, S, L and F are used to refer to the following structures: G = unsubstituted β-Glcp; X = α-D-Xyl*p*-(1 → 6)-β-D-Glc*p*; S and L = X with α-L-Ara*f*-(1 → 2)- and β-D-Gal*p*-(1 → 2)- attached to the non-reducing end, respectively; and F = L with α-L-Fuc*p*-(1 → 2)- attached to the non-reducing end (Figure 3.1).

In the primary cell walls of most eudicotyledons, the xyloglucans are fucogalactoxyloglucans. These xyloglucans have a XXXG core and contain the structural units G, X, L and F. Three successive glucose residues are substituted with xylose residues, but the fourth glucose is unsubstituted (Figure 3.1) (Vincken et al., 1997). Treating these xyloglucans with a (1 → 4)-endo-β-glucanase releases three major oligosaccharide subunits, XXXG, XXFG and XLFG, together with smaller proportions of XXLG, XLLG and XLXG (Harris, 2005).

By contrast, the xyloglucan in potato cell walls has a quite different structure. Ring and Selvendran (1981) isolated and partially characterized the xyloglucan in a 4 M KOH soluble fraction. This study showed that it contained the structural units G, X, L and S but no F. Further analysis by Vincken et al. (1996), using (1 → 4)-endo-β-glucanases and linkage analysis, showed that it has a XXGG core structure: two successive glucose residues are substituted with xylose residues, but the third and fourth glucose are unsubstituted (Figure 3.1). Oligosaccharide subunits present in the xyloglucan include XXGG, XSGG, XLGG, and LSGG. Xyloglucans

from the primary cell walls of other species in the same family as potato, Solanaceae, also have the same core structure and contain S units (Harris, 2005; Hoffman et al., 2005).

Xyloglucans also have acetyl groups attached to them. In fucogalactoxyloglucans, these have been found attached to D-Gal*p* residues, for example in *Arabidopsis thaliana* to C(O)6 of D-Gal*p* residues (Pauly et al., 2001). However, in arabinoxyloglucans isolated from culture filtrates of suspension-cultured cells of Solanaceae species, they have also been found attached to C(O)5 of the L-Ara*f* resides and to C(O)6 of D-Glc*p* residues; typically the unsubstituted Glc closest to the non-reducing end carries the acetyl group (Sims et al., 1996; Jia et al., 2005). The arabinoxyloglucans from the cell walls of tomato (*Solanum lycopersicum*) and sweet pepper (*Capsicum annuum*) were less acetylated, and acetyl groups were found on only the D-Glc*p* residues (Hoffman et al., 2005). There is no information about the acetylation of potato arabinoxyloglucan.

3.3.4 Heteromannans

Monosaccharide and linkage analyses of potato cell walls and cell-wall fractions indicated the presence of small proportions of heteromannans (Jarvis et al., 1981), but these have not been isolated and structurally characterized. However, heteromannans in the form of galactoglucomannans (Harris and Stone, 2008) have been characterized from the primary walls of suspension-cultured cells of tobacco (*Nicotiana tabacum*) (Eda et al., 1985), blackberry (*Rubus fructicosus*) (Cartier et al., 1988) and kiwifruit (*Actinidia deliciosa*) (Fischer et al., 1996). These galactoglucomannans have a backbone of alternating β-D-Man*p* and β-D-Glc*p* residues, with either single α-D-Gal*p* residues or β-D-Gal*p*-(1 → 2)-α-D-Gal*p* residues attached at C(O)6 of the Man*p* residues (Figure 3.4). A similar galactoglucomannan, but with a Glc:Man:Gal ratio of 1:1:0.5, has been isolated and characterized from primary cell walls obtained from the outer pericarp of kiwifruit (*Actinidia deliciosa*) (Schröder et al., 2001). The heteromannans in potato cell walls may have similar structures to these polysaccharides. Although heteromannans in lignified secondary cell walls and in culture filtrates of suspension-cultured cells are known to have acetyl groups attached to them (Sims et al., 1997; Harris and Stone, 2008), this is not known for the galactoglucomannans of primary cell walls.

3.3.5 Heteroxylans

Small proportions of 4-linked Xyl*p* residues were found in linkage analyses of potato cell-wall preparations (Ring and Selvendran, 1978) and of 1 M and 4 M KOH soluble extracts (Ring and Selvendran, 1981). At least some of these residues may be present in heteroxylans of primary cell walls. Such heteroxylans are known to occur in small proportions in the primary cell walls of other eudicotyledons, but their structure has been characterized in detail only for those in the walls of suspension-cultured sycamore (*Acer pseudoplatanus*) cells (Darvill et al., 1980). These heteroxylans are glucuronoarabinoxylans with a backbone of β-D-Xyl*p* residues linked

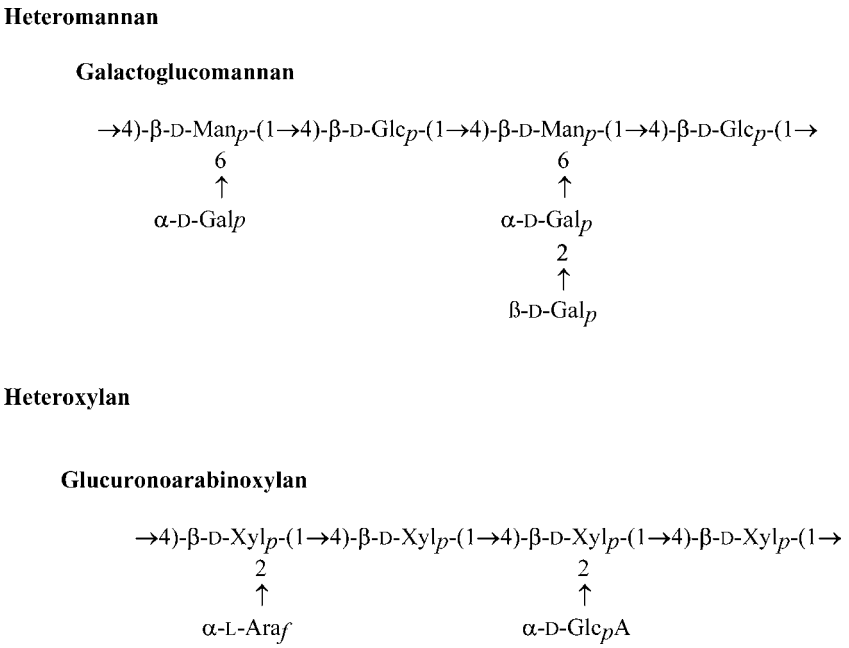


Figure 3.4: Structures of the galactoglucomannan (a heteromannan) known to occur in the primary cell walls of several species of eudicotyledons and the glucuronoarabinoxylan (a heteroxylan) known to occur in the primary cell walls of sycamore (*Acer pseudoplatanus*). The structures of the heteromannans and heteroxylans in potato cell walls are unknown.

(1 → 4)- and side chains of single α-L-Ara*f* and α-D-Glc*p*A residues linked at C(O)2 of the xylose residues (Figure 3.4). However, some of the 4-linked Xyl*p* residues in the potato cell-wall preparation may have their origin in heteroxylans in the lignified secondary walls of the few xylem tracheary elements present in potato tubers.

3.4 Genetic Manipulation of Cell-wall Polysaccharide Compositions

The polysaccharide compositions of potato cell walls have been genetically manipulated for two main reasons: first to attempt to understand the functions of the different cell-wall polysaccharides, and second to improve the value of potato pulp waste. The yield of pectin extracted from the pulp would be improved by decreasing the proportion of cellulose in the walls. The gelling quality of this pectin would also probably be improved by reducing the proportion of RG-1 side chains (Sørensen et al., 2000).

The concentrations of cellulose in potato cell walls were modified by up- and down-regulating four potato cellulose synthase genes (*StCESA1, 2, 3* and *4*) known to be involved in encoding cellulose synthases in cells making primary walls (Oomen et al., 2004). It was found that

cellulose concentrations could be increased, but mostly decreased (up to 40%) without affecting plant development.

Because of the paucity of knowledge about genes encoding enzymes involved in the biosynthesis of pectic polysaccharides (Mohnen et al., 2008), a highly innovative approach was used to manipulate these polysaccharides *in planta* in potato. This approach entailed generating transgenic potatoes that over-expressed genes encoding glycanases that degrade specific pectic polysaccharides. Genes from the fungus *Aspergillus aculeatus* that encode an endo-α-(1 → 5)-L-arabinanase (Skjøt et al., 2002), an endo-β-(1 → 4)-D-galactanase (Sørensen et al., 2000) and a rhamnogalacturonan lyase (*e*RGL) (Oomen et al., 2002) were over-expressed using the granule-bound starch synthase promoter, which is highly active in potato tubers. The arabinanase and galactanase respectively hydrolyze the arabinan and galactan side chains of RG-I and the *e*RGL cleaves the backbone into small fragments. Trangenic plants were obtained in which the *e*RGL and the galactanase were specifically located in the potato apoplast, whereas the arabinanase was in the Golgi apparatus. Compared with those of the wild type, the tuber cell walls of transgenic plants expressing the eRGL had a lower content of RG-I, but a higher content of HG (Oomen et al., 2002). The tuber cell walls of transgenic plants expressing the arabinanase and galactanase had a reduction of 69% and 76%, respectively, in the arabinan and galactan content of pectic RG-I compared with those of the wild type (Sørensen et al., 2000). Although there were histological differences from the wild type in transgenic tubers expressing *e*RGL, transgenic tubers expressing the arabinanase and galactanase showed no differences from the wild type. However, a subsequent study showed that the transgenic tubers expressing the arabinanase and galactanase were more brittle than wild type when subjected to uniaxial compression, indicating that the arabinans and galactans influence the physical properties of the tuber cell walls (Ulvskov et al., 2005).

3.5 Molecular architecture of the cell walls

3.5.1 Cell-wall models

The first model for primary cell walls rich in pectic polysaccharides, such as those of potatoes, was developed for the walls of suspension-cultured cells of sycamore (*Acer pseudoplatanus*) (Keegstra et al., 1973). This showed the pectic polysaccharides covalently linked to the xyloglucans and to the glycoprotein extensin, whereas the xyloglucans were depicted as being hydrogen-bonded to the cellulose microfibrils. At the time, there was little evidence for covalent linkages, although such linkages between xyloglucans and the pectic polysaccharide RG-I have recently been demonstrated (Popper and Fry, 2008). Instead, a type of model was developed in which a pectic polysaccharide network and a cellulose–xyloglucan network are co-extensive, but independent, and a third network, composed of extensin, is also sometimes present (McCann and Roberts, 1991) (Figure 3.5). The pectic polysaccharide network is thought to determine wall porosity and the cellulose–xyloglucan network is thought to be the main load-bearing structure.

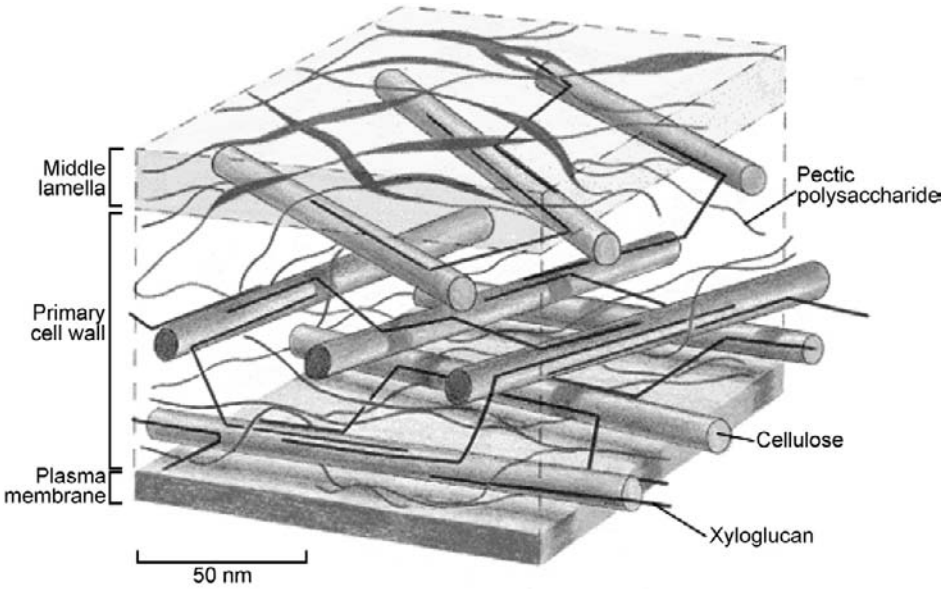


Figure 3.5: A simplified model of the molecular architecture of a primary cell wall rich in pectic polysaccharides, such as a potato cell wall. Two co-extensive, but independent polysaccharide networks are shown: a cellulose–xyloglucan network and a pectic–polysaccharide network. The middle lamella is located between the primary cell walls of adjacent cells and is responsible for cell–cell adhesion. Reprinted with permission from McCann and Roberts (1991).

This type of model is often referred to as a 'tethered or sticky network model'. Evidence for this model came from the observation of cross links between cellulose microfibrils by transmission electron microscopy of pectin-rich, primary cell walls that had been prepared using fast-freeze, deep-etch and rotary showing (McCann et al., 1990). These crosslinks were interpreted as being composed of xyloglucans (McCann et al., 1990), which are often described as coating the surface of the cellulose microfibrils (Cosgrove, 1999). However, in experiments using solid-state ^{13}C NMR spectroscopy, it was calculated that, in the primary cell walls of mung bean (*Vigna radiata*) seedlings, a maximum of only 8% of the surface of the cellulose microfibrils had xyloglucan adsorbed on to it (Bootten et al., 2004). Nevertheless, xyloglucans are assumed to play an essential role in the molecular architecture of primary cell walls.

The results of a recent study have called this into question. The primary walls of a double mutant of *Arabidopsis thaliana* in which two xyloglucan xylosyltransferase genes were not expressed were found to contain no detectable xyloglucan (Cavalier et al., 2008). Compared to the wild type, the double mutant was slightly smaller and had abnormal root hairs, yet there was no catastrophic effect on cell-wall integrity as would be predicted from current cell-wall models. A possible explanation is that the pectic polysaccharide network between the

cellulose microfibrils is able to function alone in maintaining cell-wall structure and functionality. Recent evidence has been obtained from the cell walls of potato and sugar beet that some of the arabinan and galactan side chains of RG-I may be bound to the surface of the cellulose microfibrils (Zykwinska et al., 2005, 2006, 2007). This would provide a mechanism of linking the pectic–polysaccharide network to cellulose in addition to the linkage via RG-1 covalently bound to xyloglucan. Within the pectic polysaccharide network, crosslinks between RG-II molecules by 1:2 borate-diol esters and between unesterified stretches of HG molecules by calcium ions are probably important in maintaining mechanical functionality (Ryden et al., 2003; O'Neill et al., 2004).

3.5.2 Immunolabeling studies of the location of polysaccharides

The detailed distribution of polysaccharides within cell walls can be determined by immunolabeling sections of plant tissues with appropriate antibodies (Knox, 2008). Such studies also show the distribution of polysaccharides in the middle lamella (Figure 3.5), which develops from the cell plate, formed at cell division, and is responsible for cell–cell adhesion. Cell corners (tricellular junctions) and the corners of the intercellular spaces can be regarded as extensions of the middle lamella. They are where stresses that tend to separate plant cells are concentrated and have been referred to as reinforcing zones (Jarvis et al., 2003). These zones and the middle lamella are rich in pectic polysaccharides, but contain no cellulose microfibrils (Jarvis et al., 2003).

The distribution of different pectic polysaccharide domains has been investigated in sections of mature potato tubers using immunolabeling with a range of monoclonal antibodies (Bush et al., 1999). The locations of the different epitopes were determined both within the walls (and associated middle lamella and reinforcing zones) of a parenchyma cell in a particular tissue, and among walls of parenchyma cells in different tissues (e.g. cortex and perimedullary zone). The antibodies JIM 5 and JIM 7, which recognize $\leq$ 50% methyl-esterified HG and $\geq$ 35% methyl-esterified HG, respectively, showed similar labeling patterns; they labeled the walls, middle lamellae and reinforcing zones of cells in all the different tissues. However, the monoclonal antibody 2F4, which recognizes a calcium cross-linked dimer of HG ('egg-box' structures) (Liners et al., 1989; Liners and Van Cutsem, 1992), labeled only the middle lamella and reinforcing zones; labeling density was greater in the cells of the cortex than the perimedullary zone. In contrast, the RG-I side chains galactan and arabinan, detected with the antibodies LM 5 and LM 6, respectively, were generally found in the cell walls, but not the middle lamella and reinforcing zones. The only exception was the finding of the arabinan epitope in the middle lamella of the cortical cells. The galactan epitope was found throughout the walls of the perimedullary zone cells but in a zone adjacent to the plasma membrane in the cortical cells. Changes in the location of the different epitopes during the development of potato tubers were also studied (Bush et al., 2001).

Although not investigated in potato, the occurrence of two further pectic polysaccharide epitopes in parenchyma cells of a range of species of flowering plants has been determined by immunolabeling. A polyclonal antibody against a borate–RG-II complex detected this epitope in cell walls, but not in the middle lamella or reinforcing zones (Matoh et al., 1998). However, the epitope of the monoclonal antibody LM 7 was detected only in the reinforcing zones (Willats et al., 2001b). This antibody recognizes partially methyl-esterified HG generated by non-blockwise de-esterification.

3.6 Effects of Heating on Cell-wall Polysaccharides

A number of complex changes occur during the cooking of potatoes which influences their texture (Jarvis et al., 2003; Waldron et al., 2003; Taylor et al., 2007). Starch granules swell as they gelatinize and generate pressure within the cells, resulting in the cells becoming rounded and separating from one another (Jarvis et al., 1992). Pectic polysaccharides, in the cell walls, middle lamellae and reinforcing zones are also degraded and their solubilities increased, assisting cell separation. The extent of cell separation is higher in mealy than non-mealy cultivars (Jarvis and Duncan, 1992). In a study of the effects of cooking on cell-wall polysaccharides, it was found that more HG (unbranched pectic polysaccharide) was solubilized than RG-1 (branched pectic polysaccharide) (van Marle et al., 1997b). The authors concluded that the solubilized HG may result from the degradation of partly methyl-esterified HG by β-elimination which results in cleavage of the HG chain next to a methyl-esterified uronic acid residue. They also found that the solubilized pectic polysaccharides from two cultivars with contrasting textures were different. The pectic polysaccharides solubilized from cv. Irene, which has a mealy texture, contained a higher proportion of RG-1 (branched pectic polysaccharide) and the HG was more methyl-esterified and acetylated than from cv. Nicola, which did not have a mealy texture.

In the potato-processing industry, preheating (blanching) at temperatures below 100°C is commonly done before the final cooking, to inactivate enzymes and gelatinize the starch (van Dijk et al., 2002b). Preheating may result in cooked potatoes that have a firmer texture, and there is indirect evidence that this is because pectin methylesterase is not completely inactivated (van Dijk et al., 2002b). During preheating, this enzyme removes the methyl esters from HG, which is now less susceptible to β-elimination, and calcium cross-linked junction zones can form that result in a firmer texture. In an earlier study using a different cultivar, it was found that preheating resulted in a firmer texture only if the potato tissue was pre-soaked in a dilute solution of calcium chloride, suggesting that the concentration of calcium ions was limiting (Ng and Waldron, 1997). An experimental measure of the susceptibility of cells to separate, vortex-induced cell separation, showed that without calcium chloride treatment, the cells separated after cooking, with and without preheating. However, after calcium chloride treatment, no cell separation occurred in fresh, preheated alone, or cooked (with and without preheating) cells (Ng and Waldron, 1997). These studies indicate that calcium complexed HG located

in the middle lamella and reinforcing zones probably plays an important role in cell adhesion (Parker et al., 2001). However, treating fresh potato tissue with the chelating agent CDTA (*trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid) did not result in vortex-induced cell separation, indicating that other components are also involved in cell–cell adhesion (Ng and Waldron, 1997; Jarvis et al., 2003). The partially methyl-esterified HG epitope, which occurs in the reinforcing zones and is recognized by the monoclonal antibody LM 7, may be of particular interest in this respect.

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CHAPTER 4

Structure of Potato Starch

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4.1 Introduction

Starch is the major component of potato tubers amounting approximately to 15–20% of its weight. As an effect, starch is considered to be a major factor for the functionality of the potato in food applications. For industrial applications, the processed starch from potato is considered very pure as compared to most other starch types. Compared to other commodity starches potato starch also has some unique properties that are directly attributed to its granular and molecular structures including very large and smooth granules, a high content of covalently linked phosphate, long amylopectin chains and high-molecular-weight amylose. These characteristics combined make potato starch a tremendous source of functional biopolymer for food and materials science. Especially, potato starch finds many applications in the wet-end for the manufacturing of high-quality paper (Blennow et al., 2003) and for the generation of viscous hydrocolloid systems (Wiesenborn et al., 1994). Moreover, as compared to most cereal starches, the well-ordered and dense structure of the native potato starch granule renders it very resistant to enzymatic degradation by hydrolytic enzymes, such as amyloglucosidases and α-amylases (e.g. Sun et al., 2006).

In the potato tuber, starch is found as distinct granules having a size of approximately 10–100 μm in diameter (Hoover, 2001). The granules are built up by two polysaccharides consisting exclusively of glucose as the monomer component. The glucopyranosyl residues are connected through α-D-(1,4)-linkages forming chains that through α-D-(1,6)-branches at the reducing end-side are linked to similar other chains. Amylopectin is the major component in starches in general and in potato it normally constitutes 70–80% by weight (Hoover, 2001; Yusuph et al., 2003) regardless of the size of the granules (Noda et al., 2005). Approximately 4–6% of the linkages are of the α-D-(1,6)-type, making it extensively branched. The weight-average molecular size of amylopectin is in the order of 10^7 daltons (Aberle et al., 1994; Ratnayake and Jackson, 2007) and, as a result, the macromolecule consists of a huge number of relatively

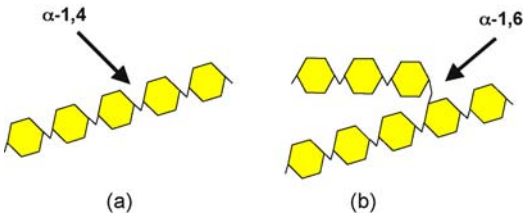

Figure 4.1: Schematic representation of the structures of (a) linear amylose and (b) branched amylose and amylopectin.

short chains with an average degree of polymerization (DP) of 21–28 residues (Zhu and Bertoft, 1996; McPherson and Jane, 1999). The minor component of starch is amylose. It is considerably smaller than amylopectin and is essentially a linear polymer consisting of chains of DP in the order of 2000–5000 residues (Hoover, 2001). However, a few branches are found in the structure and principally two types of this component exist, namely linear and branched amylose (Figure 4.1).

Besides the polysaccharide components, potato starch consists of very low amounts of material of non-carbohydrate nature. Less than 0.5% of the granules are proteins (Yusuph et al., 2003), apparently mostly being involved in starch synthesis. Lipids are virtually absent in potato starch, which is in common with some other tuber and root starches (Hizukuri et al., 1970; Hoover, 2001), but in contrast to many other starches, especially from cereals. Potato starch contains also phosphorus in the form of phosphate covalently linked to the amylopectin component (Hizukuri et al., 1970). It is considered as an important factor contributing to potato starch properties. Most other starches also contain covalently bound phosphate though in only minute amounts. Phosphorus is also a component in cereal starches. However, it is there found in the form of lysophospholipids that are forming non-covalent complexes with the amylose component (Morrison, 1995). Trace amounts of different cations, mostly potassium (Blennow et al., 2005a; Noda et al., 2005), were also described as minor components in potato starch granules, apparently coordinated to the phosphate groups.

This chapter considers the recent view on molecular structures of the amylose and amylopectin components in potato and how they are organized to form characteristic structures inside the starch granules. The phosphorylation of starch, and the synthesis of its components in normal and genetically modified potatoes, is also discussed.

4.2 Polysaccharide Components of Potato Starch

Amylose constitutes the minor, smaller and mostly linear components of starch. The molecular size-distribution of amylose expressed as weight-average molecular weight (M_w), is $0.2–3.9 \times 10^6$, whereas number-average degree of polymerization (DP_n) ranges between 840–21 800 (Hizukuri and Takagi, 1984). The polydispersity index DP_w/DP_n (or M_w/M_n) is

1.29–6.9. The slightly branched nature of amylose was known already in the 1950s, but it was only comparatively late that methods to calculate the actual molar fractions of branched and linear amylose were obtained (Takeda et al., 1987). The exo-acting enzyme β-amylase repetitively hydrolyzes maltose residues from the non-reducing end of the glucosyl chains. If the molecule is branched, the enzyme stops at the vicinity of the branches, which it cannot by-pass, resulting in a β-limit dextrin containing the reducing end. Thus, the molar amount of branched amylose was detected as tritium labeled β-limit dextrins (Takeda et al., 1992). In potato, the β-amylolysis limit of the amylose fraction is 68–88% (Hizukuri et al., 1981; Takeda et al., 1984) and the molar ratio of branched:linear amylose is 0.38 (Takeda et al., 1992). The average number of chains per molecule of the whole amylose fraction is only 7.3–12.2 (Hizukuri et al., 1981; Takeda et al., 1984). As this figure includes both the linear and branched components, the actual chain number of the branched amylose is higher. Though the size of the branched component is unknown (it was detected as the β-limit dextrin), it was shown that the degree of branching increases with size (Takeda et al., 1984).

The molecular weight of amylopectin is, as already mentioned, much larger than that of amylose. For potato amylopectin, M_w was reported to be 60.9×10^6 and the radius of gyration (R_g) 224.3 nm (Aberle et al., 1994). Number average values are considerably lower. Gel-permeation chromatography of fluorescent-labeled amylopectin gave a DP_n value of 11 200 (corresponding to $M_n \sim 1.8 \times 10^6$) (Takeda et al., 2003). Moreover, the size-separation technique revealed that the amylopectin component constitutes three molecular species with DP_n values around 16 100, 5500, and 2100, respectively. The molar proportion of the large component predominates (61%). The multiple size-components of amylopectin are not unique for potato; similar components are also found in maize, rice, and sweet potato (Takeda et al., 2003).

The chains of amylopectin are arranged to facilitate self-organization of discrete functional crystalline and amorphous sections. Hence, dissecting pools of chain populations and clustering of branch points provides important information on starch architecture at the molecular level. Generally the unit chains of starch are divided into two groups of long and short chains. This, together with the structure of components in acid-treated starch granules, gave rise to the cluster model (French, 1972; Robin et al., 1974; Nikuni, 1978). In the model the short chains are found in clusters and the long chains interconnect the clusters (Hizukuri, 1986). The chains in amylopectin were originally divided into A-chains (chains not substituted by other chains), B-chains (substituted with other chains), and the C-chain, which carries the sole reducing-end residue in the macromolecule (and is a special kind of B-chain) (Peat et al., 1952). In the nomenclature of Hizukuri (1986), the B-chains are subdivided. B1-chains are short B-chains that together with the A-chains form the major group of short chains. The long chains are subdivided into B2- (spanning two clusters) and B3-chains (spanning three clusters), etc. Details of the cluster model, and the exact differences that exist between amylopectins from different sources, remain obscure, however.

Numerous investigations describe the unit chain distribution of potato amylopectin. It is obtained by size-exclusion chromatography (Hanashiro et al., 2002), anion-exchange chromatography (McPherson and Jane, 1999) or capillary electrophoresis (Morell et al., 1998) after debranching of the molecule with the debranching enzymes isoamylase and/or pullulanase. In potato, the distribution of the short chains ranges from DP 6 to 35 or 36, their average chain length is 14–17 residues, and in the obtained chromatograms a peak is obtained at DP 13–14. The shortest chains at DP 6–8 possess typically a profile with highest amounts of DP 6 and lowest of DP 8 (McPherson and Jane, 1999; Hanashiro et al., 2002; Bertoft, 2004; Noda et al., 2005). The DP of the long chains (DP $\geq$ 36) is 48–57 and the molar ratio of short to long chains is 5.3–6.5 (Hanashiro et al., 2002; Bertoft, 2004; Noda et al., 2005). The long chains consist of at least two subgroups with DP_w approximately 58 (B2) and 75 (B3-chains) (Hizukuri, 1986). Their relative molar amounts are 9–14 and 2–3%, respectively (Hizukuri, 1986; Bertoft, 2004). In addition, trace amounts of very long chains with DP > 100 were reported (Noda et al., 2005). By debranching of fluorescent-labeled amylopectin, it was possible to analyze the size-distribution of the C-chain in preparations from starches of different botanical origin (Hanashiro et al., 2002). The chains cover the DP-range 10–130 and the presence of a peak at DP 42 and a shoulder at DP 25 for potato starch suggests that the C-chains mostly correspond to the lengths of B2- and B1-chains, respectively. It was noted, however, that one third of the molecules in the labeled C-chain fraction were not debranched and, therefore, the average length of the C-chains is probably even shorter (Hanashiro et al., 2002). Thus, the C-chain, from which the synthesis of the macromolecule supposedly begins, is surprisingly short.

In potato amylopectin the molar ratio of A:B-chains is 1.1–1.5 (Bender et al.,1982; Zhu and Bertoft, 1996; Bertoft, 2004) The size-distribution of the B-chains in the limit dextrin of amylopectin, in which the external chains extending from the outermost branches are largely removed, shows the internal unit chain profile of the amylopectin. It was found that the long internal B-chains in a range of different starches are sub-divided into the same groups as the long chains of the whole amylopectin (Bertoft et al., 2008). They are also found in practically equal amounts as in the whole amylopectin, which suggests that the long chains of amylopectin are generally comprised of only B-chains. However, in an amylose-free potato sample a small amount (2.6 mole%) of long A-chains were apparently also present (Bertoft, 2004). The short internal B-chains consist also of sub-groups. One has internal lengths from 2–6 and is quantitatively a minor group. The other possesses DP 7–34 and is the major group. Both groups are found in other starches as well and are therefore not unique for potato. The molar ratio of the short B-chain groups is, however, different in different starches (Bertoft et al., 2008). As the chains are part of the clusters, it suggests differences in the structure of the clusters in different amylopectins.

The size of the clusters was estimated indirectly from the ratio of short to long chains, which for potato suggests that a single cluster comprises ~6 chains (Hanashiro et al., 2002). Depending on

the size of the amylopectin, a single amylopectin macromolecule was subsequently estimated to consist of 15–117 clusters (Takeda et al., 2003). Clusters were also isolated from limit dextrins of the macromolecule using endo-acting enzymes. With cyclodextrin transferase from *K. pneumoniae*, Bender et al. (1982) found clusters in potato of three distinct sizes with DP 40–140, whereas Finch and Sebesta (1992) only found clusters of the larger size (corresponding to M_n 23 000) when using a tetraose-forming amylase from *P. stutzeri*. With α-amylase from *B. amyloliquefaciens* (also referred to as the liquefying α-amylase from *B. subtilis*) the clusters were found to have DP 33–70, corresponding to 5–10 chains (Zhu and Bertoft, 1996).

When discussing the molecular fine structure of amylopectin in more detail, it is useful to refer to three different structural levels. The lowest level, *building blocks*, considers very small, tightly branched units and concerns the internal organization of the chains in the second level of *clusters*. A *domain* is the third level and considers smaller or larger groups of clusters, how they are interconnected and whether clusters of different structures are found in different domains. In a recent series of experiments (Bertoft, 2007a, b), all three structural levels were isolated from amylose-free potato starch using the α-amylase from *B. amyloliquefaciens* and their structures analyzed. During initial hydrolysis of amylopectin, the macromolecule depolymerizes rapidly, and domains containing groups of clusters were isolated when the reaction was interrupted. The domains were then subjected to a further hydrolysis with the enzyme until the reaction rate became low (Bertoft, 2007a). At this stage no internal chains long enough to fill all 9 subsites of the enzyme active site remained, and the clusters had been released from the domains. In the form of limit dextrins, the clusters of the amylose-free potato possess two major sizes with DP around 54 and 75 (Bertoft, 2007a), which is similar to that estimated (with the same enzyme) for amylopectin in normal, amylose-containing potato starch (Zhu and Bertoft, 1996). Because the external chains roughly contribute with 50% of the structure, the approximate size of the intact clusters in the amylopectin is DP 100–150. Only small differences in the cluster composition were found in different domains, suggesting that the structure of potato amylopectin is rather homogenous (Bertoft, 2007a). The number of long chains found in small domains containing only 2 clusters suggested that a single long chain is involved in their interconnection, which is in accordance with previous hypotheses (Hizukuri, 1986). However, as the number of clusters increased, a surplus of long chains tended to be present. This suggested that at higher structural levels alternative models are valid (Bertoft, 2007a). One alternative that possibly can accommodate more long chains is a backbone cluster model, in which the long chains form a backbone to which the clusters are bound, rather than being integrated parts of the clusters (Bertoft, 2004) (Figure 4.2).

The composition of branched building blocks in the isolated clusters of the amylose-free potato starch, was analyzed by treating the clusters with excess α-amylase (Bertoft, 2007b). Under such conditions, the enzyme is forced to continue the attack at internal chains shorter than ∼9 residues inside the clusters. As a result the building blocks, which practically resist further attack

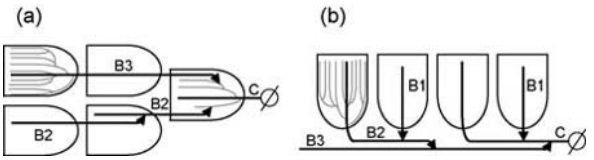

Figure 4.2: The mode of interconnection of clusters in amylopectin through (a) the traditional cluster structure and (b) the two-directional backbone structure. Boxes symbolize clusters with short chains (gray lines) indicated in some clusters. Black lines are chains involved in cluster interconnection. In (b) the B1-chains are the longest types of B1-chains with DP 25–35. The C-chain carries the reducing end-residue (∅).

(i.e., they are near-limit dextrins), are released. The blocks in potato amylopectin possess very short average internal chain lengths (ICL) of ∼2.5 residues between the branches (Bertoft, 2007b). Each cluster consists of 5–7 building blocks, depending on its DP. The smallest blocks, which are in the majority (∼60 mole%), constitute a group at DP 5–10 and are singly branched. Blocks at DP 10–14 are doubly branched dextrins, whereas larger blocks are multiply branched and found only in small number in the clusters (∼20 mole%). The clusters possess slightly different densities of building blocks, making it possible to divide them into two groups with slightly different structural characteristics. The building blocks are interconnected through chains with ICL 7–8. On this basis it was suggested that a cluster generally is defined as a group of chains interconnected by internal chains shorter than 9 residues (Bertoft, 2007b). A hypothetical model of the building block structure of potato amylopectin clusters is shown in Figure 4.3.

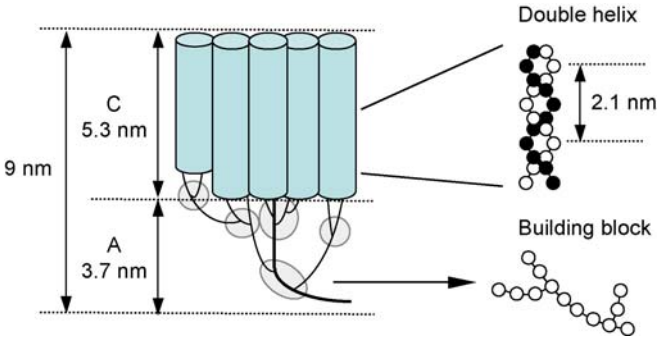

Figure 4.3: The building block structure of potato amylopectin clusters. Branched building blocks (encircled) are mainly found inside amorphous lamellae (A) of semi-crystalline rings in starch granules. Double helices (symbolized as cylinders) extend from the building blocks into the crystalline lamellae (C). Enlargements of a double helix segment, in which the single strands are parallel and left-handed, and a building block are shown to the right.

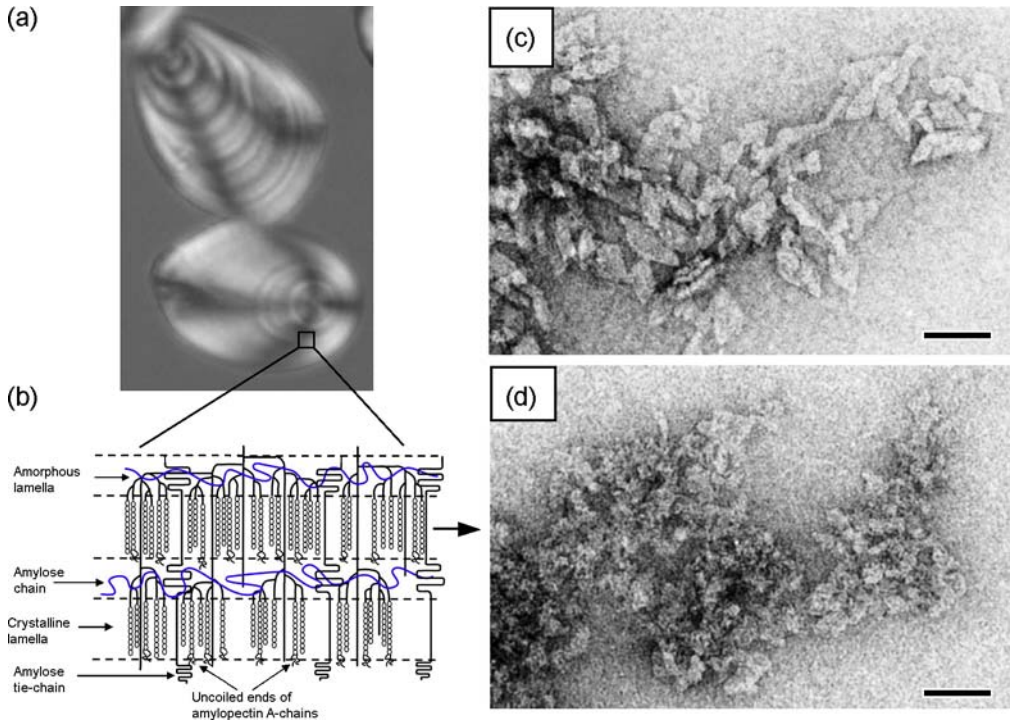

Figure 4.4: Structures in potato starch granules: (a) Potato starch granules observed under polarized light showing the characteristic 'Maltese crosses' and alternating amorphous and semi-crystalline rings (courtesy of J. Wikman, Åbo Akademi, Turku). (b) 'Enlargement' showing the structure of a semi-crystalline ring with amylose molecules embedded both inside the amorphous lamellae and between the double helices of the amylopectin in the crystalline lamellae (Kozlov et al., 2007). (c) Transmission electron microscopy image of the crystalline residue obtained after hydrolyzing amylopectin-rich native A-type waxy maize starch granules with 2.2 N HCl during 18 days at 36 °C and (d) B-type potato amylopectin starch granules. The preparations were negatively stained with uranyl acetate. Scale bars: 50 nm. (Images courtesy of: J.L. Putaux, CERMAV-CNRS, Grenoble).

4.3 Starch Granules in Potato

Despite a large variety in sizes and shapes of starch granules from different plants, their internal organization is remarkably similar. The starch macromolecules are organized in amorphous and semi-crystalline granular rings or shells, commonly called 'growth rings' (Figure 4.4) (Jenkins et al., 1993). The rings are 100–400 nm thick.

It is generally believed that the physical state of the amylose component is amorphous and therefore it is found in the amorphous parts of the granules. It is, however, not separated from the amylopectin component. By chemically cross-linking the polymers, it was shown that amylose

became cross-linked to amylopectin, and therefore the two components are found side-by-side inside the granules (Jane et al., 1992). The exact organization of the amylose may differ between granules from different plants and is dependent on the content of amylose in the granules (Kozlov et al., 2007).

The principal component of the semi-crystalline rings is amylopectin that, through its comparatively short chains, contributes to characteristic stacks of thin, alternating crystalline and amorphous lamellae (Figure 4.4). The repeat distance of the lamellae is 8.8–9.2 nm, of which the crystalline part is 5.3–5.5 nm (Jenkins et al., 1993; Kozlov et al., 2007). The amorphous lamellae contain most of the branched, inner structure of the clusters of short chains, whereas the external chains of the clusters form left-handed double helices (Figure 4.3). The pitch distance (one turn of a single strand) is 2.1 nm and consists of six glucosyl residues. In the crystalline lamellae, the double helices are packed in parallel fashion (Imberty et al., 1991). In potato granules a so-called B-type crystalline allomorph is found (Srichuwong et al., 2005). In this, the double helices are ordered into a hexagonal structure. The internal part of the structure contains a cylindrical room filled with water coordinated to the hydroxyl groups of the carbohydrate residues (Imberty et al., 1991). The B-allomorph is not unique for potato, but typically formed in starch granules containing amylopectin with comparatively long unit chains (Hizukuri, 1985). If starch granules are observed in polarized light under the microscope, a typical ‘Maltese cross’ is seen (Figure 4.4). Starch granules have been shown to be positively birefringent (Blennow et al., 2003), which shows that the amylopectin chains are radially oriented inside the granules. Using a microfocus X-ray diffraction beam, it was shown that the components in the core of the potato granules are only partially oriented, but highly oriented towards the periphery, which seems to contribute to a very compact surface structure on the potato granules. In contrast, the crystallites in cereal grain starch granules, like those found in wheat, possess so-called A-type crystals, and are only weakly, if at all oriented (Buléon et al., 1997). Observations by atomic force microscopy showed a surface full of round structures with a diameter of 10–50 nm, from which larger protrusions (50–300 nm) extended (Baldwin et al., 1998). The structures, known as blocklets (Gallant et al., 1997), are also found inside the granules (at least in maize granules) (Ridout et al., 2002) and their dimensions suggest that they possibly are identical to the amylopectin molecules. Images obtained by cryo electron diffraction and optical tomography from potato granules were interpreted as very complex super-helices of the amylopectin component (Oostergetel and Bruggen, 1993). The super-helices form an apparently delicate network around which the granules are built. Later, a super-helical structure of potato amylopectin was suggested based on small angle X-ray microfocus scattering observations (Waigh et al., 1999). The super-helix was pictured as containing pie-shaped units, possibly clusters. The pitch of the helix is 9 nm, i.e., corresponding to the lamellar repeat distance, and the outer and inner diameters are 19 and 8 nm, respectively (Waigh et al., 1999). Depending on the molecular structure, there are principally two possibilities to form a super-helix from amylopectin. With the traditional cluster model (Figure 4.2) a super-helix would be a co-operative structure composed of several amylopectin molecules lined

in parallel to each other. With the backbone model a super-helix can be formed from a single amylopectin macromolecule (Bertoft, 2004). The connection between the super-helix model and the blocklets is uncertain to date. It is, however, possible that a blocklet represents a visual form of the super-helix.

The measurable crystallinity of potato starch granules depends on their water content. In the dry granule the amylopectin component exists in a glassy nematic state with disorganized double helices. Upon hydration the amorphous backbone becomes highly plasticized and the double helices are reorganized into a smectic structure equivalent to the 9-nm lamellar repeat distance (Waigh et al., 2000). Differential scanning calorimetry shows that the melting (or gelatinization) of the crystals appears at a temperature interval from about 59–70 °C, with a peak temperature (Tp) at ~63 °C (Yusuph et al., 2003). (The exact interval depends on the sample and experimental conditions.) If the granules are treated at a temperature slightly below the onset temperature of gelatinization (T_o), the double helices reorganize into more perfect structures resulting in an increase in the melting temperature and a narrower interval (Jacobs et al., 1998). This phenomenon, known as annealing, results from a reorganization of the double helices through movements of the amorphous internal chains, to which they are connected (Tester and Debon, 2000).

By treatment of the granules in diluted hydrochloric acid or sulfuric acid, the amorphous parts of the granules are hydrolyzed (a process named lintnerization) (Robin et al., 1974). The resulting acid-resistant material (consequently called lintners) consists of the remnants of the crystalline lamellae. Analyses of the molecular composition of the lintners show that they consist of the double-helices of the former clusters (Jacobs et al., 1998). The length of the chains in potato lintners is 14–15, which closely corresponds to the thickness of the crystalline lamellae (Srichuwong et al., 2005; Genkina et al., 2007). Only few branches were found in the lintners from potato starch and other B-crystalline granules in comparison to A-crystalline granules, which suggests that only little branches are scattered into the crystalline lamellae in potato starch (Jane et al., 1997). When observed by transmission electron microscopy, the crystalline remnants from A-crystalline granules possess nanocrystals with amazingly regular parallelepiped blocks with acute angles of 60–65° (Putaux et al., 2003), in close correspondence with theoretical molecular models (Angellier-Coussy et al., 2008). Similar remnants in the lintners of potato granules are comparatively irregular and fragmented (Figure 4.4). This is somewhat surprising, as the overall structural orientation of the components in potato granules is high (Buléon et al., 1997), as noted above.

4.4 Phosphorylated Potato Starch

Most native starch types are slightly phosphorylated with phosphate groups monoesterified to the glucose residues (Blennow et al., 2002). The presence of phosphate esters in starch has been known for more than a century (Fernbach, 1904). The content of phosphate esters in starch

is very low. Nevertheless, the effects of these groups in the plant, as well as in the purified starch, are remarkable. In the plant, phosphate is required for complete degradation of the starch as described in the next section. Moreover, phosphorylated starches have a tremendous hydration capacity producing clear and highly viscous pastes (Wiesenborn et al., 1994; Viksø-Nielsen et al., 2001). The most phosphorylated starches such as those extracted from potato tubers with modified starch only have 1 out of 200 glucose residues substituted (Schwall et al., 2000; Blennow et al., 2005b). The phosphate esters are found as monoesters linked at the C-6 and C-3 positions of the glucose units (Posternak, 1935; Hizukuri et al., 1970; Tabata and Hizukuri, 1971; Bay-Smidt et al., 1994). The C-3-bound phosphate is a remarkable feature since phosphorylation at this position is rarely seen in nature.

Phosphate monoesters are present almost entirely in the amylopectin fraction (Samec, 1914), preferably in the long chains (Tabata and Hizukuri, 1971; Takeda and Hizukuri, 1982; Blennow et al., 1998). This is interesting since long-chain amylopectins preferably form the B-type crystalline polymorph found in tubers and in green photosynthetic tissue of the plant indicating that there are specific requirements for structuring these types of starches. Supposedly this is linked to the ability of these starches to be degraded by specific enzymes in the highly hydrated organs, as opposed to dry seeds storing starch with considerably less phosphate.

The presence of phosphate groups in crystalline regions of the starch granule has been indicated by effects on starch granule crystallinity as demonstrated by differential scanning calorimetry (DSC; Muhrbeck and Eliasson, 1991), its presence in so-called Nægeli dextrins representing the crystalline lamella of the starch granule (Blennow et al., 2000), its tight positioning in one of the groves in the double helix as shown by molecular models (Engelsen et al., 2003), its inability to form complexes with copper by electro paramagnetic resonance (EPR; Blennow et al., 2006), and its low mobility as recently demonstrated by NMR (Larsen et al., 2007). Considerable amounts are also indicated to be present in amorphous parts of the starch granule (Blennow et al., 2000). However, the effects of phosphate monoesters on starch molecular packing have not yet been clarified.

The distribution of phosphate in the native starch granule is seemingly not random. Lack of direct evidence for such distributional data stems from the difficulty in analyzing molecular entities and elements in semi-crystalline matrices like those found in the starch granule. As deduced from particle-induced X-ray emission (PIXE) data, phosphate is concentrated to the center of the starch granule (Blennow et al., 2005a). These findings were supported by a chemical gelatinization study (Jane and Shen, 1993), in which lower phosphate concentrations where found at the granule periphery than in the center. In contrast, a confocal laser light scanning microscopy (CLSM) study using a phosphate-specific fluorescent probe found phosphate at the surface, specifically at a rim close to the surface or spread out through the starch granule dependent on the plant genotype (Glaring et al., 2006).

4.5 Potato Starch Synthesis

Functionally, starch can be considered as a polysaccharide synthesized in a manner permitting its efficient degradation. Hence, biosynthesis of the starch granule is a delicate balance between efficient packing of the glucan chains and the possibility of breaking these structures at degradation. To complete this enzymatically catalyzed process in the potato tuber, a multitude of different enzyme activities are required.

These include activation of the glucose residue, elongation of the glucan chain and transfer of linear backbone chains forming branched structures. This process is generally more complex as compared to glycogen biosynthesis involving a multitude of different homologous enzymes supposedly responsible for synthesizing specific structures of the starch granule in different tissues and at different developmental stages. The paper by Ball and Morell (2003) can be consulted for a comprehensive review on this issue and for details as describe below.

4.5.1 Sugar activation and regulation of synthesis

Plants synthesize starch by a pathway starting with the activated sugar adenosine diphospho glucose (ADP-glucose) from glucose 1-phosphate and ATP in a reaction catalyzed by the enzyme ADP-glucose pyrophosphorylase (AGPase; Ball and Morell, 2003). This reaction is the rate-limiting step in starch synthesis. In chloroplasts, AGPase is allosterically activated by 3-phosphoglycerate, the first product of photosynthesis, and inhibited by free phosphate. The rationale for this regulation is the sensing of high photosynthesis (high levels of 3-phosphoglycerate) requiring starch biosynthesis as a means to store assimilated carbon. Likewise, low photosynthesis is revealed by high free phosphate in the cell.

4.5.2 Chain elongation

Amylopectin is produced at the surface of the granule by soluble starch synthase (SSS) and starch branching enzyme (SBE). SSS catalyzes the elongation of the chain at the non-reducing end in a reaction where ADP of the ADP-glucose molecule is displaced by the terminal hydroxyl group of the growing glucan chain creating an elongated linear α-(1,4)-glucan chain. For amylose synthesis only one enzyme is required, the granule-bound starch synthase (GBSS). Just as with SSS, GBSS adds the glucose unit from ADP-glucose to the non-reducing end of a glucose chain. GBSS is completely bound to the starch granule and elongates the glucan chains processively, i.e., without diffusion between the different substrate chains.

4.5.3 Branching and maturation de-branching

The formation of α-(1,6)-linkages in starch is catalyzed by the starch branching enzyme (SBE). In this reaction an α-(1,4)-linkage within the chain is cleaved and an α-(1,6)-linkage is formed between the reducing end of the cleaved glucan chain and a C-6 linked oxygen of an adjacent

chain. SBE activity exists as multiple enzyme isoforms. Family A isoforms use preferentially shorter glucan chains during branch formation than family B. Hence, the two isoforms seem to play distinct but interdependent roles in amylopectin synthesis. The action of debranching activities during biosynthesis seems to be very important for the correct assembly of the starch granule. Such reactions catalyzed by debranching enzymes trim off branches by hydrolysis that are not correctly positioned in the molecule generating a branching structure appropriate for crystallization. In the absence of specific debranching enzymes in plant mutants highly branched *phytoglycogen* is accumulated. Due to the branched structure of phytoglycogen it cannot form crystalline arrays and is therefore soluble. The two types of debranching enzymes in plants belong to the isoamylase-type and the pullulanase-type debranching enzymes. Both types efficiently hydrolyze the α-(1,6)-linkage of amylopectin. The balance between branching and de-branching most likely forms the fundament for the generation of the well-structured domains, clusters and building blocks as explained in detail above, but the detailed enzyme actions taking place during synthesis of these are not known.

4.5.4 Starch phosphorylation

For tuber starches generally, and for potato starch especially, the B-type crystalline polymorph is the major crystalline unit. Although this crystalline type consist of channels with structured water, this type of starches is generally very well ordered, compact and has a smooth starch granule topography. This is apparently a result of the relatively long chains in the amylopectin clusters efficiently filling out the crystallites. As a result these types of granules are very resistant to amylolytic degradation (Jane et al., 1997). In order to facilitate and regulate degradation of the starch in the tuber during its germination, the starch granules are phosphorylated (Blennow et al., 2002). Not until recently was the enzyme catalyzing the phosphorylation discovered (Lorberth et al., 1998; Ritte et al., 2002). The phosphate groups have some intriguing effects on how the starch granule is metabolized. Seemingly, the phosphate groups provide molecular signals for starch degradation in the plant. It has been suggested (Blennow et al., 2002) that the phosphate groups itself by re-structuring of the starch granule affecting the granule degradability. This speculation has not yet been fully demonstrated. However, very recently data were provided to indicate that starch phosphate esters stimulate hydrolytic enzyme activity in vitro (Edner et al., 2007) confirming that phosphate esters can themselves re-structure the starch granule stimulating degradation.

4.6 Conclusions

Potato starch, and tuberous starch in general, has some unique properties as compared to cereal starches. The most important ones include long amylopectin chains forming hydrated and ordered B-type crystallites and the presence of phosphate esters. The clusters of potato amylopectin are comparatively small, comprising 5–10 short chains. The internal part of the clusters is organized into branched building blocks mainly found in the amorphous lamellae of the

semi-crystalline granular rings. The external segments of the chains constitute double helices ordered into the characteristic hexagonal structure of the B-type allomorph. In the inner parts of the granules the order of crystalline orientation is low, but it increases towards the periphery. Certain details regarding the structure of potato starch remain, at present, unclear. These include the actual mode of interconnection of the cluster units of the amylopectin component and the connection between the proposed super-helical structure and the blocklet structures in the granules. The synthesis of starch is complex and involves a range of enzyme groups. Through genetic engineering it is possible to control the synthesis and to develop potato starches with altered structures and functionality. Of special interest is the glucan water dikinase, providing the only way hitherto to biochemically substitute starch directly in the plant.

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CHAPTER 5

Potato Proteins, Lipids, and Minerals

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5.1 Introduction

The past few years have seen a significant development in the tools that can be used to characterize biological specimens. This has had a great impact in the amount of detail emerging about the chemical nature of the potato tuber as well. One compound group that is reviewed here is the proteins. The wide variety of proteins present in the tuber has been recently explored with proteomics. On the other hand, recent advances in the isolation of the major proteins from the side stream of starch manufacture are opening new possibilities to develop value-added products. Against this background it is important to consider the safety of potato proteins, particularly since they are commonly referred to as being low-allergenic compared to the plant proteins that are currently widely used in food applications. The second group of compounds discussed here is the lipids. More information has been gained recently on the detailed composition of the membrane lipids that make up the predominant fraction of lipids in potato tuber. In addition, ceramides and glucocerebrosides have been explored in detail for the first time. Genetic modification is providing means to re-direct the metabolite flow from glycoalkaloids to sitosterol and stigmasterol. Understanding is also increasing about the chemical structure of potato skin and the contribution of lipids to its functionality. The importance of a variety of oxylipins is slowly emerging. The third group considered here is the minerals. The longer history of studies on potato minerals becomes apparent from the review that covers the accumulation and distribution of the elements, as well as the effects of genetic and environmental factors on the elemental levels. The common conception of declining crop mineral contents is challenged, and the difficulty in predicting the effects of various agricultural practices on tuber minerals is highlighted. Because potato is an important staple crop worldwide, it is important to consider its nutritional value. It is commonly claimed that potato is a source of high-quality proteins, but is the quantity of protein sufficient to justify any claims? Since potato is considered as a low-fat food, how much potato should one eat for an adequate intake of essential fatty acids? Potatoes are known as an excellent source of minerals, but what is the contribution of one potato to the

dietary reference intake? In this review, the contribution of potato proteins, lipids, and minerals to human diet is discussed.

5.2 Proteins

5.2.1 The contribution of potato proteins to human diet

The Recommended Dietary Allowance of good-quality protein is 0.80 g/kg body weight/day, and the Acceptable Macronutrient Distribution Range for protein is 10–35% of energy for adults (Food and Nutrition Board, 2005). The best protein sources for humans are animal products. Potato tuber contains 20 g protein (range 6.9–46.3 g) per kg on a fresh-weight basis (OECD, 2002). The flesh of one boiled potato cooked in skin without salt has 2.54 g protein (1.87 g/100 g) (USDA, 2007). Potato consumption thus adds only a relatively small part to the total daily protein consumption. Even then, root-tuber crops such as potato and sweet potato represent a major non-cereal plant source of dietary protein worldwide (Davies, 1996). Potato protein is of high nutritional value because it contains high levels of the essential amino acids lysine, methionine, threonine, and tryptophan (OECD, 2002). The effect of storage of whole potatoes on protein content depends on the potato cultivar and the particular proteins being examined (Pots et al., 1999). Increased amounts and/or quality of protein in potato tubers by genetic modification have been reported in a few cases (Chakraborty et al., 2000; Ryan et al., 2005).

5.2.2 Overall protein composition – potato tuber proteome

Proteomic profiling by two-dimensional electrophoresis (2-DE) can give an overview of the protein complement of potato tuber (Figure 5.1). Although the number of proteins that can be analyzed is still limited considering the predicted number of proteins present in the entire plant proteome, 2-DE remains the most widely used tool for high-resolution separation and quantification of proteins. However, there are only a few reports of potato tuber proteins being resolved by 2-DE, and little information about the natural variation caused by genetic background, environmental influence or other factors. Despite extensive research, the molecular changes that occur throughout the tuber life cycle are not yet fully resolved. A recent study by Lehesranta et al. (2006) gives the first comprehensive picture of changes in the proteome from tuberization, through tuber development, dormancy, and storage into the sprouting phase. Altogether 150 proteins showing highly significant differences in abundance between specific stages in the life cycle were highlighted, and 59 of these were identified. Characteristic to the developmental process was the accumulation of patatin isoforms and enzymes involved in disease and defense reactions. Furthermore, enzymes involved in carbohydrate and energy metabolism and protein processing were associated with development but decreased during tuber maturation. Varietal differences in potato proteins have been studied traditionally by

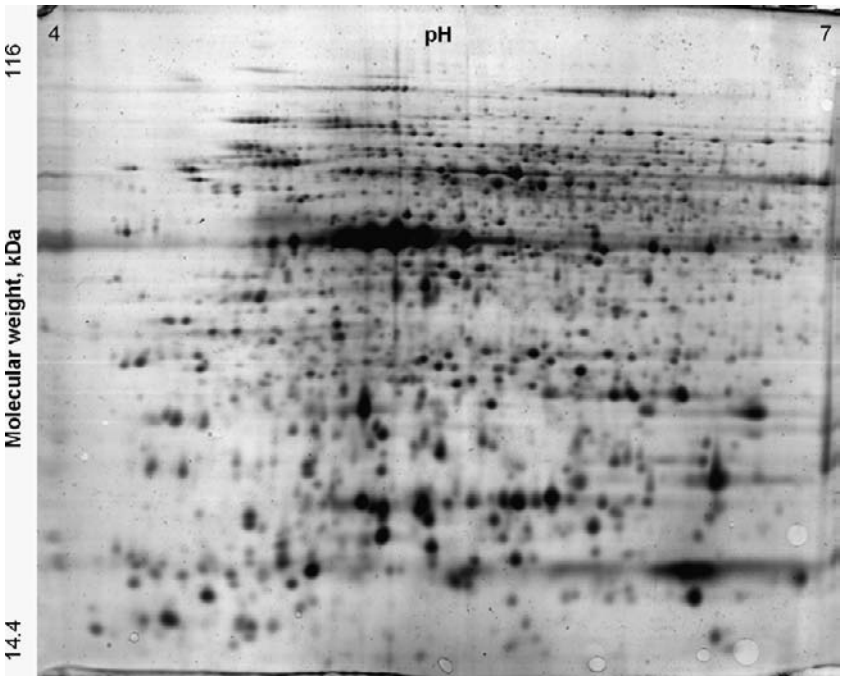

Figure 5.1: Two-dimensional electrophoresis separates potato proteins based on their isoelectric points and molecular weights. Using a wide-scale pH gradient and large gels, 1000–2000 quantifiable proteins can be separated. The gel stained with SYPRO Rubi shows soluble tuber proteins of potato cultivar Santé.

one-dimensional SDS-polyacrylamide gel electrophoresis and selected isoenzyme analyses. The power of 2-DE to reveal the differences was demonstrated by Lehesranta et al. (2005), who carried out an extensive analysis of tuber protein profiles of 32 potato genotypes representing a range of genetic variation: 21 named cultivars of tetraploid potato, eight landraces, and three diploid genotypes, including accessions and named cultivars of *Solanum phureja*. Genotypic variation was extensive, with 97% of the proteins showing significant qualitative and quantitative differences between one or more varieties and landraces. Of the nearly 2000 polypeptides detected, only 34 did not appear to differ significantly between the genotypes. Some of the most striking differences occurred in the various isoforms of patatin. A majority of the 77 proteins identified from the cultivar Désirée were present in relatively high amounts; many of them were involved in energy metabolism, protein destination, and storage or disease/defense responses. Very limited information is available on the effects of farming systems and their key components on the protein composition of plants. In a recent study, Lehesranta et al. (2007) used 2-DE to identify and quantify the effects of fertility management methods, crop protection practices, and rotational designs used in organic, low input and conventional production systems on the protein profiles of potato tubers. The differences observed between the alternative regimes were

relatively small, and much smaller than, e.g., the differences between cultivars (Lehesranta et al., 2005). Only fertilization practices (organic matter vs. mineral fertilizer) had a significant effect on protein composition. Quantitative differences were detected in 160 of the 1100 tuber proteins. The proteins identified were involved in protein synthesis and turnover, carbon and energy metabolism and defense responses, and suggested that organic fertilization leads to an increased stress response in potato tubers. The differences could, at least in part, be explained by differences in nitrogen availability.

5.2.3 Major proteins of potato tubers

Potato tuber protein complement is of interest to the potato starch industry, because high quantities of proteins can be purified from the potato juice by-product. Thus a number of studies on potato tuber proteins have been performed on cultivars grown for industrial starch production, such as Elkana in The Netherlands and Kuras in Northern Europe. A few studies have been performed on, e.g., cvs Désirée and Bintje, which are commonly used for human consumption in Europe. The soluble proteins of potato tuber have been classified broadly into three groups: patatins, protease inhibitors, and other proteins (Pots et al., 1999). Patatins and protease inhibitors are well characterized, whereas quite limited information has been available about the other major proteins.

Patatins constitute a group of homologous proteins of a size range 40–45 kDa. Patatin genes occupy a single major locus that consists of both functional and non-functional genes (Stupar et al., 2006). Patatin isoforms represent ca. 40% of total soluble proteins in potato tuber (Shewry, 2003). The patatin profiles vary markedly between potato cultivars (Lehesranta et al., 2005; Figure 5.2). Lehesranta et al. (2005) identified nine patatin forms from the tuber of cv. Désirée.

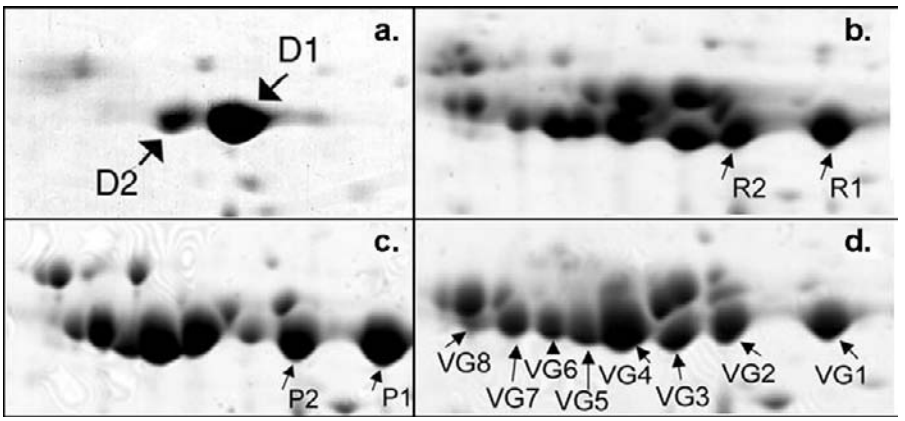

Figure 5.2: Patatin profiles of potato cultivars a. Désirée, b. Record, c. Pito, and d. Van Gogh. The two-dimensional gels were stained with Coomassie Brilliant Blue.

In the tuber of cv. Kuras, 17 patatin forms were identified (Bauw et al., 2006). Patatins are the primary storage proteins in potato tubers (Shewry, 2003). They exhibit antioxidant activity (Liu et al., 2003) and possess a wide substrate specificity as acyl hydrolases but also show β-1,3-glucanase activity (Rydel et al., 2003; Shewry, 2003). Patatins appear to play a significant role in plant defense against pests and fungal pathogens, the galactolipase and β-1,3-glucanase activities possibly being the contributing factors (Shewry, 2003; Sharma et al., 2004). Potato tubers contain several protease inhibitors that inhibit the activity of trypsin, chymotrypsin, and other proteases, thus decreasing the digestibility and the biological value of the ingested protein. However, the protease inhibitors are largely inactivated by boiling and other thermal processes, and serious anti-nutritional reactions only occur if raw or inadequately cooked potatoes are consumed or fed (OECD, 2002). In contrast to patatins, the protease inhibitors are a more heterogeneous group of proteins. Potato tuber contains inhibitors of five families of protease inhibitors (Rawlings et al., 2004; Bauw et al., 2006). These small proteins, ranging in size from 5 to 25 kDa, represent about half of the total soluble proteins in the potato juice of cv. Elkana (Pouvreau et al., 2001). Of these, ca. 70% belong to the Kunitz family (Pouvreau et al., 2003). These have been divided further into three major groups A, B, and C, to which Bauw et al. (2006) recently added two more groups (K and M). The potato tuber protease inhibitors are active against serine proteases, cysteine proteases, aspartate proteases, and metalloproteases (Pouvreau et al., 2001, 2003; Heibges et al., 2003). Protease inhibitors serve as storage proteins and regulate the activity of endogenous proteases. In addition, they can be active against herbivorous insects and phytopathogenic fungi (Bayés et al., 2006; Hermosa et al., 2006). Wounding and water stress may cause secretion of protease inhibitors from potato tubers (Ledoigt et al., 2006). Little information on the relative quantity of other proteins present in the tuber or tuber juice is available. Lipoxygenase (ca. 10% of total protein), defensin (5%) and starch phosphorylase L-1 (4%), as well as annexin, glyoxalase I, enolase, catalase and UDP pyrophosphorylase appear to be quite abundant in the tuber juice of cv. Kuras (Bauw et al., 2006; Jørgensen et al., 2006). Of those proteins, enolase was among the more prominent ones in cv. Désirée also (Lehesranta et al., 2005, 2006). Heat shock proteins and pathogenesis-related proteins appeared to be rather abundant in cv. Désirée, but quantitative comparison of different proteins in 2-DE gel should be interpreted with caution due to different staining capacity of the proteins.

5.2.4 Potential applications of potato proteins

5.2.4.1 Potato protein concentrates and isolates

While potato tuber is an important source of starch, it also contains 30–35 g buffer-extractable protein per kg dry weight (Pots et al., 1999). Protein yield per hectare of potatoes has been estimated as 500–1000 kg. The aqueous solution remaining after industrial potato starch manufacture, i.e., the potato fruit juice, contains approximately 1.5% (w/v) of soluble protein, mainly

composed of patatin and protease inhibitors. Potato protein concentrates are traditionally prepared by precipitating the proteins with a combined acidic heat treatment (thermal coagulation) of the fruit juice (Strolle et al., 1980; OECD, 2002). After centrifuging and drying, the precipitate contains more than 85% crude protein. Potato protein isolated by thermal coagulation is highly unstable and insoluble and has lost functionality for food applications (e.g., formation and stability of foam). Several efforts have been made to recover the proteins in a way that retains their native properties. The effects of various combinations of ionic strength, pH and temperature on conformational stability, activity and solubility of potato protein fractions were studied by van Koningsveld et al. (2001). Potato proteins were shown to unfold between 55 and 75°C, decreasing enzyme activities and causing loss of solubility. At mildly acidic pH the solubility was dependent on ionic strength and the presence of unfolded patatin. The use of organic solvents combined with a moderate lowering of pH resulted in potato protein precipitates with good solubility characteristics at neutral pH (van Koningsveld et al., 2002a, c). However, the presence of ethanol significantly reduced the denaturation temperature of potato proteins, implying that the isolation should be performed at low temperature in order to retain a high solubility. Largely due to these solubility problems, potato protein concentrate has been considered as by-product of low value despite the high nutritional value of the undenatured protein (Kapoor et al., 1975; Liedl et al., 1987). The traditional use is as cattle and pig feed (OECD, 2002). There is an interest to develop alternative feed materials, in particular those of vegetable origin, for salmonid feed, which is usually based on fish meal. However, conventional potato protein concentrate results in severe appetite loss in rainbow trout because of the bitter-flavored glycoalkaloids (1.5–2.5 mg/g concentrate). Refstie and Tiekstra (2003) tested a concentrate from an industrial process that almost completely removes the glycoalkaloids during the manufacture. The concentrate was well utilized and digestible by Atlantic salmon even when it replaced 40% of the fish meal protein. Since pancreatic proteases of salmonids are highly sensitive to protease inhibition, it may be necessary to ensure that the protease inhibitors are inactivated during the manufacture of the concentrate. For the aforementioned reasons, potato protein concentrates have not been used in food applications. Efforts have been made, however, to understand some of their properties relevant in food products. The ability to form and stabilize foams is an important functional property of food proteins. In whipping tests less foam was formed from untreated patatin than from the protease inhibitors, but patatin foam was much more stable (van Koningsveld et al., 2002b). The foam-forming properties of patatin could be improved greatly by partial unfolding of the protein, suggesting that the way the protein is isolated influences the foaming properties. Proteins are also used in many food products as emulsifiers and emulsion stabilizers. According to van Koningsveld et al. (2006), patatin-rich potato preparations were the most promising ones for food emulsion applications, provided the strong lipolytic activity can be diminished. Recent efforts to improve the protein quality for food applications have focused on the development of more gentle separation processes to replace the thermal coagulation method. Expanded bed adsorption (Anspach et al., 1999; Lihme et al., 2003) was first used as a larger-scale technique for the recovery of patatin from potato fruit

juice by Strætkvern et al. (1999). Besides better functionality, an advantage of the expanded bed adsorption process is that it removes a major part of glycoalkaloids (Løkra et al., 2007). The drying techniques following the extraction are important to maintain protein functionality (Claussen et al., 2007; Løkra et al., 2007). Atmospheric freeze drying was shown to be a more gentle drying process than spray drying and vacuum freeze drying. On the other hand, in some applications at least, it may be desirable to eliminate the enzymatic activities, particularly those of the protease inhibitors to ensure good digestibility of the protein-fortified diet. The Dutch potato starch group Avebe introduced the adsorbent processing platform with proprietary mixed mode ligand chemistry in 2007 in its subsidiary Solanic to extract high-performance proteins for the food and pharmaceutical industries. The process separates two fractions. The high molecular fraction, mainly composed of patatin, results in a dry food ingredient with protein content of 90–95%. The low molecular fraction comprises the protease inhibitors in liquid product. The company claims that potato protein scores better than soy protein and has the functionality of an animal protein (FLEXNEWS, 2007). This is the first effort to extract proteins at the mass production quality levels required for human consumption, and may lead in the future to better appreciation of this under-utilized resource.

5.2.5 Some suggested applications for protease inhibitors

Potato protease inhibitors have a number of potential applications. These include treatment for weight loss, peri-anal dermatitis, infections, thrombotic disease, and cancer. The reduction of food intake is based on the finding that potato protease inhibitors can elevate plasma cholecystokinin levels (Hill et al., 1990; Hu et al., 2006). This gastrointestinal hormone is involved in satiety and food intake regulation as well as blood glucose control in humans, and its increased levels in plasma delay gastric emptying, induce feeling of fullness and reduce food intake. Peri-anal dermatitis can be induced by increased levels of proteases in the feces. Potato tuber proteins can inhibit human fecal proteolytic activity efficiently (Ruseler-van Embden et al., 2004), which opens up the possibility of using topical application of potato protease inhibitors in preventing and treating this condition. Potato carboxypeptidase inhibitor can inhibit plasma carboxypeptidase activated thrombin-activatable fibrinolysis inhibitor (Nagashima et al., 2000; Bouma and Meijers 2003; Schneider and Nesheim 2003; Walker et al., 2003), and has been proposed as a potential fibrinolytic agent for treatment and/or prevention of thrombotic disease. A significant antithrombotic activity of potato carboxypeptidase inhibitor has been demonstrated recently in mice (Wang et al., 2006). Protease inhibitors might also be useful in anti-invasive and anti-metastatic treatment, because proteases are implicated in the malignant progression of human and animal tumors. Potato cysteine proteinase inhibitor PCPI 8.7 partially inhibited rodent B16 melanoma cell invasion (Sever et al., 2002). In addition, Blanco-Aparicio et al. (1998) showed that potato carboxypeptidase inhibitor competes with epidermal growth factor for binding to the receptor and has antitumoral properties; the epidermal growth factor receptor signal transduction pathway plays a prominent role in the development of carcinomas. This is the

first human epidermal growth factor antagonist described, and has therapeutic potential in the treatment of carcinomas (Sitjà-Arnau et al., 2005). Potato tuber protease inhibitors also interfere with hydrogen peroxide formation (Frenkel et al., 1987), and processes resulting from solar UV radiation (Huang et al., 1997). A series of antimicrobial Kunitz type serine protease inhibitors (AFP-J, PT-1, Potide-G) have recently been isolated from potato tubers (Park et al., 2005; Kim et al., 2005, 2006). AFP-J has potent antifungal activity against human fungal pathogens, e.g., *Candida albicans*, which is the most common cause of candidiasis (Park et al., 2005). PT-1 strongly inhibited pathogenic microbial strains, including *C. albicans*, the plant pathogenic fungus *Rhizoctonia solani*, and the plant pathogenic bacterium *Clavibacter michiganense* (Kim et al., 2005). Potide-G potently inhibited growth of a variety of human or plant pathogenic bacterial (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *C. michiganense*) and fungal (*C. albicans*, *R. solani*) strains (Kim et al., 2006), and has also potent antiviral activity against potato virus Y infection (Tripathi et al., 2006). The authors suggested that these proteins may serve as useful candidates for the development of novel anti-infective agents or agrochemicals.

5.2.6 Aspartic proteases

Cesari et al. (2007) demonstrated on bovine and human sperm in vitro that potato aspartic proteases 1 and 3 are effective spermicidal compounds. Spermicidal activity is due to membrane permeabilization and is not related to the proteolytic activity. Cesari et al. (2007) suggest vaginal contraception as a possible application.

5.2.7 Potato protein hydrolysates

Potato protein has a high content of amino acids with hydrophobic functional groups, in particular, with branched (isoleucine, leucine, and valine) and aromatic (phenylalanine and tyrosine) side chains (Refstie and Tiekstra, 2003). These may lead to bitter-tasting peptides in hydrolysis. Hydrolyzed potato protein has not been used as a food ingredient. Kamnerdpetch et al. (2007) aimed at a high degree of hydrolysis by using four different enzymes. The highest degree of hydrolysis was 44%. By this the authors aimed to increase the nutritional value of the product. Wang and Xiong (2005) showed that limited enzymatic hydrolysis of heat-coagulated potato protein increased its solubility by 14–19-fold. Since hydrolyzed animal and plant proteins are known to inhibit oxidation of bulk lipid or free unsaturated fatty acids, and since patatin and a potato protein hydrolysate were previously reported to exhibit antioxidant activity (Liu et al., 2003), Wang and Xiong (2005) evaluated the antioxidant activity of the hydrolysate. The hydrolyzed potato protein was able to improve the oxidative stability of cooked ground beef patties during refrigerated storage and might thus serve as a potential antioxidant for food quality preservation.

5.3 Safety aspects of potato proteins

One of the arguments often presented in favor of potato proteins as replacements of other plant-based proteins in food uses is that allergy to potato proteins is much less common than allergy to currently used plant proteins. For this reason, it is particularly relevant to address the safety issues of potato proteins more closely. Potato may elicit allergic reactions either by skin or inhalation contact with raw potatoes or when consumed as food. Five percent of the ca. 800 annually tested infants suspected of food allergy have been reported to show positive skin prick test reactions to potato, while the corresponding figures for eggs and cow's milk were 15% and 9%, respectively (Majamaa et al., 2001). The sensitization rate for potato in the general population has been estimated to be around 1.2% based on the value of 5.7% for Sol t 1 (patatin) in a study of 1886 Korean patients with various allergic disorders (Lee et al., 2006). Raw potatoes have been the allergen source in a number of documented cases. The reactions can be caused by skin contact with potato flesh (Carmichael et al., 1989; Iliev and Wüthrich 1998; Seppälä et al., 1999) or inhalation of aerosols during potato scraping or peeling (Pearson, 1966; Nater and Zwartz 1967; Jeannet-Peter et al., 1999). In children, allergy to raw potato has been reported only in a few cases, with immediate onset of symptoms (Dreborg and Foucard 1983; Wahl et al., 1990; Delgado et al., 1996; Beausoleil et al., 2001). Allergy to raw potato has mainly been described in adults, most frequently as a manifestation of oral allergy syndrome in patients with pollen allergy (Pearson, 1966; Quirce et al., 1989). This is generally caused by heat-labile allergens displaying immunoglobulin E cross-reactivity with tree pollen but also to grass and *Artemisia* pollen (Calkohven et al., 1987; Bircher et al., 1994; Ebner et al., 1995). The patients experience itching, rhinoconjunctivitis and, in some cases, asthma during the peeling of potatoes (Juhlin-Dannfelt, 1948; Pearson, 1966; Calkohven et al., 1987; Quirce et al., 1989). Contact dermatitis has also been described in food handlers with reactions to raw potato (Niinimäki, 1987). Cooking results in a partial or total loss of potato allergenicity (Nater and Zwartz 1967; Dreborg and Foucard 1983). Allergic reactions to cooked potatoes are considered to be fairly uncommon and have been reported in children only (Castells et al., 1986; Majamaa et al., 2001; De Swert et al., 2002, 2007), some with immediate and others with late reactions. Cooked potato can be allergenic in infants with atopic dermatitis (Majamaa et al., 2001). De Swert et al. (2002) evaluated symptoms before and after elimination of potato from the diet in atopic children and concluded that allergy to cooked potato might be responsible for severe, chronic allergic disease, especially eczema, in young children. Presenting symptoms in children with allergy to cooked potatoes are eczema, gastrointestinal complaints, urticaria and/or angioedema, wheezing/rhinitis, and anaphylaxis (De Swert et al., 2007). Most children develop tolerance at a mean age of four years. These aspects could be taken into consideration when developing food applications for potato proteins. An interesting question is which potato proteins are the main allergens. Patatin (Sol t 1) has been identified as a major food allergen among atopic children (Seppälä et al., 1999) and in persons with latex-associated potato allergy (Seppälä

et al., 2000; Schmidt et al., 2002). However, an oral sensitizing study in a Brown Norway rat model suggested that purified Sol t 1 is a weak allergen compared to a strong peanut allergen and to an intermediate allergen purified from shrimp (Knippels and Penninks, 2003). Because heat treatment of potato results in decreased allergenicity, it has been of interest to study the possible mechanism. Patatin is a glycosylated protein that when isolated denatures upon heating (Pots et al., 1998). However, denaturation does not substantially affect the affinity for IgE (Koppelman et al., 2002). Aggregation of isolated patatin results in non-reversible unfolding and a concomitant 25-fold decrease in affinity for IgE. While this cannot still fully explain the heat lability of the allergenicity of patatin, aggregation in the presence of other potato proteins (protease inhibitor pool in the studied case) results in an even more pronounced (110-fold) decrease of affinity for IgE. In addition to patatin (Sol t 1), four potato proteins belonging to the family of Kunitz-type soybean trypsin inhibitors have been identified as allergens in atopic children with positive skin prick test responses to raw potato (Seppälä et al., 2001). These proteins are Sol t 2 (cathepsin D proteinase inhibitor), Sol t 3.021 and Sol t 3.02 (cysteine proteinase inhibitors), and Sol t 4 (aspartic proteinase inhibitor). Potato can also elicit non-allergic hypersensitivity, i.e., a non-immunological reaction in which a substance in food triggers the mast cells/basophils directly or with the involvement of non-specific IgE antibodies. Purified potato lectin has been found to activate basophils and mast cells of atopic subjects by its interaction with chitobiose core of cell-bound non-specific immunoglobulin E (Pramod et al., 2007). Lectin is resistant to heat, base and acids, apparently due to its high content of carbohydrate residues and disulfide bonds, retaining nearly half of its activity in heat-processed potato extract (Matsumoto et al., 1983). The lectin content of raw and cooked potato tubers is ca. 6.5 mg and 0.5 mg per 100 g material, respectively. Higher intake of potato may thus increase the clinical symptoms as a result of non-allergic food hypersensitivity but the biological relevance of the finding of Pramod et al. (2007) is yet to be proven. Feeding digestion-resistant potato protein to rats was recently shown to increase the incidence and number of small intestinal neoplasms, including adenocarcinomas (Le Leu et al., 2007). The potato protein was prepared from potato juice by traditional steam coagulation at pH 5.0–5.5, and the digestibility was 84%, whereas it was 95% for casein. While consumption of high-protein diets has previously been shown to increase genetic damage in the colon, it was not clear why indigestible protein promoted tumorigenesis in the small intestine but not in the colon. It is of interest that potatoes have recently been recognized as a safe plant-derived food for LPT-allergic patients (Asero et al., 2007). LPT (lipid transfer protein) is a widely cross-reacting plant pan-allergen, e.g., in Rosaceae, tree nuts, peanut, beer, maize, mustard, asparagus, grapes, mulberry, cabbage, dates, orange, fig, kiwi, lupine, fennel, celery, tomato, eggplant (aubergine), lettuce, chestnut, and pineapple. Taken together, the safety aspects and other pros and cons of potato proteins need to be carefully considered when designing novel products for human use. It is also advisable to consult relevant authorities concerning the legal obligations and possible requirements for safety assessment of the products developed.

5.4 Lipids

5.4.1 The contribution of potato lipids to human diet

Potatoes are not regarded as an important source of lipids, because the lipid content of the tuber is very low, ranging from 0.2 to 2 g (1.2 g on average) per kg on a fresh weight basis (OECD, 2002). The flesh of boiled potato cooked in skin without salt contains about 0.1 g total lipids, 0.03 g total saturated fatty acids, 0.002 g total monounsaturated fatty acids, and 0.043 g total polyunsaturated fatty acids per 100 g (USDA, 2007). The Adequate Intake determined for the essential *n*-6 and *n*-3 polyunsaturated fatty acids is for adults 11–17 and 1.1–1.6 g per day, respectively (Food and Nutrition Board, 2005).

5.4.2 The lipids in potato tubers

The membrane lipids, in particular phospholipids and galactolipids, make up the predominant fraction of lipids in potato tubers, and only trace amounts of triacylglycerols are present. For example, in the tuber of cultivar Désirée, the membrane lipids constitute 92.8 mol-% of total lipids, whereas triacylglycerol amounts to only 1.1 mol-% (Klaus et al., 2004).

5.4.2.1 Simple lipids

The lipids are synthesized in potato tubers from sucrose (Klaus et al., 2004). After phloem unloading, sucrose is converted to UDP-glucose and fructose in tuber cells. A minor amount of carbohydrate is converted to acetyl-CoA and malonyl-CoA for fatty acid synthesis in the amyloplast. Fatty acyl groups are exported to the endoplasmic reticulum for lipid biosynthesis. Seventeen fatty acids have been detected in quantifiable amounts from raw potato tubers (Dobson et al., 2004). Total fatty acid content ranged from 569 to 723 μg per g of fresh weight. The predominant fatty acid is linoleic acid [18:2(*n*-6)], which encompasses ca. 50% of total fatty acids, followed by α-linolenic acid [18:3(*n*-3)] and palmitic acid (*n*-16:0), each having a ca. 20% share. Oxylipins are oxygenated fatty acids with diverse roles in the plant, such as antimicrobial or insecticidal function, or regulation of defense mechanisms (Blée, 2002). Oxylipins are derived from α-linolenic and linoleic acids. Lipoxygenases catalyze the oxidation of these fatty acids to highly reactive hydroperoxides, which are quickly metabolized into a series of oxylipins (Feussner and Wasternack, 2002). Since free fatty acids appear to be the preferred substrates, phospholipases first break down the complex lipids. However, lipoxygenases can also react with esterified fatty acids in lipids. Jasmonates are oxylipins derived from linoleic acid and have important signaling functions in plants (Weber, 2002). The tuber-inducing factor in potato, tuberonic acid or its glucopyranosyl derivative is derived from jasmonic acid. Other oxylipins are, e.g., traumatin, a plant wound hormone, and phytoprostanes that mediate defense reactions in response to oxidative stress. Triacylglycerols are the most abundant forms

of storage lipids. However, their amount is very low in potato tubers. One of the limiting factors for storage lipid accumulation in potato tuber is the product of acetyl-CoA carboxylase reaction, i.e., malonyl-CoA, which is the substrate for elongation during fatty acid synthesis (Klaus et al., 2004). Overexpression of acetyl-CoA carboxylase in the amyloplasts of potato tuber led to a 30% increase in total fatty acid content and a five-fold increase in the amount of triacylglycerol (from 11.6 to 58 g per kg fresh weight). Sterols can occur as free sterols, steryl esters, steryl glucosides, and acylated steryl glucosides (Bergenstråhle et al., 1996). Sterols are synthesized in potato from acetyl-CoA through mevalonate and squalene (Arnqvist et al., 2003; Krits et al., 2007). A branch point metabolite, cycloartenol, can be used for the synthesis of glycoalkaloids through cholesterol intermediate, or can be metabolized to brassinolide, or to stigmasterol through isofucosterol and sitosterol. Potato tuber contains a number of free sterols and steryl esters (Bergenstråhle et al., 1996). Fresh tubers of potato cultivar Désirée contain ca. 60, 50, 30, and 7 mg per kg of isofucosterol, sitosterol, stigmasterol, and cholesterol as free sterols, and ca. 3.3, 7.7, 4.6, and 1.8 mg per kg of the same compounds as steryl esters (Arnqvist et al., 2003). In an attempt to reduce the glycoalkaloid levels, Arnqvist et al. (2003) overexpressed soybean type 1 sterol methyltransferase in potato. In addition to decreased glycoalkaloid levels, the modification resulted in increased total sterol level in the tubers. The main increase in total sterols was due to higher levels of free and esterified sitosterol. This suggested that conversion of sitosterol to stigmasterol is a rate-limiting step in sterol biosynthesis in potato tuber. Tocopherols are substituted benzopyranols. α-Tocopherol (vitamin E) is one of the main antioxidants in potato tuber. Potato tubers contain 0.7–4.5 μg α-tocopherol per g fresh weight (Ducreux et al., 2005; DellaPenna and Last 2006). The amounts of α-tocopherol found in Andean potato tubers, ranging from 2.73–20.80 μg per g dry weight, were higher than those reported for commercial varieties (Andre et al., 2007). The lipophilic biopolymer suberin is a major constituent of the inner part (periderm) of potato tuber skin (Vogt et al., 1983; Graça and Pereira, 2000; Graça and Santos, 2007; Franke and Schreiber, 2007). It is a complex polyester mainly based on glycerol (22%) and aliphatic long-chain α,ω-diacids (32%) and ω-hydroxyacids (14.8%) (Graça and Santos, 2007). Small quantities of 1-alkanoic acids (8.4%) and 1-alkanols (2.5%) are also present. A small amount of ferulic acid (1%) is linked to the primary hydroxy group of ω-hydroxyacids (Graça and Pereira, 2000). Compared to other plants, suberin in potato tuber periderm has a high proportion of unsaturated monomers (Graça and Santos, 2007). The chain lengths for monomers range from C_{16} to C_{32} and increase in native periderm during tuber storage (Schreiber et al., 2005). An attempt was recently made toward the identification of the interunit covalent linkages among the aliphatic ester, phenolic, and carbohydrate moieties in suberized potato tissues (Arrieta-Baez and Stark, 2006). Suberin functions as a barrier to pathogen attack. The soluble lipids associated with suberin are also responsible for the low water permeability of periderm membrane, thus preventing water loss. Schreiber et al. (2005) studied wax and suberin development of native and wound periderm of potato tuber of cv. Désirée. Within one month of storage, the amount of suberin in the polymer increased two-fold in the native periderm (to 180 $\mu g\,cm^{-2}$), whereas in the wound periderm

about 75.0 μg cm^{-2} suberin polymer was newly synthesized. Native potato tuber periderm developed a very efficient transport barrier for water within one month of storage, while the water permeability of wound periderm remained much higher. However, from their studies Schreiber et al. (2005) concluded that the low water permeability of potato periderm is nearly exclusively determined by waxes and not by the suberin polymer itself. The wax content of potato tuber periderm (cv. White Rose) has been determined as 2 μg cm^{-2} and consists of hydrocarbons (31%; major alkane C_{25}), fatty alcohols (24%; major components C_{26} and C_{28}), fatty acids (11%), wax esters (7%) and other unknown components (Espelie et al., 1980). The wax content of potato tuber periderm is very low compared to, e.g., sweet potato (32 μg cm^{-2}) and carrot (29 μg cm^{-2}).

5.4.2.2 Complex lipids

Membrane lipids, in particular phospholipids and glycoglycerolipids, make up the predominant fraction of lipids in potato tubers (Pun et al., 1980). Phosphatidylcholine is the major phospholipid (30.7 mol-% of the total polar or complex lipids), followed by phosphatidylethanolamine (19.6%), phosphatidylinositol (9.3%), phosphatidic acid (3.2%), phosphatidylserine (1.5%), phosphatidylglycerol (1.2%), and diphosphatidylglycerol (cardiolipin) (0.7%) (Dobson et al., 2004). Of the glycoglycerolipids, the most abundant one in potato tuber is digalactosyldiacylglycerol (12.2 mol-%), followed by monogalactosyldiacylglycerol (8.7%), sulfoquinovosyldiacylglycerol (1.4%), and putative polygalactosyldiacylglycerol (0.8%) (Dobson et al., 2004). There is also a substantial amount of acyl sterol glycoside (9.0 mol-%). Dobson et al. (2004) also analyzed the fatty acid composition of the different complex lipid classes in tubers from the cultivar Cara. Sphingolipids are the most diverse and complex class of lipids, and are common constituents of cellular membranes. Sphingolipids were recently analyzed from tubers of several potato varieties by Bartke et al. (2006). The potatoes contained ca. 0.1–8 μg per kg single ceramides and up to 197 μg per kg glucocerebrosides, with C16:0h-glucosyl-4,8-sphingadienine as the major component (ca. 80% of all detected cerebrosides). The major long-chain base was 4,8-sphingadienine ($d18:2^{\Delta 4,\Delta 8}$) (ca. 80–85% of the long-chain bases of all detected cerebrosides), with lower amounts of 4-hydroxy-8-sphingenine ($t18:1^{\Delta 8}$) and 8-sphingenine ($d18:1^{\Delta 8}$). These three compounds were acylated with 22 different saturated and unsaturated hydroxy and nonhydroxy fatty acids with 16–26 carbon atoms to form the individual ceramides and glucocerebrosides. 2-(α-)Hydroxypalmitic acid (C16:0h) was the major fatty acid acylated to the long-chain bases.

5.4.3 The effects of storage on potato tuber lipids

The effects of storage on lipids depend on potato variety (Mondy et al., 1963). Tuber storage at 4°C resulted in an initial small increase in total fatty acid content (Dobson et al., 2004). Prolonged storage resulted in a fall to the initial values detected close to harvest. The content

of linoleic acid decreases and α-linolenic acid increases in tubers during storage (Spychalla and Desborough 1990; Dobson et al., 2004). The higher level of fatty acid unsaturation may mitigate increases in tuber membrane permeability during storage, thus positively influencing the processing quality of stored potato tubers. The different changes in linoleic and α-linolenic acids might also relate to oxylipin metabolism, because the major lipoxygenase in potato tuber preferentially uses linoleic acid as substrate (Royo et al., 1996). Fauconnier et al. (2003a) demonstrated that, during aging and sprouting in potato tubers stored at 20°C, the lipoxygenase pathway was activated. Galactolipases and phospholipases liberated free fatty acids, which were peroxidized to fatty acid hydroperoxides by lipoxygenase. The 9-hydroperoxide of linoleic acid was the main oxylipin formed. The hydroperoxides were then converted to colneleic acid (9-oxa-8-trans,10-trans,12-cis-linoleic acid), which was degraded to 9-oxo-nonanoic acid. Potato tubers contain about 3 μg colneleic acid per g fresh weight (Fauconnier et al., 2003b).

5.4.4 Lipids and potato flavor

Raw potato contains low levels of flavor volatiles. Cooking influences flavor generation. Fatty acid oxidation products contribute to the volatile profile and flavor of boiled potatoes (Petersen et al., 1998; Oruna-Concha et al., 2002). The straight-chain volatiles are derived by oxidation of unsaturated fatty acids (mainly linoleic and α-linolenic acids) which produces a range of flavor-active volatile aldehydes, ketones, alcohols, and alkyl furans. Dobson et al. (2004) suggested that the higher amount of α-linolenic acid in *Solanum phureja* may partially explain the higher intensity of earthy flavor in boiled potatoes compared to *S. tuberosum*. This may be due to 4*Z*-heptenal derived from an oxidation product of α-linolenic acid. The predominance of unsaturated fatty acids in the lipids is a critical factor in the manufacture and storage of dehydrated potato products because of their easy oxidation. Lipid degradation and production of oxylipins is a major cause of off-flavors during storage. Laine et al. (2006) studied the precursors responsible for off-flavor formation during storage of fresh potato flakes for 6 months at 25°C. Hexanal was identified as the main compound responsible for off-flavors. The authors suggested that hexanal was a degradation product of linoleic acid formed through linoleic acid hydroperoxide cleavage.

5.5 Minerals

5.5.1 The contribution of potato minerals to human diets

Humans require at least 22 mineral elements for their wellbeing (White and Broadley, 2005a). Potatoes are an excellent source of these elements (Storey and Davies, 1992; White et al., 2009). A single, medium-sized potato weighing 200 g fresh weight can provide about 26% of the US

Table 5.1: The mineral content of 200 g fresh weight of potatoes and its potential contribution to the US diet calculated as a percentage of the US Dietary Reference Intake (DRI).

	DRI	US potatoes	UK potatoes	% DRI
N (mg)	ns		660	–
S (mg)	ns		60	–
K (mg)	4700	850	720	18.1
Cl (mg)	2300		132	–
Ca (mg)	1000	22	10	2.2
P (mg)	700	118	74	16.8
Na (mg)	1500	12	14	0.8
Mg (mg)	420	45	34	10.6
Fe (mg)	8	1.4	0.8	18.1
Zn (mg)	11	0.6	0.6	5.5
Mn (mg)	2.3	0.3	0.2	13.0
Cu (μg)	900	231	160	25.6
I (μg)	150	37	6	24.9
Se (μg)	55	0.8	2	1.4

Mineral contents are means of four potato varieties available in the USA (USDA, 2006) or the UK (FSA, 2002), with the exception of US potato I, which was taken from True et al. (1978). The DRI values are those for a 31–50-year-old male (Food and Nutrition Board, 2004) ns = not specified. Adapted from White et al. (2009).

Dietary Reference Intake (DRI) of Cu, 17–18% of the DRI of K, P, and Fe, and between 5 and 13% of the DRI of Zn, Mg, and Mn (Table 5.1). Potatoes are generally not rich in Ca, but can be a valuable source of trace elements, such as Se and I, if fertilized appropriately (Eurola et al., 1989; Poggi et al., 2000; Turakainen et al., 2004; Broadley et al., 2006). Moreover, since potato tubers have relatively high concentrations of organic compounds that stimulate the absorption of mineral micronutrients by humans, such as ascorbate (vitamin C), protein cysteine and various organic and amino acids (USDA, 2006), and low concentrations of compounds that limit their absorption, such as phytate (0.11–0.27% dry matter; Frossard et al., 2000; Phillippy et al., 2004) and oxalate (0.03% dry matter; Bushway et al., 1984), the bioavailability of mineral elements in potatoes is potentially high.

5.5.2 The accumulation of mineral elements by potato tubers

Mineral elements are not synthesized by the plant. They must be acquired from the soil solution by the plant roots (Figure 5.3). Although tubers have associated roots, with the possible exception of Ca, these roots appear to supply only small amounts of minerals to tubers (Kratzke and Palta, 1985, 1986; Busse and Palta, 2006; Sowokinos, 2007). Most of the minerals present in potato tubers appear to have been taken up originally by the main roots that deliver them first to the shoot via the xylem. From the shoot, these mineral elements must be loaded into the phloem for

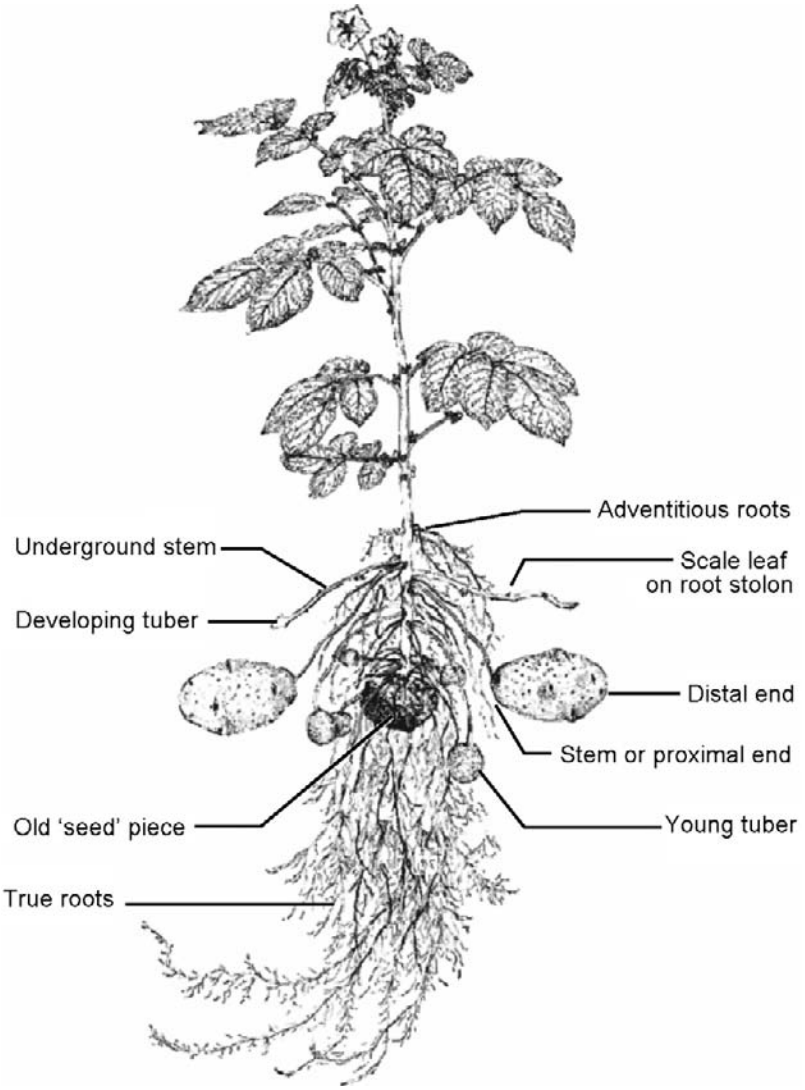

Figure 5.3: Diagrammatic drawing of the potato plant. Redrawn from Kehr et al. (1964).

translocation to the tuber. One consequence of this is that, even if the shoot receives adequate amounts of all minerals for its growth and development, elements that are not readily translocated in the phloem may have low concentrations in tubers. It is thought that Ca and B have extremely low mobility in the phloem and that Cu, Fe, Mn, Mo, and Zn have restricted mobility (Epstein, 1972). Thus, tuber mineral composition is not related simply to shoot composition (Figure 5.4). In the context of differences in the phloem mobility of mineral elements, it is noteworthy that mean tuber Ca and Mn concentrations were less than 3% those of diagnostic leaves, mean tuber

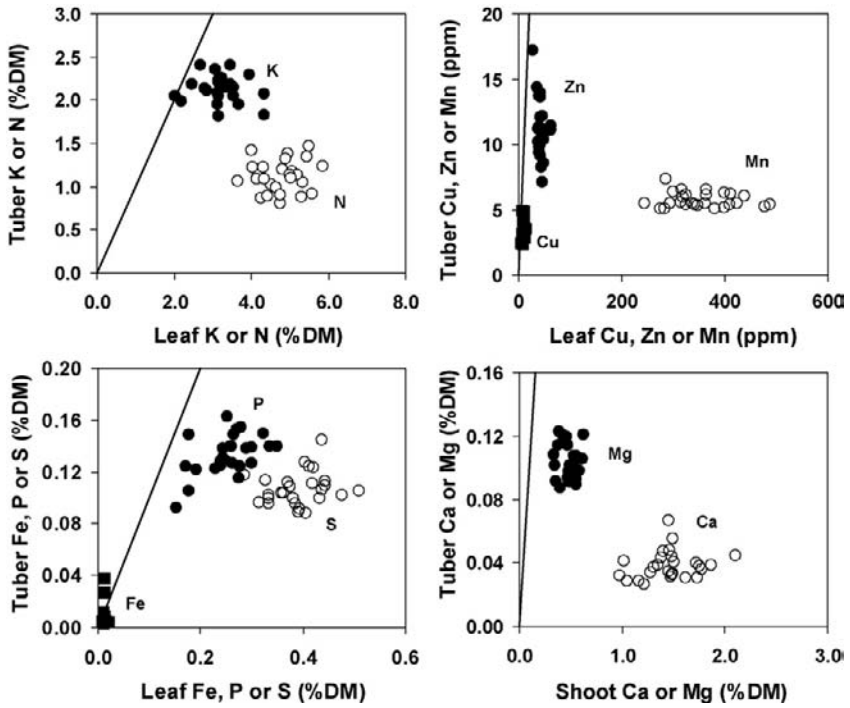

Figure 5.4: Relationships between leaf and tuber K, N, Fe, P, S, Cu, Zn, Mn, Mg, and Ca concentrations among a core collection of 26 commercial *Solanum tuberosum* varieties trialed in the field at the Scottish Crop Research Institute in 2006. Data are means of two replicate plots each containing eight plants at 40 cm spacing (P. J. White, J. E. Bradshaw, M. F. B. Dale and J. P. Hammond, unpublished data).

B, Cu, Mg, N, S, and Zn concentrations were between 20 and 50% those of diagnostic leaves and mean tuber Fe, K, and P concentrations were greater than 50% those of diagnostic leaves of plants grown in the experiment shown in Figure 5.4.

5.5.3 Distribution of mineral elements within potato tubers

The concentrations of mineral elements in tissues of potato plants change with plant age (Harris, 1992). Tuber concentrations of many mineral elements change dramatically in the weeks following tuber initiation (Harris, 1992; Turakainen et al., 2004), but their concentrations remain relatively constant during the final stages of crop development (Soltanpour, 1969; Kolbe and Stephan-Beckmann, 1997). The distribution of mineral elements also varies within the potato tuber (Figure 5.5). Variations in concentrations of mineral elements can exist between the stem end and the distal end of the potato tuber (Hughes and Swain, 1962; Bretzloff and McMenamin, 1971; De Kock et al., 1979; Ereifej et al., 1998; Sowokinos, 2007), and some elements, such as the cations K, Ca, Mg, Fe, Zn, Mn, and Cu, are more concentrated in the potato skin relative to

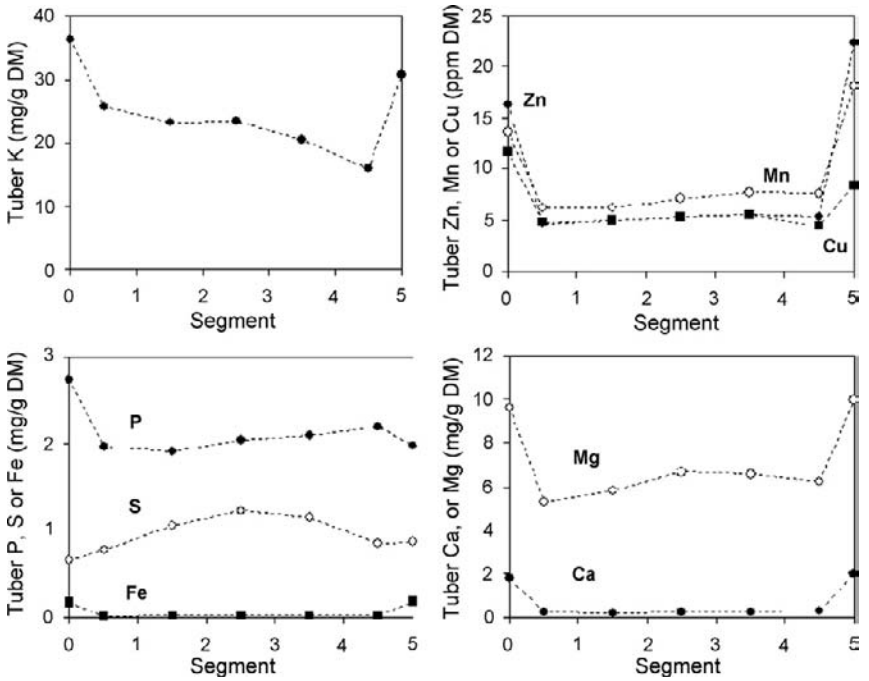

Figure 5.5: Distribution of mineral elements within potato tubers. Data show longitudinal profiles of elements from the distal (Segment 1) to the stem end (Segment 5) of tubers from Stirling plants (N. Subramanian, G. Ramsay, M. R. Broadley and P. J. White, unpublished data).

the flesh (Bretzloff and McMenamin, 1971; McGuire and Kelman, 1984, 1986; Ereifej et al., 1998; Wszelaki et al., 2005; Sowokinos, 2007).

5.5.4 Varietal differences in tuber mineral composition

There is considerable variation in tuber mineral concentrations among genotypes of cultivated potatoes (*Solanum tuberosum* and *S. phureja*; White et al., 2009). When grown under identical conditions, *S. tuberosum* genotypes have been shown to differ in tuber N, P, S, K, Ca, Mg, Fe, Zn, Mn, and Cu concentrations (Fitzpatrick et al., 1969; Augustin, 1975; Rexen, 1976; Randhawa et al., 1984; Workman and Holm, 1984; McGuire and Kelman, 1986; Tzeng et al., 1990; Van Marle et al., 1994; Ereifej et al., 1998; Allison et al., 2001; Dampney et al., 2002; Trehan and Sharma, 2003; Brown et al., 2005; Tekalign and Hammes 2005; Karlsson et al., 2006; White et al., 2009) and systematic differences in tuber K, Mg, Fe, Zn, Mn, and Cu concentrations have been observed between potato varieties obtained commercially (Casañas Rivero et al., 2003; Di Giacomo et al., 2007). Some of this variation can be attributed to the ratio of skin to flesh and/or the distance between the stem and the distal ends, both of which are

genetically determined, but other genetically determined properties also influence tuber mineral concentrations significantly. These observations suggest that it should be possible to breed for increased mineral concentrations in potato tubers.

5.5.5 The effects of agronomy on tuber mineral composition

There has been some concern that mineral concentrations in edible produce have declined over the last 60 years (Davis et al., 2004; White and Broadley, 2005b; Davis, 2006; Ekholm et al., 2007). This has been attributed to both a decrease in the mineral elements available to crops, because soils can become depleted in these elements, and/or 'growth dilution' as a consequence of increasing yields through agronomic and/or genotypic improvement (Davis et al., 2004; White and Broadley, 2005b; Davis, 2005). However, there is little evidence that these factors have affected the concentrations of mineral elements in potato tubers (White et al., 2009). Yields of potatoes have increased significantly over the last 50 years, due to the deployment of higher-yielding varieties together with the widespread use of fungicides, fertilizers, and irrigation (Harris, 1992; White et al., 2007). Although occasional studies have reported reduced tuber concentrations of some mineral elements when yields are increased by improved irrigation (Simpson, 1962; Asfary et al., 1983) or by growing higher-yielding varieties (Randhawa et al., 1984; Allison et al., 2001; Trehan and Sharma 2003; Tekalign and Hammes 2005), this is not universally observed (White et al., 2009). Furthermore, it has been suggested that any decrease in tuber mineral concentrations might be rectified through the application of appropriate fertilizers (White et al., 2009). The mineral composition of potato tubers is determined to a great extent by the phytoavailability of mineral elements in the soil. It will, therefore, depend upon local geology (True et al., 1978) and agronomic practice, such as conventional or organic production (Warman and Havard 1998; Wszelaki et al., 2005). It is generally observed that the application of mineral fertilizers increases tuber concentrations of the minerals supplied. This is true for fertilizers containing both macronutrients, such as N, P, K, Ca, and Mg (reviewed by White et al., 2009), and micronutrients, such as B, Zn, Fe, Mn, Cu, Mo, Se, and I (Eurola et al., 1989; Karam et al., 1998; Poggi et al., 2000; Turakainen et al., 2004; Broadley et al., 2006). However, in addition to increasing tuber concentrations of the elements supplied, the application of mineral fertilizers often affects tuber concentrations of other mineral elements (reviewed by White et al., 2009). This is the result not only of complex interactions between minerals in the soil, and their consequences for the uptake of mineral elements by plants, but also the effects of tissue mineral composition on the redistribution of elements within the plant (White et al., 2009). At present, the precise effects of fertilizers on the mineral content of tubers are difficult to predict and likely to depend upon a multitude of environmental, soil, fertilizer, and management factors. Nevertheless, it should be possible to increase tuber mineral concentrations by combining genotypes that have naturally higher tuber mineral concentrations with appropriate fertilization strategies to deliver more minerals to human diets without compromising yield.

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CHAPTER 6

Analysis and Biological Activities of Potato Glycoalkaloids, Calystegine Alkaloids, Phenolic Compounds, and Anthocyanins

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6.1 Introduction

This limited overview on the analysis of four classes of the following secondary potato metabolites is, except for anthocyanins, largely limited to our own studies of glycoalkaloids, calystegine alkaloids, and phenolic compounds. Because interest in these potato constituents arises from potential health benefits and occasional toxicity, we also include in this overview a brief discussion of these aspects that relate to composition and a description of experimental methods. The interested reader should consult the cited references for an entry into the extensive worldwide literature on the diverse analytical and biological aspects for these metabolites.

6.2 Glycoalkaloids

Steroidal glycoalkaloids are naturally occurring, secondary plant metabolites that are found in a number of foods including potatoes, tomatoes, and eggplants (reviewed in Friedman, 2002; Friedman and McDonald, 1997, 1999a, b). Although in high doses they are toxic, glycoalkaloids may also have beneficial effects. These include lowering of blood cholesterol (Friedman et al., 2000a, b), protection against infection by *Salmonella typhimurium* (Gubarev et al., 1998), and chemoprevention of cancer (Cham, 1994; Lee et al., 2004; Friedman et al., 2005, 2007).

In commercial potatoes (*Solanum tuberosum*) there are two major glycoalkaloids, α-chaconine and α-solanine, both trisaccharides of the common aglycone solanidine. These two compounds comprise about 95% of the glycoalkaloids in potato tubers. Their hydrolysis products, the β and γ forms and solanidine, may also be present in relatively insignificant concentrations. The structures of these glycoalkaloids and their hydrolysis products are presented in Figure 6.1.

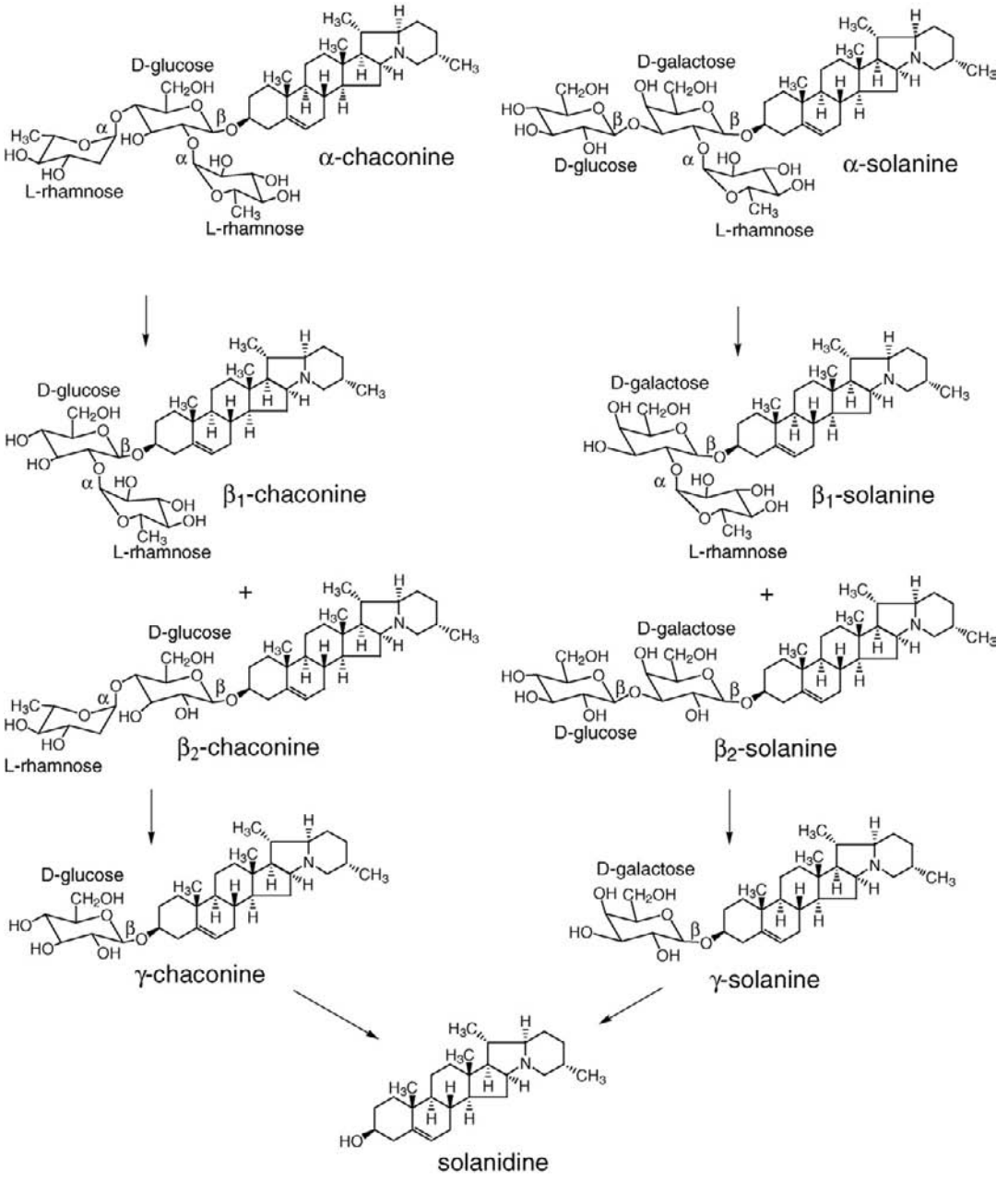


Figure 6.1: Structures of potato glycoalkaloids α-chaconine and α-solanine, and hydrolysis products (metabolites).

6.2.1 Toxicity

The toxicity of glycoalkaloids at appropriately high levels may be due to such adverse effects as anticholinesterase activity on the central nervous system (Abbott et al., 1960; Heftmann, 1967), induction of liver damage (Caldwell et al., 1991), and disruption of cell membranes adversely affecting the digestive system and general body metabolism (Keukens et al., 1995). At lower doses, the toxicity of glycoalkaloids in humans causes mainly gastrointestinal disturbances such as vomiting, diarrhea, and abdominal pain. At higher doses, it produces systemic toxicity, including symptoms such as fever, rapid pulse, low blood pressure, rapid respiration, and neurological disorders. Several cases of lethal poisoning have been reported (Korpan et al., 2004). There is some concern that dietary glycoalkaloids, due to their anticholinesterase properties, may adversely influence the actions of anesthetic drugs that are metabolized by acetylcholinesterase and butyrylcholinesterase (Krasowski et al., 1997). We found that solanidine exhibited estrogenic activity in an in vitro assay (Friedman et al., 2003a), with unknown health effects. Other complicating factors regarding the glycoalkaloid content of the diet that must be taken into account are: (a) α-chaconine appears to be more biologically active by a factor of about three to ten than is α-solanine; and (b) certain combinations of the two glycoalkaloids can act synergistically (Rayburn et al., 1995b; Smith et al., 2001).

These considerations have led to the establishment of informal guidelines limiting the total glycoalkaloid concentration of new potato cultivars to 200 mg/kg of fresh weight. As discussed elsewhere, these guidelines may be too high (Friedman, 2006; Friedman and McDonald, 1997). In one short-term clinical trial with human volunteers, one test subject experienced gastrointestinal disturbances after consuming mashed potatoes containing glycoalkaloids at the recommended limit, 200 mg/kg (Mensinga et al., 2005).

There have been few human studies, most of them anecdotal, so the susceptibility per individual variation and influence of other factors is not well established. The incidence of glycoalkaloid poisoning may be underreported, probably because physicians are more likely to implicate foodborne pathogens or general viral infections as the causative agents of gastrointestinal illness. Even these more common illnesses are known to be underreported. We therefore have no real basis to determine the frequency of poisoning caused by glycoalkaloids (Hopkins, 1995).

Toxicity may be influenced by other factors such as diet and general health. Glycoalkaloids are not well absorbed. However, the damaged intestinal wall may allow a spillover effect, causing them to be absorbed at a much faster rate after the mucosal cells have become compromised. This would account for the differences in symptoms observed for low (gastrointestinal) and for high (acute systemic) toxicity. It is worth noting that the apparent non-toxicity of the tomato glycoalkaloid α-tomatine appears to be due to complex formation with cholesterol in the digestive tract (Friedman et al., 2000b, 2007). Potato tubers of somatic hybrids whose progenies were the cultivated potato *Solanum tuberosum* and the wild-type *Solanum acaule* contained four glycoalkaloids, including tomatine, derived from the fusion parents (Kozukue et al., 1999).

These considerations suggest the possibility of developing high-tomatine potatoes for safety and health-promoting qualities (Friedman et al., 2007; Kozukue et al., 2008).

Other dietary factors, such as fiber, may also affect absorption in the gut. Hydrolysis products, the β-, γ-, and aglycone forms, are less toxic than the α-form (Rayburn et al., 1994). Therefore, processes that induce hydrolysis of the α-forms, such as exposure to *Aspergillus niger* (Laha and Basu, 1983) or to acid pH, may decrease toxicity. Other components and nutrients in the blood stream my affect toxicity as well. Folic acid, glucose-6-phosphate, and nicotine adenine dinucleotide (NADP) are reported to protect frog embryos against α-chaconine-induced developmental toxicity (Rayburn et al., 1995a; Friedman et al., 1997). Folic acid is now widely consumed by pregnant women to protect the fetus from neural tube malformations. It is also worth noting that Renwick (1972) found an epidemiological correlation between the congenital neural malformations anencephaly and spina bifida in fetuses and consumption of blighted potatoes by their mothers.

6.2.2 Analysis

Glycoalkaloids appear to be largely unaffected by food processing conditions such as baking, cooking, and frying. The content of glycoalkaloids can vary greatly in different potato cultivars (Friedman and Dao, 1992). Additionally, growing conditions and post-harvest exposure to light, mechanical injury, and storage can enhance glycoalkaloid levels (Kozukue et al., 1993; Machado et al., 2007).

The complex nature of the glycoalkaloid–dietary relationship suggests the need for accurate methods to measure the content of individual glycoalkaloids and their metabolites in fresh and processed potatoes as well as in body fluids such as plasma and tissues such as liver. HPLC methods are now widely used to determine the concentrations of individual glycoalkaloids, as well as glycoalkaloid hydrolysis (glycolysis) products in fresh and processed potatoes and in different parts of the potato plant such as leaves and sprouts (Bushway, 1982; Bushway et al., 1986; Carman et al., 1986; Kozukue et al., 1987, 1999, 2001; Saito et al., 1990; Dao and Friedman, 1992, 1994, 1996; Friedman and Levin, 1992; Hellenäs et al., 1992; Friedman et al., 1993; Houben and Brunt, 1994; Friedman and McDonald, 1995; Kubo and Fukuhara, 1996; Panovska et al., 1997; Friedman et al., 1998; Brown et al., 1999; Kuronen et al., 1999; Nitithamyong et al., 1999; Bodart et al., 2000; Simonovska and Vovk, 2000; Sotelo and Serrano, 2000; Väänänen et al., 2000; Fragoyiannis et al., 2001; Esposito et al., 2002). Other possible methods include ELISA and the use of biosensors. We will now describe selected methods for analysis of glycoalkaloids.

6.2.2.1 HPLC analysis

The glycoalkaloids and their hydrolysis products, with the exception of the aglycone, are analyzable in a single isocratic run. Most analyses were done on reverse phase columns. Separation

on reverse phases is based on differences in polarity of the molecules provided by the sugar(s) attached to the aglycone. The most polar α-glycoalkaloids elute first, followed by the β- and γ-glycoalkaloids. The aglycone elutes very late, if at all under isocratic conditions. Analysis of all compounds can be achieved in a single HPLC run using gradient elution.

Previously, we found that silanol moieties remaining on silica packing after bonding the C18 or C8 functional groups, and after endcapping, strongly influenced retention and separation of potato glycoalkaloids (Friedman and Levin, 1992). Because the secondary interactions of the packing with these compounds improved the separation allowing resolution of α-solanine, α-chaconine, and hydrolysis products to baseline, we selected columns with less endcapping and more acidic characteristics. Recent advances in column packing technology have led to multifunctional columns that take advantage of these mixed mode separations, finely controlling the mix between hydrophobic, hydrophilic, and specialized column interactions. Better amino functional group (NH2) columns are now also available. In the following 'Methods' section, we describe two HPLC methods. The first method was developed in our laboratory using a highly acidic C18 column, and the second was developed by our colleague in Korea using an amino column. Both methods produce good separation and have different advantages. Although the amino column technique has longer run times, it is more adaptable to HPLC/MS.

6.2.2.2 ELISA analysis

A simple method that can analyze a large number of samples in a reasonably short time is needed to meet the needs of plant breeders, plant molecular biologists, food processors, and scientists interested in better defining the role of glycoalkaloids in the plant, in the diet, and in medicine. A reliable immunoassay that correlates with HPLC may provide the answer. An ELISA kit for glycoalkaloids that meets these criteria should find widespread use. To meet this need, we evaluated a prototype ELISA kit produced by EnviroLogix, Inc., Portland, Maine, based on antibodies we developed (Stanker et al., 1994, 1996, 1997). Our results show a good correlation between HPLC and the ELISA for glycoalkaloids in potato tubers and processed products (Table 6.1). ELISA is a simple method that can be used routinely by a broad range of users (Sporns et al., 1996; Friedman et al., 1998).

6.2.2.3 Biosensors

Researchers (Benilova et al., 2006; Arkhypova et al., 2008) are developing a biosensor-based pH-sensitive field-effect transistor technology for rapid determination of glycoalkaloids. The test takes advantage of the anticholinesterase activity of the glycoalkaloids. These tests could hold great promise, analogous to the ELISA test mentioned above.

6.2.3 Methods

The following is a description of the methods we used to analyze glycoalkaloids.

Table 6.1: Comparison of glycoalkaloid content of the same potatoes and potato products analyzed by HPLC (sum of α-chaconine and α-solanine) and ELISA

Sample	Assay method	
	HPLC	ELISA
Whole potatoes:	fresh (mg/kg)	Fresh (mg/kg)
Russet, organic	5.8	5.1
Russet	22	24
Yukon Gold	40	38
Purple, small	45	37
Red, small	101	128
Gold, small	105	113
White, large	125	132
White small	203	209
Potato plant parts:	dehydrated (mg/kg)	dehydrated (mg/kg)
Flesh, Red Lasoda	45.6	51.6
Peel, Shepody	1432	1251
Sprouts, Shepody	7641	6218
Leaves	9082	8851
Processed potatoes:	original (mg/kg)	original (mg/kg)
French fries, A	0	1.2
French fries, B	24.1	22.7
Chips, low-fat	15.2	22.7
Skins, A	43.3	35.0
Skins, B	37.2	41.0

6.2.3.1 *Extraction of glycoalkaloids from freeze-dried potatoes for HPLC*

Freeze-dried potato powders (17 mg–1 g, depending on availability) were extracted (Friedman et al., 2003c) with 40 mL of 5% acetic acid accompanied by ultrasonication for 10 min at room temperature. After filtration through a 3G3-glass filter, the residue was rinsed three times with 30 mL of 5% acetic acid each time. The washings were combined with the original filtrate. The filtrate was transferred to a 200-mL Erlenmeyer flask to which was added 10 mL of concentrated NH_4OH to precipitate the glycoalkaloids. The basic solution was placed in a 70°C water bath for 50 min and then refrigerated overnight. The precipitate was collected by centrifugation at 18 000 ***g*** for 10 min at 1°C and washed twice with a 2% solution of NH_4OH. The pellet was dried at 30°C under reduced pressure, then dissolved in 1 mL of a mixture of tetrahydrofuran/acetonitrile/20 mM KH_2PO_4, and centrifuged at 18 000 ***g*** for 10 min at 1°C. The supernatant (50 μL) was used for HPLC.

6.2.3.2 *HPLC technique, NH2 column*

Figure 6.2a shows the HPLC chromatogram using the NH2 column (Friedman et al., 2003c). HPLC chromatography was carried out with the aid of a Hitachi liquid chromatograph model

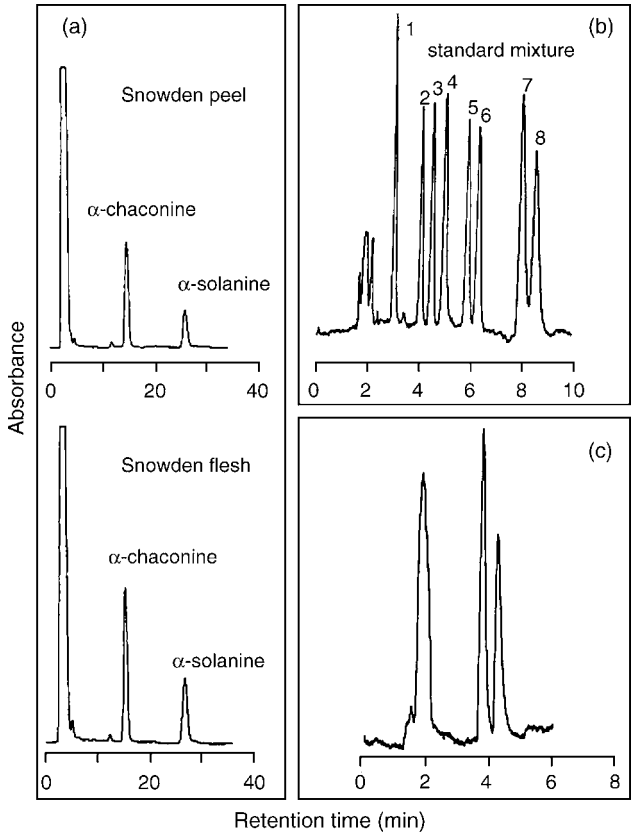


Figure 6.2: A comparison of HPLC separation methods. (a) HPLC of α-chaconine and α-solanine in the flesh and the peel of one variety of potato. Conditions: column, Inertsil NH2 (5 μm, 4.0 x 250 mm); mobile phase, acetonitile/20 mM KH_2PO_4 (80:20, v/v); flow rate, 1.0 mL/min; column temperature, 20°C; UV detector, 208 nm; sample size, 20 μL. (b) HPLC chromatogram of approximately 1 μg of each of potato glycoalkaloids and their hydrolysis products 1, solasonine (internal standard); 2, α-solanine; 3, α-chaconine; 4, β2-solanine; 5, β1-chaconine; 6, β2-chaconine; 7, γ-solanine; 8, γ-chaconine. Conditions: column, Resolve C18 (5 μm, 3.9 x 300 mm); mobile phase, 35% acetonitrile/100 mM ammonium phosphate (monobasic) at pH 3; flowrate, 1.0 mL/min; column temperature, ambient; UV detector, 200 nm; sample size. (c) HPLC chromatogram of the aglycones solanidine and solasodine. Conditions: column Supelcosil C18-DB (3 μm, 4.6 x 150 mm); mobile phase, 60% acetonitrile/10 mM ammonium phosphate pH 2.5; flowrate, 1.0 mL/min; column temperature, ambient; UV detector, 200 nm.

665A-11 equipped with a model 655-40 autosampler and a UV detector (Hitachi model 655A UV monitor) set at 208 nm. Column temperature was controlled with a Coolnics model CTR-120 device (Komatsu Electronics, Tokyo, Japan). Chromatogram peak areas were integrated with a Hitachi D-2500 chromato-integrator. The column was an Inertsil NH2 (5 μM, 4.0 × 250 mm)

(GL Science, Japan). The mobile phase was acetonitrile/20 mM KH_2PO_4 (80:20, v/v). For the aglycon solanidine, the mixture consisted of acetonitrile/2.5 mM KH_2PO_4 (93:7, v/v). The flow rate was 1 mL/min at a column temperature of 20°C. The concentrations of α-chaconine and α-solanine in the potato extracts were calculated by comparison with the integrated peak areas of known amounts of the standards by a Hitachi chromato-integrator.

6.2.3.3 HPLC technique, C18 column

Figures 6.2b and 6.2c show HPLC chromatograms using the C18 column. HPLC was carried out with the aid of a Beckman Model 334 liquid chromatograph with a 427 integrator and a 165 UV-visible variable wavelength detector set to 200 nm. The column was a Resolve C18 (5 μm, 3.9 × 300 mm) (Waters, Milford, MA). The mobile phase was 35% acetonitrile and 100 mM ammonium phosphate (monobasic) adjusted to pH 3.5 with phosphoric acid at a flow rate of 1 mL/min and ambient temperature. Concentrations of α-chaconine and α-solanine in the potato extracts were calculated by comparison of integrated peak areas with the peak of a solasonine using a Beckman 427 integrator. Conditions for solanidine were different due to the fact that it bound strongly to the C18 column and exhibited the significant peak tailing sometimes seen when basic compounds separated on acidic columns (Friedman and Levin, 1992). We used a Supelcosil Cl8 deactivated base, 3 μm, 4.6 × 150 mm column (Supelco Inc., Bellefonte, PA) with the following conditions: eluent, 60% acetonitrile/10 mM ammonium phosphate, pH 2.5.

6.2.3.4 Identification of glycoalkaloids

The two potato glycoalkaloids in the potato extract were identified as follows: (a) comparison of thin-layer chromatography of standards α-chaconine and α-solanine and of samples of each peak collected from the HPLC column containing the individual glycoalkaloids; and (b) HCl hydrolysis of the HPLC samples into sugars and the aglycon solanidine. These were identified by GLC-MS (Kozukue et al., 1999, 2008; Kozukue and Friedman, 2003).

6.2.4 Results

Table 6.2 contains a survey of glycoalkaloid content in potatoes analyzed using the above HPLC NH2 column technique. None of the whole potatoes exceeded the 200 mg total glycoalkaloids per kg of potatoes (see A + B column). However, this was not the case for potato peel. Five of the eight samples exceeded this benchmark. The high content of peels should not be of concern, unless consumers ate large amounts of peel, as they sometimes do in some commercial products, such as potato skin appetizers.

Researchers have proposed making use of that peel waste. Rodriguez de Sotillo proposed making use of the high antioxidant content of potato peel as an antimicrobial (Rodriguez de Sotillo et al., 1998). To prevent possible poisonings, care must be exercised when using the waste in human or animal food (Kling et al., 1986), or when releasing it into the environment as industrial waste.

Table 6.2: Glycoalkaloid and calystegine content of potato flesh, potato peel, and whole potatoes of eight potato cultivars (in mg/kg)

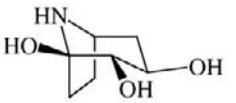
Potato cultivar		Glycoalkaloids				Calystegines			
		α-chaconine (A)	α-solanine (B)	A + B	A/B	calystegine A_3	calystegine B_2	$A_3 + B_2$	B_2/A_3
Atlantic	flesh	4.7	2.9	7.6	1.6	1.1	1.5	2.6	1.4
	Peel	8.8	3.6	12.4	2.4	31.2	141	172	4.5
	whole	5	8	8	1.7	3.5	12.9	16.4	3.7
Dark Red Norland	flesh	3.4	1.3	4.7	2.6	0	1.3	1.3	–
	Peel	128	60.3	188	2.1	6.4	33.3	39.7	5.2
	whole	16.8	7.7	24.5	2.2	0.7	4.7	5.4	6.7
Ranger Russet	flesh	12.8	7	19.8	1.8	1.1	2.3	3.4	2.1
	Peel	230	110	340	2.1	87.1	380	467	4.4
	whole	34.3	17.2	51.5	2	9.6	39.7	49.3	4.1
Red Lasoda	flesh	4.4	2.8	7.2	1.6	1.4	4.3	5.7	3.1
	Peel	134	96.6	231	1.4	10.5	24.83	35.3	2.4
	whole	16	11.2	27.2	1.4	2.2	6.1	8.3	2.8
Russet Burbank	flesh	25.9	21.4	47.3	1.2	11.1	56.5	67.6	5.1
	Peel	182	103	285	1.8	6.6	67.8	74.4	11.8
	whole	36.3	26.8	63.1	1.4	10.8	57.3	68.1	5.3
Russet Norkota	flesh	0.74	0.54	1.3	1.4	0.2	0.8	1	4
	Peel	48.1	23	71.1	2.1	33.6	129	163	3.9
	whole	4.8	2.5	7.3	1.9	3	11.9	14.9	4
Shepody	flesh	1.8	1.1	2.9	1.7	2.2	9.1	11.3	4.1
	Peel	282	147	429	1.9	44	299	343	6.8
	whole	25.1	13.2	38.3	1.9	5.6	33.1	38.7	5.9
Snowden	flesh	91.5	56.5	148	1.7	0.8	0.8	1.7	1
	Peel	372	171	543	2.2	54.2	96.3	150	1.8
	whole	119	67.9	187	1.8	5.8	10.2	16	1.8

6.2.4.1 Ratio of α-chaconine to α-solanine

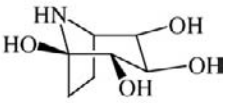
The ratios of α-chaconine to α-solanine for the potato samples as seen in Table 6.2 ranged from 1.2 to 2.6. The ratio for peel, generally in the range of about 2, was much higher than for flesh with values near about 1.5. Since, as mentioned earlier, α-chaconine is more toxic than α-solanine, it is desirable to have this ratio as low as possible. We can only speculate about possible reasons for the wide variations in these ratios. Since the two glycoalkaloids, which share the common aglycone solanidine but not the same trisaccharide side chain (Figure 6.1), appear to be synthesized via distinctly different (discrete) biosynthetic channels (Choi et al., 1994), it is possible that the rates of biosynthesis of the two glycoalkaloids in the different channels are cultivar-dependent. Another possible rationalization for the varying ratios is that the rate of metabolism of the two glycoalkaloids is also cultivar-dependent. These considerations imply that alteration of the genes encoding enzymes involved in the biosynthesis of α-chaconine and/or α-solanine could have unpredictable results.

6.3 Calystegine Alkaloids

Calystegines are polyhydroxylated nortropane alkaloids present in potatoes (Richter et al., 2007; Kvasnicka et al., 2008). These water-soluble alkaloids were first discovered in 1988 from transformed root cultures of the non-food plant *Calystegia sepium* (Tepfer et al., 1988). Their structures were elucidated in 1990 (Goldmann et al., 1990). Since then, they have been found several other plant families, including the Solanaceae; specifically in *Solanum melongena* (eggplant) and *Solanum tuberosum* (potato), reported to contain calystegine A_3 and calystegine B_2 shown in Figure 6.3 (Nash et al., 1993; Asano et al., 1997; Keiner and Dräger, 2000; Bekkouche et al., 2001; Keiner et al., 2002). At least eight calystegines are currently known and many exhibit potent specific inhibition of glycosidases that are universally required for normal cell function. These polyhydroxylated alkaloids act as sugar mimics and inhibit glycosidases because of a structural resemblance to the sugar moiety of the natural substrate (Asano et al., 2000). Although no human toxicity data for calystegines have been reported,



calystegine A_3



calystegine B_2

Figure 6.3: Structures of potato calystegines.

polyhydroxylated alkaloids are reported to have therapeutic effects in the treatment of cancer, diabetes, bacterial and viral infection, and to stimulate the immune system (Asano et al., 2001; Watson et al., 2001). Biosynthetically, calystegines appear to be derived from the tropane alkaloids atropine and scopolamine also present in some Solanaceae plants such as *Datura stramonium* (Jimsonweed) (Dugan et al., 1989; Friedman and Levin, 1989; Keiner et al., 2000).

6.3.1 Methods

We validated and improved a GC-MS method to measure the two major potato calystegines A_3 and B_2 in freeze-dried potato peel and potato flesh samples (Friedman et al., 2003c). We used this method to analyze the same potatoes that we previously analyzed for glycoalkaloids (Table 6.2).

6.3.1.1 Extraction and isolation of hydrophilic alkaloid fractions

The procedure was adapted from that of Nash et al. (1993). Weighed samples (1.0 g) of powdered potato flesh and peel were stirred at room temperature for 24 h with methanol/water (4:1, v/v, 25 mL). Each sample was vacuum filtered through a pad of Celite diatomaceous earth to remove solids. The filtrate was concentrated to ~3 mL by rotary evaporation at 45°C. The residue was transferred quantitatively to a 10-mL beaker with deionized water and the pH adjusted to 4.0 with HCl. The more or less cloudy solution was introduced directly onto a 150 mm long × 12 mm diameter bed of cation-exchange resin (Dowex AG 50W × 8, Bio-Rad, Hercules, CA) at a flow rate of ~1 mL/min. After the column had been rinsed at the same flow rate with 55 mL of deionized water (~3 bed volumes), 0.5% NH_4OH (55 mL) was introduced and the alkaline eluent was collected and concentrated to 2–6 mL by rotary evaporation at 45°C. The resulting solution was transferred quantitatively to a 10-mL volumetric flask and made up to 10 mL with deionized water from which 1.0-mL aliquots were transferred to 4-mL borosilicate glass screw cap vials, frozen in liquid nitrogen and freeze-dried.

6.3.1.2 Preparation of trimethylsilyl (TMS) ether derivatives

To each vial was added dry pyridine (45 μL), N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) (Pierce, Rockford, IL) (45 μL), and as internal standard 10 μL of a solution of 250 μg of perseitol (Aldrich) in 50 μL each of pyridine and MSTFA that had been previously warmed for 1 h at 100°C. The vials were then heated for 1 h in a Reacti-Therm block heater (Pierce).

6.3.1.3 GC-MS analysis

The derivatized samples were analyzed on a Hewlett-Packard 5890 series II GC (helium carrier gas) coupled to a Hewlett-Packard 5971 mass-selective detector (MSD). A 60 m, 0.32 mm i.d., 0.25-im film, SE-30 fused silica capillary column (J&W Scientific, Folsom, CA) was installed in the GC, and an on-column injector (SGE model OC1-3) held at ambient temperature was fitted to the column inlet. Samples (0.5 μL) were injected directly into the column held at 105°C

for 0.2 min. The column was ramped at 30°C/min for 0.5 min, programmed from 120 to 300°C at 10°C/min, and held at the final temperature for 10 min. The MSD was operated at 70 eV in the EI mode with scans taken every 1.5 s from 75 to 600 amu. A postinjection delay of 7.0 min was set to allow solvent and derivatizing agent to elute before mass spectral data acquisition began. Retention times and mass spectra of calystegine standards confirmed the presence of the trihydroxy nortropane alkaloid, calystegine A_3, and the tetrahydroxy nortropane alkaloid, calystegine B_2, as TMS ethers in all eight of the potato cultivars examined. The amounts of the two alkaloids were calculated by comparison of the integrated total ion current peak areas with the peak area of the internal standard, perseitol-TMS.

6.3.2 Results

Figure 6.4 illustrates the separation on GC-MS total ion chromatograms of calystegines A_3 and B_2 extracted from potato powders. Results of the GC-MS analyses for calystegines A_3 and B_2 are shown in Table 6.2. For whole potatoes, there is a 12-fold variation from lowest

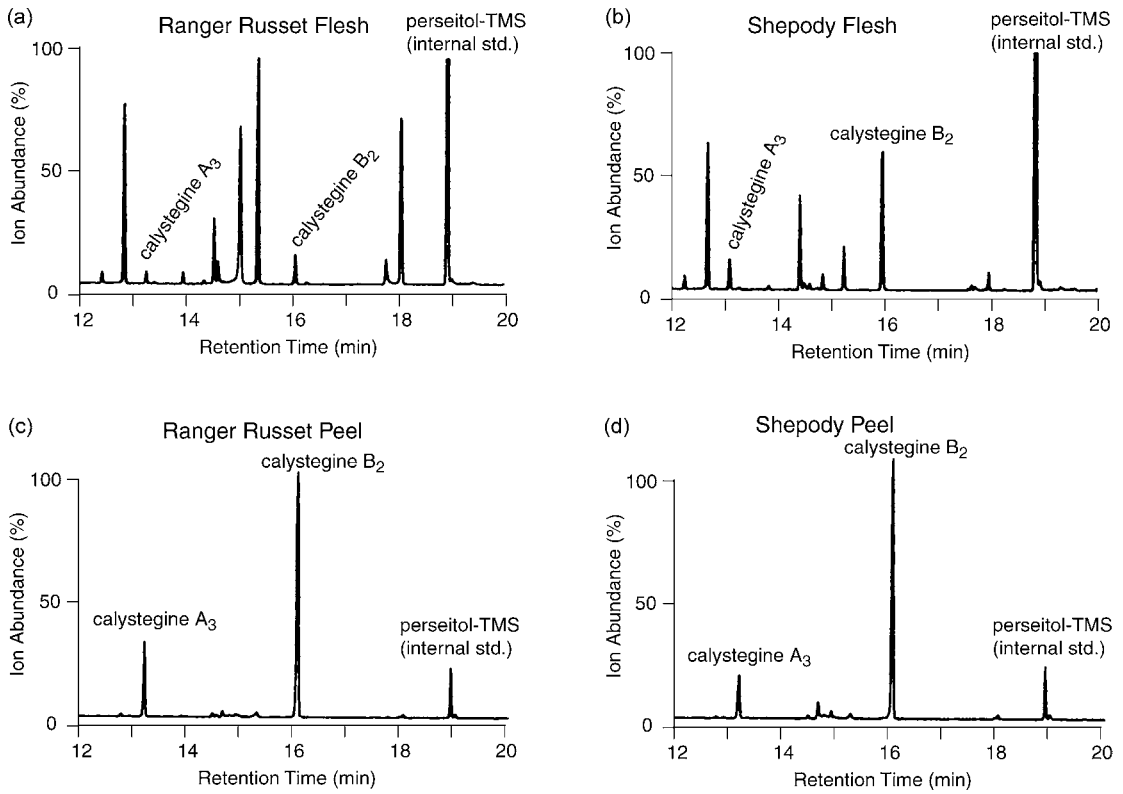


Figure 6.4: GC-MS total ion chromatograms of the hydrophilic alkaloid fraction extracted from freeze-dried potato flesh and peel.

to highest values for A_3 and a 15-fold variation for B_2. For the sum of the two calystegines ($A_3 + B_2$ column), there is a 13-fold variation from lowest to highest values. For calystegine B_2/A_3 ratios, there is a 3-fold variation from lowest to highest values. It is also instructive to calculate the following ratios of total glycoalkaloid to total calystegine content for whole potatoes: Russet Norkota, 0.49; Atlantic, 0.49; Russet Burbank, 0.93; Shepody, 0.99; Ranger Russet, 1.0; Red Lasoda, 3.3; Dark Red Norland, 4.5; Snowden, 11.7. The biosynthesis of glycoalkaloids seems to parallel that of calystegines in some varieties but not in others. The concentrations of both calystegines and glycoalkaloids are greatest in the peel. Relative levels in the flesh and peel vary widely among the cultivars evaluated. That the glycoalkaloid/calystegine ratio also varies implies that the synthesis of these two classes of secondary metabolites may be under separate genetic control. Because there is some correlation between their levels, they may respond similarly to some stress conditions. Moreover, since the individual calystegine isomers differ in their biological activities (Asano et al., 1997; Watson et al., 2001), both their ratios as well as total amounts present in different potato cultivars may be important in assessing the role of calystegines in the diet. Since the biological activities as well as the roles in host-plant resistance of both glycoalkaloids and calystegines could be interrelated, there is a need to define the levels of both glycoalkaloids and calystegines in different potato cultivars and to study individual and combined effects in animals and humans. Possible therapeutic applications of high-calystegine potato diets also merit study.

6.4 Phenolic Compounds

Phenolic compounds are secondary plant metabolites found in potatoes and other plants (reviewed in Friedman, 1997; Mattila and Hellström, 2007). In the plant, phenolic compounds function beneficially to defend against invading pathogens, including bacteria, fungi, and viruses. They also, however, participate in enzyme-catalyzed browning reactions that may adversely affect color, flavor, and nutritional quality of potatoes. Antioxidative phenolic compounds show promise as health-promoting phytochemicals as they have been shown to exhibit beneficial antimutagenic, anticarcinogenic, antiglycemic, anticholesterol, and antimicrobial properties. These considerations suggest the need for accurate analysis of phenolic compounds in potato leaves, stems, and tubers and in processed potato products.

Figure 6.5 shows the structures of *trans*-cinnamic acid and four cinnamic acid derivatives (phenolic compounds) reported to be present in potatoes. Because potatoes are one of our major food plants, we validated with the aid of HPLC and LC/MS the content and distribution of antioxidative phenolic compounds in parts of the potato plant, in potato tubers, in the peel and flesh of tubers, in potatoes sold commercially in Korea and the United States, and in home-processed potatoes. The following discussion, based on our own studies, is followed by a brief overview of analytical methods for potato phenolic compounds by other investigators.

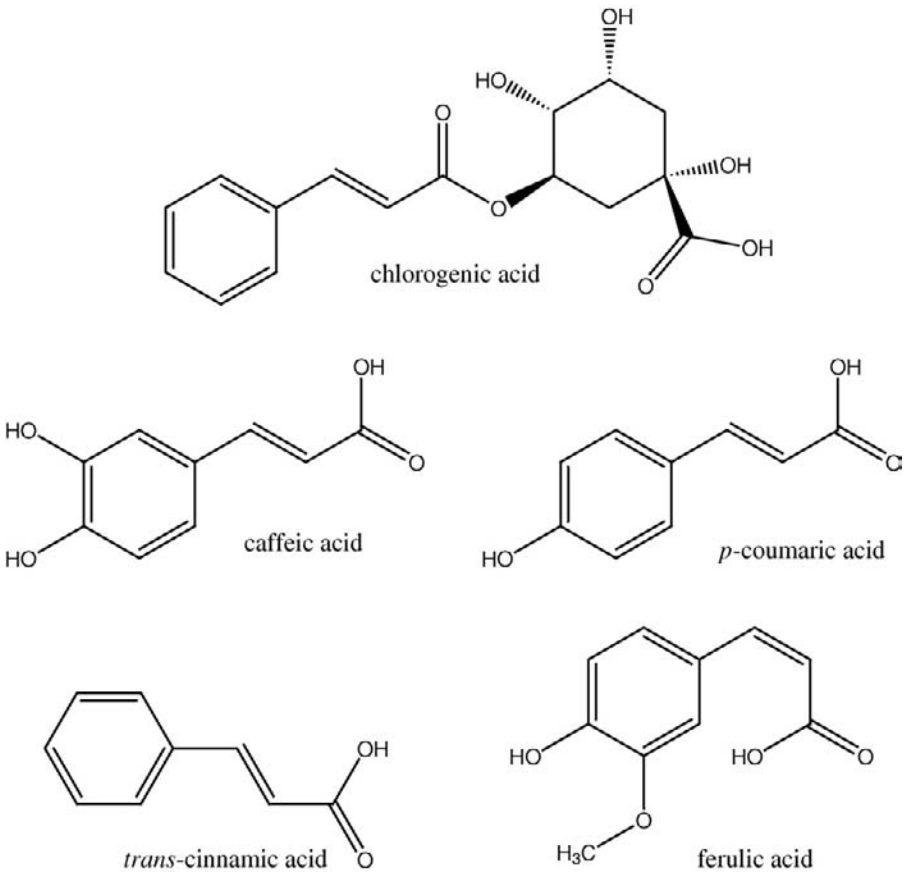


Figure 6.5: Structures of cinnamic acids derivatives commonly found in potatoes.

6.4.1 Analysis

Reported analytical methods for potato phenolics include HPLC (Percival and Baird, 2000; Tudela et al., 2002; Verde Méndez Cdel et al., 2004; Shakya and Navarre, 2006; Mattila and Hellström, 2007; Reddivari et al., 2007a), capillary electrophoresis (Fernandes et al., 1996), colorimetry/spectrophotometry (Friedman et al., 1989; Dao and Friedman, 1992; Griffiths et al., 1992; Dao and Friedman, 1994; Percival and Baird, 2000; Kanatt et al., 2005; Stratil et al., 2006), and GC/MS (Tisza et al., 1996). The HPLC and LC/MS methods we previously used to determine glycoalkaloids and vitamin C in potatoes (Friedman et al., 2003c; Han et al., 2004) were adapted to the analysis of potato phenolic compounds (Im et al., 2008). Figure 6.6 shows a chromatogram of potato flesh before (a) and after (b) spiking. Figure 6.7 shows a LC/MS chromatogram of potato peel, monitoring absorbance at 280 nm, 340 nm, and the TIC.

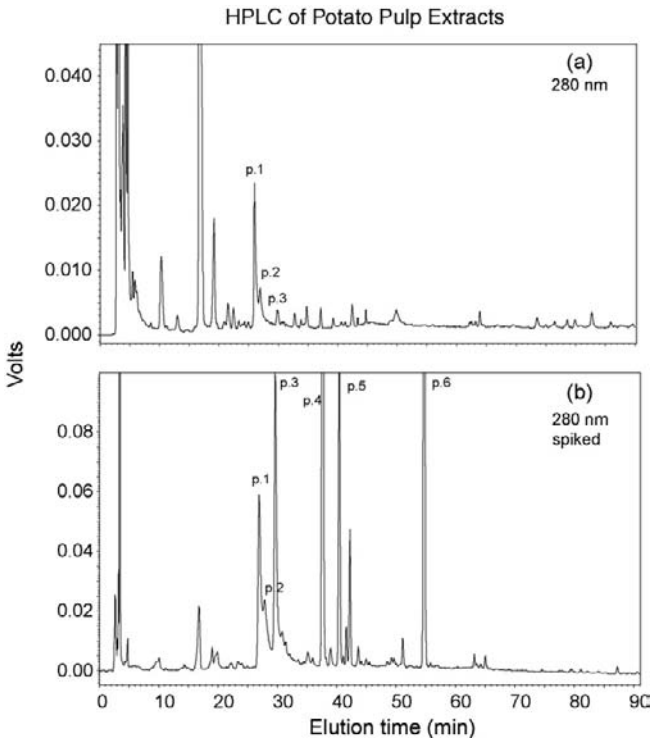


Figure 6.6: HPLC chromatogram of the extract from Superior potato flesh (a) and of the same extract spiked with standards (b). Identification; p.1, chlorogenic acid; p.2, chlorogenic acid isomer; p.3, caffeic acid; p.4, p-coumaric acid; p.5, ferulic acid; p.6, t-cinnamic acid. Column, Inertsil ODS-3 v (5 μm, 4.0 x 250 mm); flow rate, 1.0 mL/min; column temperatures, 20°C; mobile phase, acetonitrile:0.5% formic acid (gradient mode); detector, UV at 280 nm.

6.4.1.1 Extraction of phenolic compounds from peels and flesh of potato tubers

The potato tubers were each peeled to a depth of 2–3 mm. Fresh peel weights amounted to 21.1–23.9% of the total weight of the potatoes. Fresh peel and flesh were then cut with a knife into 4-mm-thick slices. Samples (10 g) of peel and flesh from each potato tuber were then placed into a 250-mL flask with a reflux condenser to which was added 50 mL of 80% ethanol, followed by heating at 80°C for 10 min. After homogenization in a Waring blender, the mixture was again transferred to a flask for re-extraction, followed by centrifugation at 12 000 ***g*** for 15 min at 5°C. The residue was extracted twice with 20 mL of 80% ethanol and centrifuged. The combined extracts were made up to 100 mL with 80% ethanol. This solution (10 mL) was evaporated under reduced pressure at 20°C and the residue was dissolved in 80% ethanol (1 mL) and centrifuged. The supernatant (20 μL) was used for HPLC.

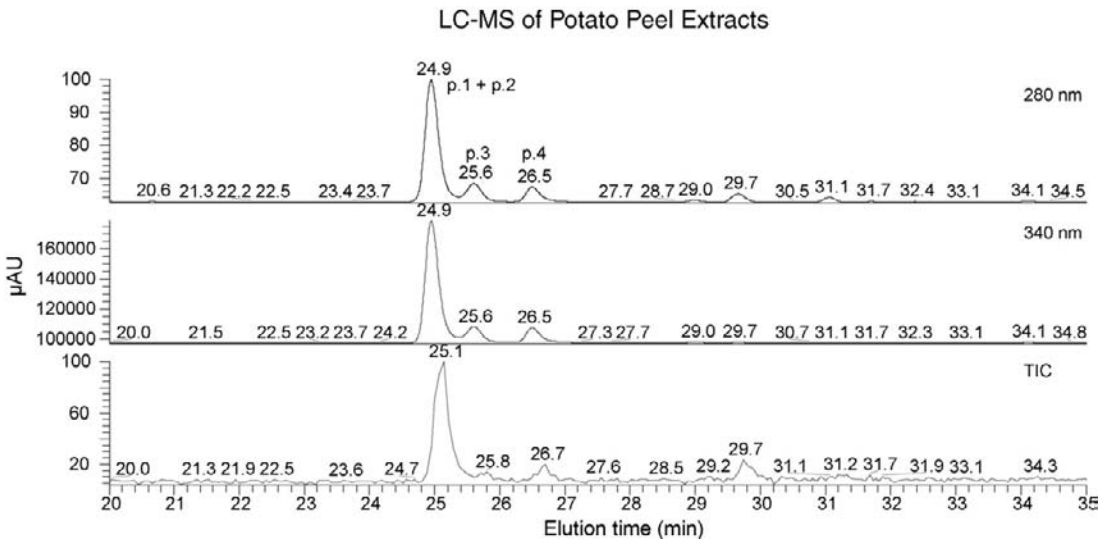


Figure 6.7: LC-MS chromatograms of an extract of Superior potato peel monitored at 280 nm, 340 nm, and TIC. Column: Inertsil ODS-3 (3 μm, 4.0 x 150 mm). Flow rate: 0.2 mL/min. Column temperature: 30°C. Mobile phase: acetonitrile: 0.5% formic acid (gradient mode).

6.4.1.2 HPLC analysis

HPLC analysis was carried out on a Hitachi liquid chromatograph model 665-II equipped with a Shimadzu UV-VIS detector (Model SPD-10Avp, Kyoto, Japan) set at 280 nm and 340 nm. Column temperature was controlled with a Shimadzu CTO-10Asvp Thermometer. Chromatogram peak areas were integrated with a Hitachi D-2500 chromato-integrator. An Inertsil ODS-3v column [5 μm, 4.0 × 250 mm (GL Science Inc., Tokyo, Japan)] was used to analyze the phenolic acids. The mobile phase of the (A/B) gradient was (A) acetonitrile and (B) 0.5% formic acid. The content of acetonitrile in the solvent was increased as follows: 5% (0–5 min); 18% (30 min); 70% (90 min); 5% (120 min). The flow rate was 1 mL/min at a column temperature of 20°C. Three separate analyses were carried out with each sample. Plots of concentration versus peak areas (calibration plots) were linear at the concentration range of 8.0 (LOD)–300 ng for ferulic acid; 4.7–400 ng for caffeic; 2.0–400 ng for *trans*-cinnamic acids; 3.2–600 ng for coumaric acid; and 16.5–800 ng for chlorogenic acid. Percent recoveries of spiked samples were as follows (n = 3): trans-cinnamic acid, 95.2; chlorogenic acid, 97.2; p-coumaric acid, 102.0; ferulic acid, 102; and caffeic acid, 107.

6.4.1.3 LC-MS/MS

Liquid chromatography/mass spectrometry analyses were performed with an ion trap mass spectrometer (LCQ, Thermo Fisher Scientific Inc., MA) equipped with an HPLC system (Agilent, CA; Model 1100) connected with a diode-array detector (DAD, G1315A). The sample solution (1–5 μL) was applied on an Inertsil ODS-3 column (2.1 × 150 mm, 3 μm, GL

Sciences Inc., Tokyo, Japan) and was separated using gradient solvent system at the flow rate of 200 μL/min. The content of acetonitrile in 0.5% formic acid was increased as follows: 5% (0–5 min); 18% (30 min); 70% (90 min). The LC eluate was introduced into the mass spectrometer after 3 min of the sample injection. The MS/MS experiments were carried out in the negative-ion modes. The parameters were optimized using a standard chlorogenic acid solution by mixing the mobile phase (50% acetonitrile) eluted from the LC system as follows: ESI spray voltages; 4.5 kV (negative-mode); capillary temperature, 50°C; capillary voltages, −42 V (negative-mode); sheath gas (nitrogen) flow rate, 64 (arbitrary unit); auxiliary gas flow rate, 55 (arbitrary unit); tube lens offset voltages, −15 V; multipole 1 offset voltages, 1.0 V; multipole 2 offset voltages, −7.0 V; intermultipole lens voltages, 14 V. Helium was used as collision gas and the relative collision energy was set at 40% for MS/MS and MS3 experiments over a selected mass window of 2 Da. Mass selection of the analyte by m/z was followed by fragmentation and analysis of the fragments.

6.4.2 Identification

Structural identification of individual phenolic compounds in extracts was performed by associating the HPLC peak of each compound with the corresponding UV (Figure 6.8) and mass spectrum (Figure 6.9). The HPLC chromatograms (Figure 6.6) demonstrate the presence of caffeic, chlorogenic acid (5-caffeoylquinic acid), and a chlorogenic acid isomer with the same molecular weight as chlorogenic acid in the potato extracts. We saw no evidence for the presence of *p*-coumaric and ferulic acids in these extracts.

6.4.3 Discussion

Chlorogenic acids (CGA) are a family of esters formed between *trans*-cinnamic acids and (-)-quinic acid (Clifford et al., 2003). At least three isomeric forms of chlorogenic acid may be present in potatoes: 3-, 4-, and 5-caffeoylquinic acids (Fernandes et al., 1996; Friedman, 1997). UV light induces the isomerization of naturally occurring *trans*-chlorogenic isomers to the *cis* form (Clifford et al., 2008). The HPLC and LC/MS data of the present study indicate the presence of two isomeric forms, 5-caffeoylquinic acid for which we had a standard, and another isomer with the same molecular weight for which we do not have a standard. The LC/MS patterns illustrated do not differentiate between the two isomers. In analogy with the observation by Fernandes et al. (1996) that the 5- and 4-caffeoylquinic isomers were present in quantifiable amounts in potatoes in a ratio of 8.5:1, it is likely that the smaller of the two isomer peaks is probably 4-caffeoylquinic acid. Table 6.3 shows the levels of phenolics we found in potato, leaves, and stems. The data also show that chlorogenic acid and its isomer constituted ∼96–98% of the total phenolic content and that the total in flowers was 2.74 times greater than in leaves and 58.5 times greater than in stems. Although we do not know the reason for the high levels in the flowers, a likely explanation is that the high amounts are needed to protect the flowers against attacks by phytopathogens.

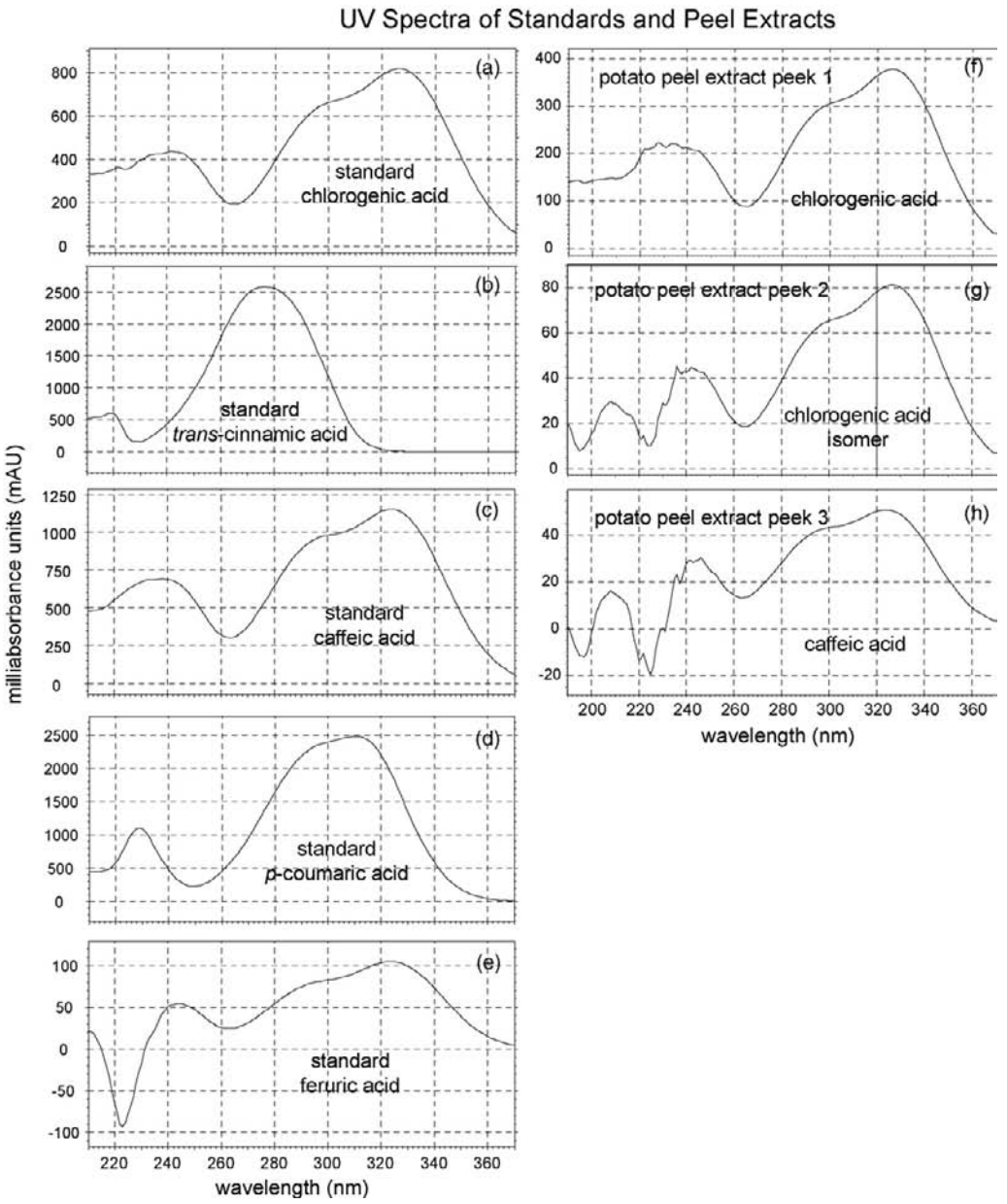


Figure 6.8: UV spectra of standard chlorogenic acid (a); *trans*-cinnamic acid (b); caffeic acid (c); *p*-coumaric acid (d); and ferulic acid (e). The spectra of peaks 1 (chlorogenic acid) (f), peak 2 (chlorogenic acid isomer) (g), and peak 3 (caffeic acid) (h) were determined with HPLC fractions isolated from extracts of Superior potato peel.

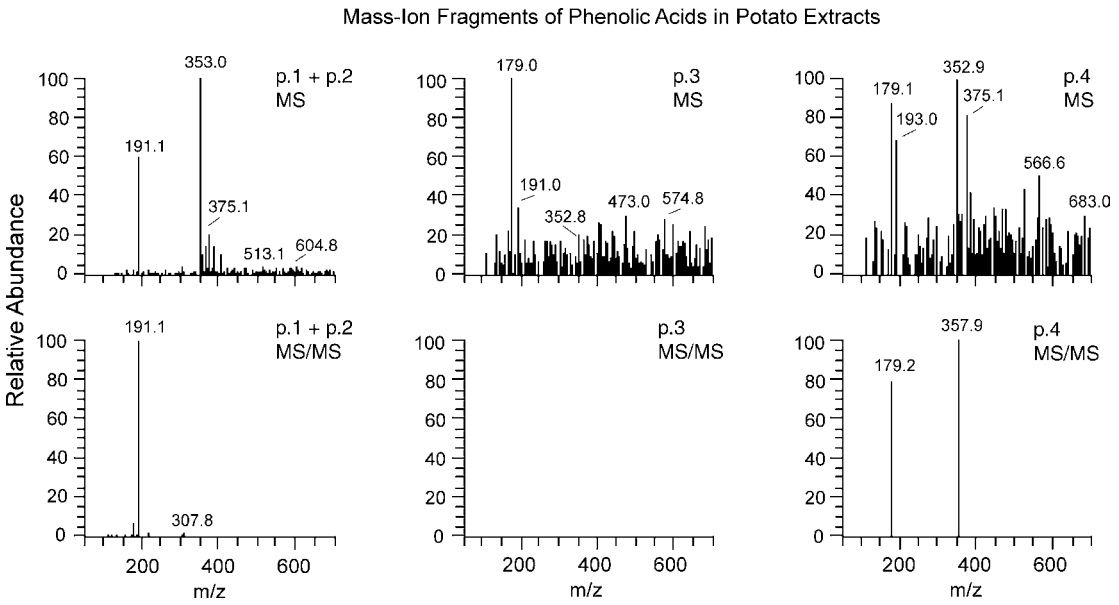


Figure 6.9: MS and MS/MS (negative ion mode) of peaks 1 and 2 (chlorogenic acid and its isomer), peak 3 (caffeic acid) and peak 4 (chlorogenic acid isomer) from isolated HPLC chromatograms of potato extracts.

Table 6.3: Distribution (in mg/100g fresh wt) of phenolic compounds in three parts of superior potato plants

Plant part	Chlorogenic acid	Chlorogenic acid isomer	Caffeic acid	Total
Flower	424	189	13.5	626
Leaf	14.8	13.3	1.2	29.3
Stem	4.2	6.1	0.4	10.7

Table 6.4 shows the distribution of the three phenolics in the peel and flesh of five commercial potato varieties grown in Korea. Noteworthy is the large variation in the ratio of peel to flesh levels ranging from 2.55 for the Jasim to 21.1 for the Jowon potatoes.

Table 6.5 shows the distribution of the three phenolics in the peel and flesh of the Korean Superior potato variety available in four sizes: large, medium, small, and very small. The data indicate that the size of the potato does not seem to influence the total phenolic content, except that the ratio of peel to flesh for the very small potatoes (7.95) is about one half the corresponding ratios of the other three potatoes. These results indicate that the distribution of phenolic compounds between peel and flesh varies widely among different potato varieties. They also suggest that

Table 6.4: Content (in mg/100 g fresh wt) of phenolic compounds in the peel and flesh of Korean potato varieties

Potato variety	Potato section	Chlorogenic acid	Chlorogenic isomer	Caffeic acid	Total	Ratio: peel/flesh
Jasim	Peel	34.0	6.8	1.2	42.1	2.6
	Flesh	12.0	4.4	0.11	16.5	
Atlantic	Peel	4.4	1.8	0.96	7.2	14.6
	Flesh	0.35	0.13	0.01	0.5	
Jowon	Peel	10.2	3.0	0.7	13.9	21.1
	Flesh	0.45	0.17	0.04	0.66	
Superior	Peel	8.7	0.99	1.2	10.9	20.2
	Flesh	0.47	0.06	0.01	0.54	
Jopung	Peel	4.9	1.3	0.39	6.6	7.6
	Flesh	0.57	0.26	0.02	0.85	

Table 6.5: Effect of potato size on the phenolic acid content of the peel and flesh of potatoes. Listed values in mg/100 g fresh wt

Superior potato size	Potato part	Chlorogenic acid	Chlorogenic acid isomer	Caffeic acid	Total	Ratio peel/flesh
Large	Peel	7.4	0.92	1.1	9.3	15.8
	Flesh	0.53	0.04	0.02	0.59	
Medium	Peel	8.7	0.99	1.2	10.9	20.2
	Flesh	0.5	0.06	0.01	0.54	
Small	Peel	5.3	0.61	1.6	7.5	16.7
	Flesh	0.39	0.05	0.01	0.45	
Very small	Peel	8.3	1.0	0.04	9.4	7.9
	Flesh	1.0	0.11	0.04	1.2	

consumers and potato processors can select from available potato varieties those with high, intermediate, or low amounts of phenolic compounds. Additional studies revealed that the total phenolic content of the vertical slices ranged from 0.79 to 2.49 mg/100 g fresh wt, a 3.15-fold variation from highest to lowest. The corresponding range for the horizontal slices was from 0.84 to 6.58 mg/100 g fresh wt, a 7.83-fold variation from highest to lowest. These results suggest that it may be possible to select, depending on need, slices with high or low amounts of phenolic compounds for the preparation of potato-based foods. Such selection should take into account possible variability of phenolic content in different tubers of the same cultivar.

Table 6.6 lists the phenolic acid content of 25 potato powders prepared by lyophilization of commercial potatoes with unknown history. For the dry lyophilized powders, the data show that chlorogenic acid levels (in mg/100 g wt) ranged from 3.28 for Kenebec potatoes to 637 for

Table 6.6: Content of phenolic compounds of freeze-dried powders prepared from fresh potatoes sold in the United States

Potato variety	Chlorogenic acid	Chlorogenic acid isomer	Caffeic acid	H_2O in fresh tubers (%)	Total phenolics (mg/100 g)	
					Dry tubers	Fresh tubers
Kennebec	3.28	0.34	0.47	75.0	4.09	1.03
Russet, baking, batch 1	18.2	1.0	1.8	79.5	21.0	4.3
White, large, batch 2	14.5	4.9	2.0	83.4	21.4	3.6
Yukon Gold, grade "A", large	14.3	4.7	4.6	76.9	23.6	5.5
Yukon Gold, grade "B", medium, batch 1	19.0	6.0	5.0	75.5	30.0	7.4
White, large, batch 1	21.6	6.7	2.8	79.9	31.2	6.3
Russet, baking, batch 2	25.9	10.7	2.5	79.6	39.1	8.0
Yukon Gold, grade "C", small, batch 1	26.7	7.5	7.9	82.3	42.1	7.5
Yukon Gold, grade "C", small, batch 2	26.0	12.3	5.3	82.7	43.6	7.5
Red, medium, organic	35.0	8.0	4.0	82.3	47.0	8.3
White, medium	34.1	12.8	6.0	79.4	53.0	10.9
Yukon Gold, grade "B", medium, batch 2	35.6	8.9	9.3	82.4	53.8	9.5
Butterball Creamer, organic, German	35.95	13.4	7.1	80.5	56.5	11.0
White Creamer, small	41.9	10.4	9.8	83.4	62.1	10.3
Ruby Red Crescent	49.5	16.4	6.5	78.9	72.4	15.3
Red, grade "A", large, batch 1	56.4	17.0	7.9	80.9	81.3	15.5
Red, grade "A", large, batch 2	64.5	16.7	10.4	83.1	91.7	15.5
Red Creamer Marble	65.6	37.2	1.1	84.2	103.9	16.4
Red, grade "C", small	73.1	22.4	15.2	81.3	110.7	20.7
Fingerling, Ozette, batch 3	92.3	44.9	10.4	79.5	147.6	30.3
Purple, large	108.6	37.9	5.3	75.3	151.7	37.5
Fingerling, Ozette, batch 1	104.6	49.7	13.8	80.0	168.0	33.6
Fingerling, Ozette, batch 2	113.5	41.9	12.7	78.5	168.0	36.1
Fingerling French	203.0	69.7	7.8	79.3	280.5	58.0
Purple Peruvian	637.3	90.5	29.3	77.3	757.0	171.8

Purple Peruvian potatoes, a 194-fold variation from lowest to highest value. The corresponding range for the chlorogenic acid isomer was from 0.34 to 90.5, a 266-fold variation; and for caffeic acid, from 0.47 to 29.3, a 62.3-fold variation. Total amounts of phenolic compounds for the dry powders ranged from 4.09 to 757, a 185-fold variation; and for fresh tubers from 1.03 to 172, a 167-fold variation. The cited observations demonstrate wide variation both in individual and total phenolic acid content of commercial potatoes. The red- and purple-colored potatoes contained the highest amounts of phenolic compounds. Our data show that commercial potatoes evaluated differ widely in their content of phenolic acids. There is a need to determine possible relationships between phenolic content and health-promoting potential of different commercial potato varieties (Rodriguez de Sotillo et al., 1998; Rauha et al., 2000; Friedman et al., 2003b; Kanatt et al., 2005; Rivera-Carriles et al., 2005; Nara et al., 2006; Huang et al., 2007).

6.4.3.1 *Home processing*

The contents of soluble phenolic acids in raw potato peels determined by HPLC varied from 23 to 45 mg/100 g fresh wt. Boiled peels contained lower amounts (Mattila and Hellström, 2007). Cooking of potatoes and other vegetables in small amounts of water retained most of the phenolic compounds (Andlauer et al., 2003). Steamed potato strips retained 42% of initial chlorogenic acid content and frying, 24% (Tudela et al., 2002). Similar decreases were observed in the content of caffeic acids following exposure of the strips to home-processing conditions. Figure 6.10 shows the effect of several home-processing methods on polyphenol content in potatoes (Im et al., 2008). Chlorogenic acid loss is greatest with boiling in 3% salt, suggesting

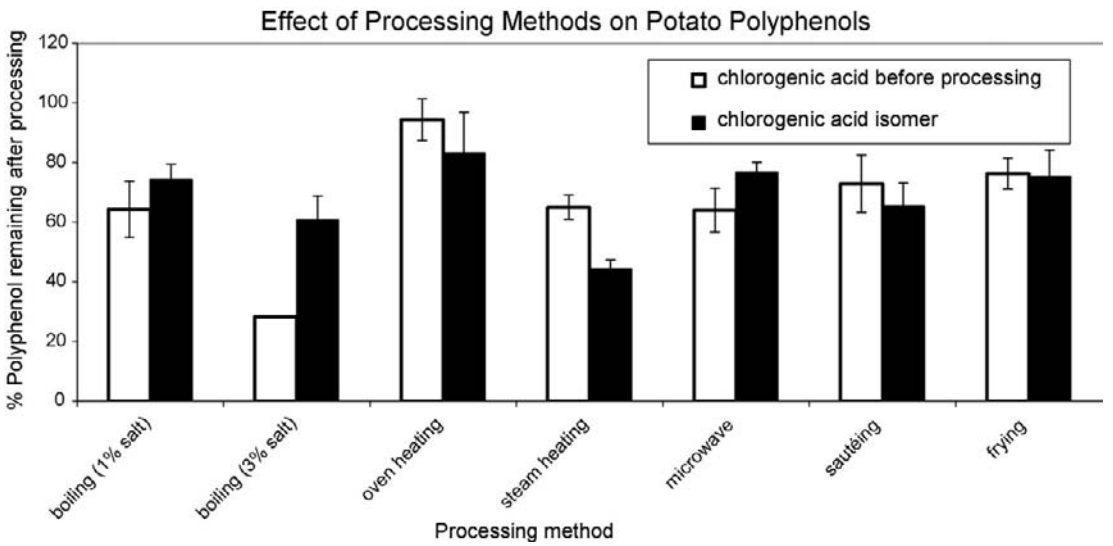


Figure 6.10: Effect of processing method on loss of phenolic compounds of Superior potato flesh. Values are averages from three separate determinations ± SD versus matched controls.

that the compound is leaching into the water. Oven heating provides the best retention of both compounds.

6.4.4 Other studies

We previously measured the chlorogenic acid content of seven potato varieties by UV spectroscopy. Tuber values ranged from 9.6 to 18.7 mg/100 g fresh wt, and leaves harvested at different times from 132 to 242 mg/100 g fresh wt (Dao and Friedman, 1992, 1994; Friedman, 1997). The chlorogenic acid content of 145 mg/100 g freeze-dried potatoes determined by electrophoresis was similar to that determined by HPLC (154 mg/100 g) (Fernandes et al., 1996). No other phenolic compounds were detected in quantifiable amounts. These authors also reported that exposure of the tubers to light resulted in significant increases in chlorogenic acid content, confirming related observations by other investigators (Dao and Friedman, 1994; Griffiths et al., 1995; Percival and Baird, 2000). Storage can cause an accumulation of phenolics. Total phenolic acid content of potatoes (in mg/100 g fresh wt) grown in India increased from 50.6 to 83.7 during storage for 120 days (Mehta and Singh, 2004). Genetic modification induced both inadvertent (Defernez et al., 2004) and purposeful (Lukaszewicz et al., 2004) increases in the phenolic acid content in some potato varieties. However, there was no increase in the transgenic potato Spunta (El Sanhoty et al., 2004).

There is considerable variability of phenolics in potatoes. The average chlorogenic and caffeic acid content of five potato varieties (in mg/100 g fresh wt) grown in the Canary Islands ranged from 21.0 to 28.3 and from 0.73 to 1.12, respectively (Verde Méndez Cdel et al., 2004). Total phenolic acid content of 74 potato cultivars grown in the Andes of South America ranged from 1.12 to 12.37 mg of gallic acid equivalents/g dry wt, an 11-fold variation from lowest to highest value (Andre et al., 2007). The total phenolic acid content of specialty potato selections grown in Texas ranged from 221 μg to 1252 μg chlorogenic acid equivalent, a 5.7-fold variation from lowest to highest value (Reddivari et al., 2007a). Purple flesh selections had the highest amounts, followed by red flesh and yellow selections. Other studies found that the caffeic acid content of different potato cultivars varied widely, ranging from 0.3 to 3.6 mg/100 g in tubers and from 18.8 to 28 mg/100 g in peels (Dao and Friedman, 1992; Dao and Friedman, 1994; Mattila and Kumpulainen, 2002; Mattila and Hellström, 2007).

The cited observations suggest that it is possible to identify potato cultivars with low or high phenolic acid content for human use and to select processing conditions that minimize losses of phenolic compounds. In summary, the methods we developed and used to determine the content and distribution of phenolic compounds in potato plant flowers, leaves, and tubers, in the peel and flesh parts of potato tubers, and in freeze-dried and processed commercial potatoes merit application in numerous studies designed to assess the role of potato phenolic compounds in host-plant resistance, plant breeding, plant molecular biology, food chemistry, nutrition, and medicine. The described wide distribution of phenolic compounds in different commercial

potato varieties and on changes in phenolic compound content during home processing of potatoes may also help consumers to select, depending on need, potatoes with low or high levels of health-promoting phenolic compounds, to use processing conditions that minimize their degradation, and to control enzymatic browning reactions (Molnar-Perl and Friedman, 1990; Friedman and Bautista, 1995) that are reported to cause undesirable discolorations and to damage nutritional quality.

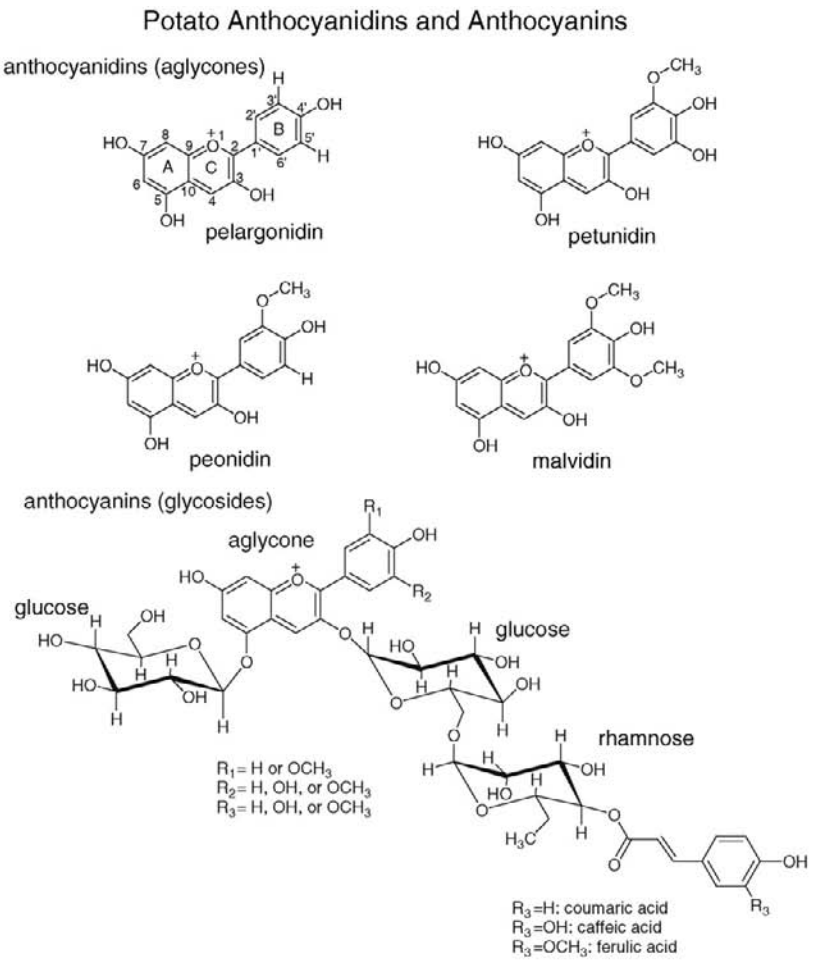
6.5 Anthocyanins

In this section, we will briefly review the chemistry, plant physiology, processing effects, composition, and biological properties of potato anthocyanins. Anthocyanins are widely distributed among plants. They are pigments responsible for many of the blue, red, and violet colors in plants. Anthocyanins have an array of health-promoting benefits primarily acting as antioxidants through a variety of mechanisms (Kong et al., 2003). They also have the potential to be used in the food-processing or the pharmaceutical industry as natural alternatives to synthetic antioxidants, stabilizers, and colorants (Wrolstad et al., 2001; Andre et al., 2007). For instance, because extracts from red- and purple-flesh potatoes at pH 3 showed similar hues as the dye FD&C Red #40, they have the potential to replace that colorant (Giusti and Wrolstad, 2003; Reyes and Cisneros-Zevallos, 2007). Because they are natural and health-giving, they are likely to gain wide consumer and regulatory acceptance.

6.5.1 Chemistry

Figure 6.11 depicts the structures of anthocyanins found in colored potatoes. Anthocyanins are 3-mono- or 3,5-diglucosides of anthocyanidins (aglycones): primarily pelargonidin, petunidin, malvidin, and peonidin in potatoes. Their chemical structure governs color, tinctorial strength, and stability. Substitutions can affect the tertiary and quaternary formations of the molecule, making the chromophore more or less susceptible to hydration, which causes color loss. Acetyl and cinnamoyl moieties are often attached to the glucosyl side chain (Brouillard et al., 2003). The presence of caffeyl residues in the anthocyanin structure not only confers color stability but also allows color diversification (Dangles et al., 1993). Acylation with cinnamic acid shifts colors to purple, increases hydration and antioxidant capacity, and decreases visual detection thresholds (Stintzing et al., 2002). Sugar substituents in the 3- and 5-position also affect these parameters. Molar absorptivities of anthocyanins ranged from 15 600 to 39 590 for pelargonidin-3-glucoside (pg-3-glu) and pg-3-rutinoside-5-glucoside acylated with p-coumaric acid, respectively. Small differences in chemical structure appear to have large effects on color and tinctorial strength of anthocyanin extracts.

Stability is also affected by pH, light, heat, and mechanical stress. Colors change depending on pH and on protonation and hydration reactions during storage. The most stable form, the flavylium cation, predominates at low pH (Torskangerpoll and Andersen, 2005). Stability is



R_1=H, R_2=H, R_3=H: pelargonidin 3-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=H, R_3=H: peonidin 3-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside; peonarin

R_1=OCH_3, R_2=H, R_3=OH: peonidin 3-[6-*O*-(4-*O*-*E*-caffeoyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=OH, R_3=H: petunidin 3-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside; petanin

R_1=OCH_3, R_2=OH, R_3=OH: petunidin 3-[6-*O*-(4-*O*-*E*-caffeoyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=OH, R_3=OCH_3: peonidin 3-[6-*O*-(4-*O*-*E*-feruloyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=OCH_3, R_3=H: malvidin 3-[6-*O*-(4-*O*-*E*-coumaroyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

R_1=OCH_3, R_2=OCH_3, R_3=OCH_3: malvidin 3-[6-*O*-(4-*O*-*E*-feruloyl-*O*-α-rhamnopyranosyl)-β-*D*-glucopyranoside]-5-*O*-β-*D*-glucopyranoside

Figure 6.11: Structures of Anthocyanins found in potatoes.

also influenced by light and temperature (Reyes and Cisneros-Zevallos, 2007). Anthocyanins in extracts of red-flesh potatoes were more stable to thermal degradation at pH 3 than were corresponding extracts from purple-flesh potatoes (Reyes and Cisneros-Zevallos, 2007). The stability of anthocyanin extracts to pH <3 and thermal degradation at pH 3 followed first-order kinetics. Changes in lightness and hue followed zero-order kinetics, while changes in chroma followed first-order kinetics.

6.5.2 Plant physiology

Anthocyanins have multiple functions in the plant (Lukaszewicz et al., 2004). These include visual attraction of pollinators, acting as feeding deterrents, photoreceptors to protect against damage by UV radiation, and as chelators of metal ions (Hamouz et al., 2006, 2007; Lachman et al., 2008). Increasing anthocyanin content in crop plants may result in self-protection against biotic and abiotic stresses and may lead to health-promoting effects after consumption. Post-harvest wounding stress induced increases in both anthocyanins and total phenolic content of purple-flesh potatoes (Reyes and Cisneros-Zevallos, 2003). Slicing induced ~60% increases in total phenolics, including anthocyanin levels, with a parallel 85% increase of antioxidant capacity. The authors suggest that controlled post-harvest abiotic stresses could induce accumulation of antioxidants, thus enhancing the nutritional and health-promoting value of potatoes and other horticultural food crops. Slicing, used in the preparation of potato chips and French fries, could be used as a tool to boost the anthocyanin content to obtain healthier products. Use of such a tool would have to be weighed against any detrimental effects of polyphenol oxidase catalyzed reactions and glycoalkaloid production. Perhaps the processes could be uncoupled. Could the high phenolic content of wounded potatoes also protect against acrylamide formation in chips and fries (Friedman and Levin, 2008)? Anthocyanin content also correlated with potato tuber resistance to bacterial infection by *Erwinia carotovora* (Lorenc-Kukula et al., 2005) and *Pectobacterium cartovorum* (soft-rot) (Wegener and Jansen, 2007).

6.5.3 Genetic manipulation

It was possible to increase the anthocyanin content of transgenic potatoes by overexpressing genes that govern the formation of the enzymes dihydroflavonol 4-reductase (DFR), chalcone synthase (CHS), user and chalcone isomerase (CHI), which all catalyze the biosynthesis of anthocyanins (Lukaszewicz et al., 2004). The resulting increases in anthocyanin and phenolic acid content correlated with increases in antioxidant activities of the potato extracts, but less than expected, inferring that other processes may be at work. Stobiecki et al. (2003) created transgenic potato plants overexpressing and repressing enzymes involved in biosynthesis of flavonoids. Overexpression of DFR with in-sense orientation resulted in an increase in tuber anthocyanins, just as the antisense orientation resulted in decreased anthocyanins. The transformation of these potato plants was also accompanied by significant changes in glycoalkaloids, although changes were not dependent on flavonoid composition, except in the transgenic plants

containing the highest and lowest flavonoids, in which case there was a positive correlation. It appears that the changes in glycoalkaloids resulted not from the gene construct used for transformation on orientation of coding sequence, nor on the site of transgene incorporation, but from chromatin stressed upon transformation. The possibility of decreasing the concentration of toxic compounds in potato tubers while increasing flavonoids appears advantageous. Transgenic studies aimed at manipulating other aspects of the potato should be wary of having a negative effect on phenolic content. In one study, the concentration of anthocyanins in potatoes was adversely affected by gene manipulation designed to increase their methionine content (Dancs et al., 2008).

6.5.4 Analytical aspects

Total anthocyanin content can be measured colorimetrically by a differential method, taking advantage of the color changes induced by pH (Shahidi and Naczk, 2004). It is common to analyze the anthocyanidins (aglycones) by acid hydrolysis of the anthocyanins and subsequent chromatography (Merken et al., 2001). HPLC is used to identify the individual anthocyanins. Using analytical HPLC, Lewis et al. (1998) identified anthocyanins, flavonoids, and phenolic acids in the skin and flesh of the tubers, flowers, and the leaves of 26 cultivars of *Solanum tuberosum* L. This seminal study demonstrates a wide variation in anthocyanin levels of skin and flesh of different-colored tubers. The amounts in tubers reached up to 5 mg/kg fresh wt. The total glycoalkaloid content of red-fleshed potato breeding clones ranged (in mg/100 g tuber) from 2.0 to 36.3. Two cultivars with highest anthocyanin content contained the lowest levels of glycoalkaloids. To minimize anthocyanins degradation during the extraction, the authors recommend precipitating the glycoalkaloids in pH 8 solvent with an efficiency of 90% rather than subjecting the extracts to pH >9.2 for 100% efficiency (Rodriguez-Saona et al., 1998, 1999).

The purple potato, *Solanum tuberosum* cv. Congo, contained the anthocyanins petanin and the novel 3-O-[6-(4-ferulyl-O-α-rhamnopyranosyl)-β-glucopyranoside]-5-O-β-glucopyranosides of petunidin and malvidin (Fossen and Andersen, 2000). Solid phase extraction, countercurrent chromatography, preparative HPLC, HPLC-DAD, and LC-ESI-MS2 methods were successfully used to separate and characterize coumaric acid anthocyanin derivatives (3-ρ-coumaroylrutinoside-5-glucosides of petunidin, pelargonidin, peonidin and malvidin) from non-acylated anthocyanins as well as chlorogenic acids of pigmented potatoes (Eichhorn and Winterhalter, 2005). Analysis of 27 potato cultivars and four breeding clones showed that on average the highest amount (in g/kg fresh wt) of anthocyanins (0.65) were present in the skin followed by whole tubers (0.31) and flesh (0.22) (Jansen and Flamme, 2006). The 'Peru Purple' variety had the highest concentration in the skin (2.96).

Rates of nitrogen fertilization, year of harvest, and location of plant growth, and post-harvest storage for 135 days did not affect anthocyanin content of the tubers. The glycoalkaloid content

ranged as follows (in mg/100 g fresh wt): skin, 17.2; whole tubers, 4.4; and flesh, 2.3. Native South American potato cultivars contain high levels of anthocyanins. However, minerals in the soil appear not to significantly influence the phenolic content of purple potatoes (Andre et al., 2007).

6.5.5 Potential health benefits

Colored potatoes can significantly contribute to anthocyanin intake in the diet. Purple-fleshed potatoes contained 17–20 and red-fleshed 20–38 mg/100 g (Brown et al., 2005). This compares to 24–25 in red wine and 10–60 in the red raspberry (one of the highest in content). The authors suggest that potatoes could provide a cheaper source for anthocyanin extracts than other sources. Antioxidative activities of anthocyanin-containing potato extracts were higher than expected from anthocyanin content, suggesting synergistic effects among anthocyanins in the mixtures of the extracts (Giusti et al., 1999).

The antiviral effect of red-fleshed potato anthocyanins results from additive or synergistic effects of each anthocyanin pigment present in the mixture isolated from fresh potato hybrid (Hayashi et al., 2003). Anthocyanin-rich extracts and steamed red potatoes fed orally inhibited the growth of stomach cancer in mice (Hayashi et al., 2003, 2006). Unlike other reported studies on caspase-dependent anthocyanin-induced cell death (Hou et al., 2004), the cytotoxic activity against prostate cancer cells of an anthocyanin fraction from potatoes is due to activation caspase-independent apoptosis (Reddivari et al., 2007b). Consumption anthocyanin-rich red potato flakes had an antioxidant effect on serum lipid peroxidation and hepatic expression of superoxide dismutase mRNA in rats and protected against oxidative stress induced by a high cholesterol diet and liver injury (Han et al., 2006, 2007a, b).

6.6 Conclusions

The described methods for the analysis of biologically active anthocyanins, calystegine alkaloids, glycoalkaloids and hydrolysis products, and phenolic compounds in commercial potatoes, in processed potato products, and in new cultivars can lead to improvements in the precision and reliability of analyses for quality control and for safety of final products. Accurate analyses will benefit growers, researchers, processors, and consumers. Analytical studies have also facilitated concurrent studies of toxicities and of beneficial health effects as well as investigations of the biosynthesis of the secondary metabolites. Although potato processors are generally interested in imparting desirable sensory (organoleptic) attributes to potato products, the discovery of health-promoting effects of potato ingredients implies that analytical methodology will be paramount in future efforts designed to enhance the levels of these compounds in the human diet. Future studies should attempt to further simplify and improve the described analytical methodologies.

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CHAPTER 7

Thermal Processing and Quality Optimization

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7.1 Introduction

Potato processing dates back to at least 200 AD, at which time potatoes were grown in the mountain areas of Peru; the natives froze them at night and thawed them during the daylight hours, then juice was expressed from the thawed potatoes. This cycle was repeated several times until the moisture content was reduced sufficiently to preserve the potatoes. Almost two thousand years later this process is being used in the preparation of dehydrated potatoes. When the Spanish explorers arrived in the New World, the potato was widely distributed throughout South and Central America, Mexico, and even parts of the present-day United States. They recognized the great value of the potato and obtained them for use in provisioning their ships. Records have been found which show that in 1573 a hospital in Seville (Spain) bought potatoes, and in 1588 there are records of potatoes being used in Italy as cattle feed. In the eighteenth and nineteenth centuries potato became the major source of food in a large part of Europe, and experiments were conducted with several types of dried potatoes in the latter half part of the eighteenth century for provisioning of ships. During World Wars I and II dehydrated potato products were manufactured for military use (Talburt, 1975), and after that considerable quantities of potato flour continued to be produced in Europe. Flakes and granules are now widely used for mashed potatoes, snacks, and baked products. Dried potato products are used in soups and salads and ground in extruded products (Gould, 1999a). Potato starch has been extensively produced since 1831 (first starch plant in USA) and is utilized in significant quantities in Europe, the USA, Canada, and Asia (Salunkhe and Kadam, 1991). Potato chips were first made in Saratoga (USA) around the middle of the nineteenth century (1853). There was some small-scale commercial production for a number of years, but chips did not really develop to any great extent until improved peeling and frying techniques were developed, and production grew rapidly during World War II. French fries were also a World War II product, first made by Belgians; potatoes were cut and par-fried in the morning then given a final fry on the street corner. The same product

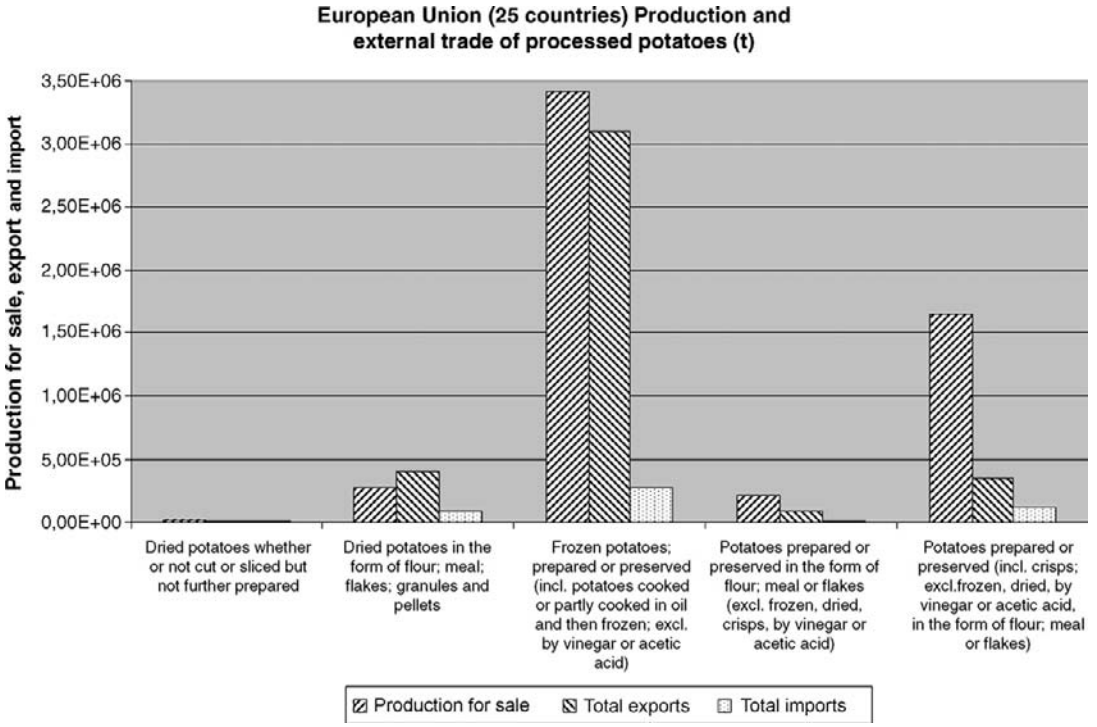

Figure 7.1: Production and external trade in processed potatoes of the European Union for 2005 (25 countries). Prepared with values obtained from Eurostat (2005).

was made in US restaurants after the War. Frozen potatoes were first produced commercially in Maine (1947), where they were blanched, fried twice, frozen, stored and finally heated in the oven to consume (Gould, 1999a).

Nowadays potato is one of the major food crops in the world. Heavy yields can be grown relatively cheaply in a wide variety of soils and climates, and potatoes are one of the mainstays in the diet of people in many parts of the world. In 2005 total EU 25 (Europe of the 25) potato production reached 58 848 073 t, with yields of 29.2 t/ha and a cultivation area of 1 985 358 ha (British Potato Council, 2007). Thus, potato processing and potato products are popular and acceptable throughout Europe. Figure 7.1 shows the 2005 production and external trade in processed potatoes for the European Union (25 countries). The main item is frozen potatoes, including prefried, at 3 414 931 t, followed by potatoes prepared or preserved as potato chips or crisps, 1 647 950 t; dried potatoes in the form of flour, meal, flakes, granules, and flakes, 280 377 t; potatoes prepared or preserved in the form of flour, meal, or flakes (excluding frozen, dried, crisps), 207 037 t; and 18 986 t of dried potatoes not further prepared (Eurostat, 2005). Frozen potato products account for more than 61% of all potatoes that are processed, crisps for

29.5%, dried potato products for 5.37%, and miscellaneous products in the form of flour, meal, or flakes for 4.1%.

Before discussing the quality of a food, it is essential to clearly define what is meant by quality, given that the term in its broadest sense embraces and is dependent on factors of varied origin and nature. Any food, whether frozen or not, is considered to be of good quality if it meets the following requirements: there must be a total absence of pathogens and compounds toxic to humans (hygiene and health quality); it must be easily digestible, with good nutritional value, meaning high concentrations of vitamins, macronutrients and minerals, and an appropriate caloric content (nutritional quality); its sensory attributes, appearance, flavor, aroma and texture must be constant, and in the case of frozen products they must be as close as possible to those of fresh produce (sensory quality); and the presentation and mode of preparation must conform to consumer preferences (commercial quality) (Canet and Alvarez, 2006).

Looking back also over the historical development of quality requirements for processed foods, freezing when properly carried out is undoubtedly the most satisfactory method for the long-term preservation of vegetable produce. The low temperatures commonly prescribed for frozen foods ($-18^{\circ}C$) can maintain initial quality and nutritional value practically unchanged, so that frozen and fresh vegetable products differ only in texture (Canet, 1989), which is however a particularly important quality attribute in potato products.

Potatoes are an excellent source of carbohydrates and contain significant amounts of phosphorus, potassium, calcium, and vitamins, especially vitamin C. Potato protein content, at over 10%, is relatively close to that of wheat flour (11%); also, thanks to their lysine, methionine, cystine and cysteine contents, potatoes are a valuable supplement to cereal proteins. For instance, potatoes provide a significant source of proteins (10–15% of total requirements), a major source of vitamin C, an important source of energy, and also minerals like iron and other vitamins such as thiamin, nicotinic acid, riboflavin, and pro-vitamin A (β carotene) (Salunkhe and Kadam, 1991).

Freezing of vegetables immediately post-harvest guarantees consumers a minimum loss of nutritional value and higher vitamin C content than could be attained by any other form of preservation and distribution. Furthermore, if properly handled before freezing and correctly processed and distributed, there is no possibility of growth of microbial contaminants, which also guarantees hygiene and health quality.

The quality of potato tubers depends upon genetic, climatic, biotic, chemical, and edaphic factors; varietal characteristics; precipitation, temperature and sunshine conditions; competition with other plants; the use of chemicals; and the physical, chemical, and biological properties of the soil, which influence the capacity of the crop to take up the necessary water and nutrients to ensure success.

The production of high-quality potato products depends on the initial quality of the tuber; on the conditions and time lapse between harvesting and processing; on preparatory operations such as washing, peeling, trimming, and cutting; in the case of frozen potato products, on pre-freezing treatments like blanching or pre-frying, freezing per se, storage temperature, time and tolerance of frozen storage, and on final preparation procedures such as boiling, cooking, or microwaving. It is this set of factors, known as P-P (Product-Processing) factors, and the achievement of optimum interaction among them, that defines quality. This quality can be considerably diminished by adverse storage times and temperatures, that is, T-T-T (Time-Temperature-Tolerance) factors. The large number of influencing factors makes it difficult firstly to optimize the final quality, and secondly to assess and quantify the loss of quality during storage.

In frozen vegetables, health quality, nutritional quality, and aspects of sensory quality like color and texture can be objectively assessed and controlled; also, in frozen potato products the effects of the thermal treatments included in the process have to be assessed due to their influence on texture, color, and nutritional value. However, in the case of overall assessment of sensory quality, only the consumer can perceive and process the overall blend of sensations that denote quality and cause consumers to prefer, accept, or reject a product.

In view of the daily growing importance of frozen potato products, after dealing with raw composition as it relates to processing and quality, technological product factors and the suitability of varieties for processing, quality assessment of raw and processed material, we review how the final quality of the potato is affected by preparatory operations such as washing, peeling, and cutting. Here, we place particular emphasis on recent findings on the way in which the thermal treatments entailed in the process – blanching and pre-frying, stepwise blanching, freezing and thawing cycles, frozen storage conditions, thawing, and cooking – affect the structure and the texture (which is considered the main attribute of potato quality), and on ways to optimize it.

7.2 Product vs. quality

The raw material used in the preparation of frozen potato products is an important conditioning factor affecting both the quality and the nutritional value of the final product. A product's suitability for processing is determined by its composition, by agro-technical practices and conditions, by the cultivar (cultivated variety) involved, and by technological factors such as ripeness and the time elapsing between harvesting and processing. Clearly then, only raw material that is clean, sound, and of high nutritional, safety and sensory quality should be selected for processing.

7.2.1 Composition

The product factors briefly mentioned in the previous section constitute an important field of research given the considerable influence they have on the final quality of potato products, a

subject comprehensively dealt with in other chapters of this book and treated here only as it relates to the quality of frozen potato products. There is an extensive literature on proximate potato composition, which depends on and varies with the variety, growing area, cultural practices, storage history, and the methods of analysis used. Talburt et al. (1975) give a proximate composition for the potato derived from various reviewers: water, average percent (ap) 77.5% and range percent (rp) 63.2–86.9%; total solids (ap) 22.5%, (rp) 13.1–36.8%; protein (ap) 2%, (rp) 0.7–4.6%; fat (ap) 0.1%, (rp) 0.02–0.96%; total carbohydrates (ap) 19.4%, (rp) 13.3–30.53%; crude fiber (ap) 0.6%, (rp) 0.17–3.48%; and ash 1% (ap) and 0.44–1.9% (rp).

The main components in terms of their importance for the final quality of frozen potato products are starch, sugars, non-starch polysaccharides, phenol, and related substances. Starch comprises between 65% and 80% of the dry weight of the potato tuber and is the most important nutritional component; its concentration and its physical, chemical, and histological characteristics are intimately related to several quality parameters of processed potato products and also influence the operational conditions in the process. Starch is present as microscopic ellipsoid granules inside the parenchyma tissue cells, measuring about 100 by 60 microns on average on the longitudinal axis. The largest granules are found in the vascular areas, and small grain sizes are associated with dry seasons, small tubers, immaturity, potassium deficiency, and prolonged post-harvest storage. Granule size and size distribution contribute to the character and quality of potato products. Starch content is accepted as a varietal characteristic; there are close correlations among specific gravity, total solids and starch content, and there is a highly significant correlation between the starch content of the raw tuber and several attributes of textural quality of potato products (mealiness, consistency, sloughing, and sogginess). When the temperature of the potato tissue rises to 50°C, the starch granules absorb the cell water and swell; in the range 64–71°C the starch begins to gelatinize and the cells tend to separate and become rounded. Excessive cell separation results in sloughing, a tendency that is important not only in the cooking of the potato but also in commercial peeling methods using heat and lye scald, where excessive rupture of cells and escaping gelled starch produces a gummy texture in the processed potato products. It is the amount of starch in the individual cell rather than the total amount of starch in the tuber that influences cell separation and texture changes. The starch cell together with the cell wall constituents, especially pectin substances, play a very important role in determining the final texture of frozen potato products, as discussed later on.

The main factors influencing the sugar content of potatoes during post-harvest storage are variety and temperature: varieties with low specific gravity tend to accumulate more sugar than varieties with high specific gravity; freshly mature tubers may contain only traces of sugars, while tubers harvested before maturity may have 1.5% sugar, and small tubers usually contain higher sugar percentages than large ones. At storage temperatures below 10°C the total and reducing sugars increase, the rate and extent of the increase being greater the lower the temperature down to freezing point. The sugar contents of tubers stored at low temperature and reconditioned at

higher temperatures gradually decrease over a period of 3–4 weeks, while the starch percentage increases during conditioning. Poor texture after cooking is associated with low-starch and high-sugar content. In potato products such as chips, French fries, and dehydrated potatoes, the final color is strongly dependent on the reducing sugar content rather than the total sugar content. Potatoes containing more than 2% reducing sugars (dry weight) are unacceptable for processing; it is general practice to keep potatoes which are poor sugar formers in storage and process them while their sugar level is low and they have not sprouted, or to condition them (2–3 weeks) cold storage tubers at room temperature, or by storing at 10°C.

Potatoes contain non-starch polysaccharides which make up the cell wall and the intercellular cementing substances, among which we may distinguish crude fiber, cellulose, pectin substances, hemicelluloses, and other polysaccharides. Dry matter after removal of all the soluble, starch, and nitrogenous constituents is considered the crude fiber (1% of dry weight), which includes cell wall components, lignin and suberin. Cellulose is part of the supporting membrane of the cell wall, a mixture of high-molecular-weight polymers of glucose residues, together with hemicelluloses and other polysaccharides (10–20% of non-starch potato polysaccharides).

Pectin substances, polymers of galacturonic acid, with the carboxyl groups more or less methylated, are usually divided into protopectin, soluble pectin, and pectic acid. Protopectin is an insoluble, highly polymerized form of pectin associated with the cell wall structure. Water-soluble pectin is the product of partial depolymerization of protopectin. Freshly harvested potatoes are relatively high in protopectin, and protopectin content decreases and the soluble pectin content increases during storage. The pectic acid fraction in the form of calcium and magnesium salts is believed to be the cementing substance in the middle lamella of potato tissues. In the development of potato product texture, swelling of the gelatinized starch is the major factor causing cell rounding and separation, a tendency which is opposed by the molecular size and calcium content of the pectic material of the middle lamella and cell wall. Heating at 70°C produces changes in the pectic substances of the middle lamella, and addition of calcium results in a firmer cooked potato. These two mechanisms, the swelling of the gelatinized starch and the precipitation of pectic substances on cell walls, both induced by thermal treatments, are the key to achieving the optimum desirable final texture for frozen potato products.

Phenolic compounds are associated with the color of raw potatoes and certain types of discoloring in processed potato products. In normal uninjured potatoes there is no oxidation of the phenolic substances such as to form discoloration products. When potato tubers are injured by bruising, cutting or peeling, the phenols are rapidly converted to colored melanins due to oxidation of phenolic compounds by enzyme phenolase; this is determined by the concentration of tyrosine, which is considered the most important factor in controlling the extent of pigmentation caused by enzymatic browning. The amount of browning is influenced by potato variety, season, cultural practices, growing and storage conditions, and the extent of pigmentation produced by

a given amount of tyrosine varies. The levels of enzymatic browning and tyrosine and phenolase activity decrease when the plants are treated with higher concentrations of potassium.

7.2.2 Varietal suitability for processing

Potatoes used for processing must possess certain quality characteristics, and as a result potato varieties have been developed specifically for the purpose of processing. As already noted, the biochemical composition, especially the dry matter (DM) content, carbohydrates (reducing and non-reducing sugars), textural characteristics and color-related alterations (discoloration of raw flesh, browning and post-cooking blackening) are the key quality parameters for processing (Kadam et al., 1991). The choice of variety is probably the most critical decision as regards matching tuber quality with intended market. Dry matter (DM) content is one of the most important factors of processing qualities over a range of uses which substantially affect the texture in practice and the potato's suitability for processing. The specific potato cultivar requirements for processing chips and French fries are moderately high DM content with low reducing sugar and large, long, oval tubers. For crisps, high DM and low reducing sugars are essential and moderate-sized oval tubers are preferred. Cultivars with high DM contents are also preferred for dehydrated potatoes. Moderately low DM and small tubers are the essential requirements for canned potatoes. And finally, medium-firm, slightly mealy potatoes which do not disintegrate are the best suited for multipurpose and domestic use.

7.2.3 Agro-technical practices and conditions

There are a number of cultural and environmental conditions which prevail during the growing season and strongly affect the processing quality of potatoes: climate, rainfall, irrigation and soil moisture, soil type, time of planting and harvesting, kind and amount of fertilizers used, and control of insects and diseases.

Climatic factors that influence the potato yield are temperature, light intensity and photoperiod, rainfall and length of growing season. Potato is classified as a 'cool-season crop.' Temperature influences the rate of absorption of plant nutrients and translocation within the plant and the rate of respiration, and it also facilitates early growth. Temperature determines to a great extent the length of the growing seasons, maturity of the tubers at harvest and also their specific gravity. At low temperatures, the rate of respiration is less than the rate of photosynthesis, resulting in more accumulation of carbohydrates in the tubers and an increase in the tuber's specific gravity; low and moderate temperatures during the growing season result in higher yields and high-specific-gravity tubers. Although other factors than temperature are involved, the influence of light on growth and yield is dependent upon light intensity and quality and day length. During the stage of tuber formation, potato plants devote a large portion of the carbohydrates produced by photosynthesis to growth of the tubers and less to vegetative growth. A long photoperiod prolongs the maturity period of plants, whereas with a short photoperiod they tend to mature

earlier. Differences in soil moisture as a result of rainfall, irrigation, and type of soil affect the dry matter content of potatoes and their quality for processing; potatoes respond to an ample soil moisture supply with an increase in yield. The most critical period as regards water requirements is at the onset of tuberization. It is desirable to have a uniform moisture supply in the soil at all times during the growing season, and in areas with low rainfall additional irrigation should be distributed to provide adequate moisture for the growth of the plants. The soil moisture should be about 60% of field capacity for optimum yield. Excessive rainfall or irrigation late in the growing season usually results in low-specific-gravity tubers which are unsuitable for processing.

The soil type in which potatoes are grown may affect the specific gravity of tubers because of its water holding capacity, drainage, aeration, structure, temperature, and fertility. To obtain high yields, the soil should be loose and friable with good drainage and aeration. Potatoes do not grow well on heavy-textured or undrained soils. Potato plants respond well in soils that have 50% or more pore or air space, sandy loam, and loamy soils rich in organic matter are the most suitable for potato cultivation. In general, loam soils produce high-specific-gravity tubers because their soil moisture, temperature, and structure are closer to optimum than more lightly or heavily textured soil. Sandy soils result in early crop maturity. The optimum soil pH for potatoes is 5.0 to 5.5 so as to limit potato scab disease, which is favored by alkalinity.

Potatoes require large quantities of mineral nutrients for maximum growth. The recommended ratios of nitrogen, phosphorus, and potassium are 1/2/1, 1/2/2, 2/3/3, and 1/1/1 (Sawant et al., 1991). Fertilizers are applied before or at planting depending on soil type, climate, variety, and moisture supply, and an additional second application may be appropriate during the growing season. The soil pH should be taken into consideration when selecting the sources of nitrogen, phosphorus, and potassium fertilizers. High-yield responses are achieved by application of nitrogen, while phosphorus is critical at early stages of growth and has some effects on dry matter. High-yield potato cultivars require large amounts of potassium for growth and development, and potassium sulfate (SO_4K_2) is recommended because it causes less reduction of specific gravity than potassium chloride (ClK) and potash (K_2O).

Micronutrients do also affect potato quality and yield; applications of B, Mn, and Zn increase the starch grain size, while applications of iodine or ammonium molybdate increase the dry matter and starch content.

7.2.4 Technological factors

The industry needs continuous supplies of raw material, which means that it is essential to properly organize the varieties used, planting times, growing zones, mechanical harvesting, transportation, and handling in order to avoid rapid loss of quality after harvesting. Because processing causes complete tissue death, potatoes that are to be processed need to be harvested

at the exact moment of ripening. Technological factors such as ripeness and storage conditions (temperature, relative humidity, and air circulation) during the time elapsing between harvesting and processing all contribute to final potato product quality. As noted above, sugar and starch contents are critical quality factors for processing potatoes; as the tubers grow and mature, the sugar content decreases, reaching its lowest point when the vines are nearing complete senescence. Sucrose or Chemical Maturity Monitoring (CMM) (Gould, 1999b) can be used to optimize harvest timing and make correct decisions on storage temperature protocols. Stark et al. (2003a) made the following suggestions for quality maintenance during production and storage.

Potatoes intended for chip production should have a reducing sugar level below 0.35 mg/g (or 0.035%) of fresh tuber weight at harvest time. Potatoes intended for processing as French fries should have less than 1.2 mg/g (or 0.12%) of tuber fresh weight. In both cases sucrose content should be below 1.5 mg/g (or 0.15) of fresh tuber weight. Potatoes with higher values than these will usually show color problems after cooking.

7.2.4.1 Maturity monitoring and storage temperature

During the last month of the growing season, fields should be sampled weekly and sugar content determined. During vine senescence prior to harvesting, sugar should fall below the levels indicated above. This is indicative of chemical maturity and suggests that harvesting can proceed with the maximum likelihood of quality being maintained in storage. At this stage, the most critical factor is the sucrose level. If it is below the indicated levels, harvesting can proceed and the tubers can be stored in a normal fashion at the appropriate final holding temperature for the variety and the intended market. Potatoes intended for chip processing are typically stored at 10–12.7°C, for French fry processing 8.3–10°C, for fresh use 5.5–7.2°C, and for seed 2.7–4.4°C.

7.2.4.2 Determination of early storage condition

If tubers come out of the field with sucrose levels above 1.0 mg/g (or 0.10%) of fresh tuber weight for chips and 1.5 mg/g (or 0.15%) of fresh tuber weight for French fries, and with glucose values above 0.35 mg/g (or 0.035%) of fresh tuber weight for chips and 1.2 mg/g (or 0.120%) of fresh tuber weight for French fries, they will in principle present cooking problems. However, storage managers may consider one of the following strategies based on manipulation of early storage temperatures. These economically critical decisions are based on levels of sucrose and glucose at harvest.

Scenario 1: Sucrose levels are acceptable (<1.5), but glucose levels are too high (chips >0.35, fries >1.2). The immediate fry color may be too dark, but the potential for long-term storage can still be good.

Action – During the wound-healing period at the beginning of storage, the temperature should be held at 15.5°C for 2 weeks or until the glucose concentrations drop to acceptable levels. The temperature can then be ramped slowly downward to 7.2–8.8°C for fries or 10–11.1°C for chips. Glucose levels should subsequently be determined at regular intervals to ensure that they remain within the acceptable range.

Scenario 2: Sucrose levels are too high (>1.5), but glucose levels are acceptable (chips <0.35, fries <0.12). The immediate fry color may be good, but long-term storage may be negatively impacted as sucrose is converted to reducing sugars.

Action – The same wound-healing conditions should be used as recommended for Scenario 1. The sucrose levels should be determined at the end of the wound-healing period. If sucrose levels are still too high, a higher than normal holding temperature (possibly 12.7°C for chipping potatoes and 10°C for frying potatoes) may be required. It may be necessary to sell these potatoes before others that have better sugar indicators.

Scenario 3: Both the sucrose and glucose levels are too high. Both the immediate fry color and long-term frying potential may be poor.

Action – The recommendations described for Scenario 2 should be followed. A wound healing temperature of 15.5°C should be maintained until both the sucrose and glucose levels are acceptable. A more intensive monitoring program will be required, with sugars being measured at least every 5 days. The storage manager should consider moving these potatoes to market as early as is feasible.

7.2.4.3 Storage maintenance

As mentioned earlier, storage conditions can cause potatoes to accumulate unacceptable quantities of sugars, even when the levels are acceptable at harvest. Sugar analysis can be used to indicate when conditions need adjustment. Sugar accumulation in storage can generally be attributed to cold temperatures, inadequate supply of air to the pile, or senescent sweetening. These conditions can be detected by sugar monitoring, usually before any obvious decline in quality.

7.2.4.4 Low-temperature stress

When storage temperatures are too cold, both sucrose and glucose levels will climb simultaneously into the unacceptable range. This can occur within a few days if the temperature is several degrees below optimum, or it can occur slowly when the temperature is only a few degrees too low. Problems with low-temperature sweetening can usually be solved with a 2–4-week period of reconditioning at 12.7–15.5°C, followed by a slow return to the desired holding temperature.

7.2.4.5 Inadequate air

Oxygen deprivation caused by inadequate air movement in storage causes sucrose levels to slowly increase. Later, the glucose levels follow the same pattern and the fry color goes off-grade. Another typical symptom of ventilation stress is that individual tubers may fry darker in the middle than around the outside. Early detection of the rise in sucrose levels can help resolve this problem. Solving the problem may be as simple as increasing the frequency or length of ventilation to the pile.

If the problem is one of inability to move air through the pile due to obstructions or dirt, more drastic measures may be required, such as early marketing of the potatoes or movement to a different storage building. If ventilation stress is the culprit, an increase in air supply will result in an immediate response to corrective action, but the return to acceptable sugar levels may be slow.

7.2.4.6 Senescent sweetening

The maintenance of acceptable sugar concentrations during the first 5–8 months of storage, followed by a slow increase in sucrose levels over the next several months, may be an indication of senescent sweetening. Senescent sweetening is a permanent condition and only gets worse with time. If no temperature stress or ventilation problems can be identified, and a sample of potatoes removed from the storage does not respond to reconditioning, then the potatoes should be marketed as quickly as possible. If an entire pile of potatoes is suspected of age-related sweetening, it is critical that no attempt be made to recondition the potatoes. Warm temperatures will only speed up the aging process and make the problem worse.

A more detailed discussion of potato tuber quality can be found in Chapter 16 of the book 'Potato Production Systems' (Stark and Love, 2003b).

7.2.5 Quality assessments of raw and processed potatoes

Only raw material of high nutritional, safety, and sensory qualities should be selected for processing. At the factory, the processor has the choice of accepting or rejecting incoming loads of raw material. The decision is made after measuring samples from each consignment against a raw material specification, which should describe precisely what is required and the extent to which the quality may deviate from the standard before it is rejected (Arthey, 1993).

The tuber samples should be selected at random and be representative of the entire batch in question. The samples should be evaluated for specific gravity and graded for size and absence of external and internal defects. A sub-sample should be fried and if the fry color is not satisfactory, a reducing sugar evaluation should be made and the strategies for storage and end use followed as recommended above. The appearance and size of tubers are important to the user, and cleaning, uniformity of shape and size, depth of eyes, flesh color, and peel are very important in terms of

processing. The size is important to determine the usage of a given lot of potatoes; medium–large round tubers are preferred by the chip industry, as their shape facilitates peeling with minimal loss; the long, oblong type of potato is preferred by the French fry industry; and size uniformity is preferred for processing in all cases to obviate the need for size grading, rejection of tubers, or cutting large specimens before processing. Shape and eye depth tend to be quite uniform within cultivars; round, oblong, elliptical, or long are the usual shapes, and shallow eyes are important for processing in order to minimize loss in peeling.

Gould (1999c) devised and presented a potato receiving inspection report, a methodology for evaluating quality and grading of potatoes for making chips and the specifications of potatoes for the chip market. He also cites the US standards for grades of potatoes for processing (1963) (Gould, 1999d), the US standards for grades of canned white potatoes (1987) (Gould, 1999e), the US standards for grades of frozen French fried potatoes (1967) (Gould, 1999f), and the US standards for grades of frozen hash brown potatoes (1976) (Gould, 1999g). In each case all these standards include definitions, product descriptions, styles, grades, size, test procedures for specific gravity, pulp temperature, glucose testing, fried color determination, texture, color, flavor and odor, defects, tolerances, damage, etc.

For frozen vegetables in general (including potato products) Canet and Alvarez (2006) cite quality indices or specifications laid down by the industry's own quality control laboratory or produced by public or private institutions. Volume 5A of the FAO/WHO Joint Food Standards Program (Codex Alimentarius) contains a set of standards for quick-frozen fruits and vegetables (Anonymous, 1995a). In addition to defining the process and form of presentation for the various products, it deals with other quality factors including definition of defects, sizes of analytical samples and defective units, criteria for acceptance of batches, levels of additives, hygiene, and labeling and packaging. Volume 13 of the Codex also presents analytical and sampling methods (Anonymous, 1995b). Between 1997 and 2003, Campden and Chorleywood Food Research Association (CCFRA), in conjunction with the UK frozen vegetable industry, compiled handbooks for many products: The Raw Material Guidelines for Quick Freezing (Anonymous, 1995–2003a), addressing quality standards, detailed sampling plans, and defects (definitions and tolerances); the Specifications for Quick Frozen Fruits and Vegetables (Anonymous, 1995–2003b), including product grades and grade defects (definitions and tolerances); and sensory assessment. There are FDA food defect action levels for vegetables and vegetable products (Anonymous, 1998a) and FDA macroanalytical methods for vegetables and vegetable products (Anonymous, 1998b).

A wide variety of methods and instruments have been employed to control the quality of raw and processed potatoes. The key characteristics of importance for potatoes for processing are dry matter, reducing sugar content, color, size, and defects. The Potatopro Newsletter (2008) Food Innovation Online (http://www.foodinnovation.biz) shows equipment (Martin Lishman Ltd,

Unit 2b, Roman Bank, Bourne, Lincolnshire, PE10 9LQ, UK) for evaluating all these parameters. Dry matter content, weight in water and specific gravity of potatoes are directly related and could be determined by a dry matter kit, a hydrometer, and/or a digital balance to measure weight in water, which directly displays dry matter and specific gravity. The minimum and maximum dry matter (%) required for potatoes to be processed as French fries are 19.7–24.1%, as potato chips 21.7–25.1% and for dehydrated products 20.7–24.1%. Reducing sugars (glucose, fructose) are an important parameter in the quality control of potatoes for processing given their influence on the final color of fried potato products. The maximum reducing sugar content for French fries is 0.5%, for chips 0.2% and for dehydrated products 0.3%; sugar contents and their influence on final color can be determined by direct measurement of the sugars, or by other methods involving the standard preparation of a product – e.g. frying a number of strips from the core of a potato tuber and determining the color by spectrophotometry and comparing with the USDA French fry color card, or the potato chip standard color reference chart. Apparatuses for direct measurement of sugar are based on the action of glucose oxidase on the glucose in potato juice; this is oxidized to gluconic acid, and the orthotolidine indicator changes from yellow to various shades of green depending upon the concentration of glucose in the juice (Kadam et al., 1991). This is a suitable method given the high correlation between the glucose determined and the resulting color of the potato product. The industry standard for instrumental color measurement of par-fried or finish-fried French fries is the Agtron-E30, a direct reading reflectance abridged spectrophotometer designed to provide the ratio of reflectance of a product in two spectral modes, near infrared and green. The ratio is automatically computed and digitally displayed as a single number. For chips the AGTRON M-SERIES with a large area (26 in^2) reflectance spectrophotometer is designed to deal with the measuring of color and process-related changes associated with foods of irregular geometry with little or no sample preparation. The benefit of instrumental measurements compared to the use of color cards is its objectivity; the Agtron equipment is therefore the preferred method for product specifications (Agtron Inc., 9395 Double 'R' Blvd. Reno, Nevada 89521). To avoid bruising and defects in the production chain and the processing line the number of drops and the drop heights should be kept to a minimum. To control potato damage and bruising, a wirelessly monitored device simulating a potato (Smart Spud) reduces storage diseases and allows the user to see, in a matter of seconds, exactly how harvesters, windrowers, grading, washing, and packing facilities are causing potato damage. The Smart Spud from Sensor Wireless (Sensor Wireless Inc, 106 E Kensington Road, Charlottetown, PE C1A 5J5 Canada) cuts down potato bruising and damage by at least 10% and improves quality control routine and record keeping by pinpointing where damage is occurring through impact, vibration, and temperature analysis.

Texture is a key component of the quality and palatability of potato products. Texture is generally quantified by measuring the resistance of a product to an applied force. A number of different rheological parameters can be used to evaluate a range of tuber characteristics such as firmness, hardness, softness, adhesiveness, fracturability, etc. There is a considerable amount

of literature on methods for objectively measuring potato and potato product texture and related chemical and sensory parameters. Due to space constraints, we can refer here only to those habitually used by the authors. To evaluate the effects of different thermal treatments included in the freezing process, like blanching, freezing, thawing, etc., Canet (1980) uses compression, shear and stress relaxation tests performed with an Instron Food Testing Instrument model 1140 (Instron, Canton, Mass., USA) on cylindrical specimens (diameter: 25.4 mm, height: 10.0 mm) of potatoes (cv. *Jaerla*) (Canet et al., 1982a, b). Alvarez (1996) characterizes the rheological behavior and establishes the softness kinetics of potato tissues (cv. *Monalisa*) by means of compression, shear testing (Alvarez et al., 1997, 1999; Alvarez and Canet, 1997, 1998a) and creep testing (Alvarez et al., 1998) on cylindrical specimens, and tensile tests (bone-shaped specimens, length 75 mm, width 20 mm, neck region 8 mm wide) using an Instron Food Testing Instrument model 4501 (Alvarez and Canet, 1998b; Alvarez et al., 1999). Also, stress relaxation and TPA tests have been performed using the TA HDi Texture Analyser (Stable Micro Systems Ltd, Godalming, UK). A recent International Technical Specification describes a method for the determination of rheological properties by uniaxial compression tests at a constant displacement rate [ISO /TS 17996 /IDF/RM 205:2006(E)]. It was devised for hard and semi-hard cheeses, but the protocol is perfectly adaptable to potato tissues. Canet et al. (2005c) studied the optimization of low-temperature blanching for maintenance of potato firmness by means of such uniaxial compression tests using a TA.HD texturometer (Stable Micro Systems, Godalming, UK) on cylindrical potato (cv. *Kennebec*) samples. For French fries, Alvarez et al. (2000) has characterized the frying process of fresh and blanched potato strips using texture profile analysis (TPA), multiple puncture and Volodkevich tests carried out with a TA HDi Texture Analyser (Stable Micro Systems Ltd, Godalming, UK).

For mashed potato texture, profile analysis (TPA) and cone penetration tests are performed with a TA HDi Texture Analyser (Stable Micro Systems Ltd, Godalming, UK). During the tests, the mashed potatoes are kept at 55°C by means of a temperature-controlled Peltier cabinet (XT/PC) coupled to a separate heat exchanger and PID control unit. For the cone penetration tests, a spreadability rig is used, consisting of a 45° conical perspex probe (P/45°C) that penetrates a conical sample holder containing 7 ± 0.1 g of mashed potatoes (Alvarez et al., 2005; Canet et al., 2005b; Fernández et al., 2006).

For more detailed discussion of potato product quality assessment and assurance, the book 'Total Quality Assurance for the Food Industries' (Gould, 2001) is essential reading, and for frozen French fried potatoes we especially recommend Hui's chapter of the 'Handbook of Vegetable Preservation and Processing' (Hui, 2004).

7.3 Processing vs. quality

Processing consists of a series of stages from reception of the products at the plant to their final dispatch. The first main stage comprises a number of preliminary operations to prepare

the product for subsequent frying, drying, canning, or freezing. These operations are: blow dry cleaning, removal of stones and washing, inspection and selection, classification, peeling, and, depending on the variety and the final use, chopping and slicing. Hygiene conditions must be absolutely strict, and care must be taken to avoid excessive wastage and mechanical damage. Especially in the case of frozen potatoes, the second stage consists of blanching (heating for a short time to inactivate the enzymatic systems responsible for off-odors and flavors and changes in color during frozen storage), plus other prefreezing treatments (use of coadjuvants to improve blanching action before frying, or pre-frying), and finally cooling and draining to prevent yield and energy loss during freezing. The third and fourth stages are freezing and frozen storage respectively. Potato products are usually frozen individually, that is, individually quick-frozen. They are then frozen-stored packaged in small containers weighing anything from 400 g to several kilograms for direct dispatch, or bulk stored in polyethylene-lined pallet boxes which can contain several hundred kilos of product, thus helping to optimize the utilization of storage space (Canet and Alvarez, 2000).

7.3.1 Main preparatory procedures

The purpose of these procedures is to take raw potatoes as received by processors and from them to make a product which once processed and frozen is 'ready-to-eat' with minimal final preparation by the consumer. Following an initial selection process to meet the quality standards required, the tubers are cleaned by vibration or air blast to remove unwanted materials (such as leaves, husks, etc.) and brushed to remove earth, stones, and excess dirt.

7.3.1.1 Washing and peeling

Washing cleans the potatoes of dirt and impurities (soil and waste matter), of pesticide residues, and of up to 90% of the microbial flora. There are different types of washers that can be used (rotary and high-pressure sprays). Light chlorination by adding gaseous chlorine or sodium hypochlorite enhances the action of water, preventing the formation of sludges of bacterial origin in the equipment and the development of unpleasant odors. Free chlorine contents in the region of 5–10 ppm do not adversely affect product flavor or corrode equipment. The effect of the heating treatment during steam peeling of potatoes was assessed by Garrote et al. (2000), who determined the minimum temperature and cooking values necessary for peeling to take place, and the consequences ensuing at cellular level, especially for pectic constituents. The results showed that for steam peeling to take place the thermal treatment must satisfy the following conditions: the cooking value must reach at least 17 s at 100°C, and the temperature must reach at least 100°C.

Peeling, one of the most delicate pretreatments, is achieved industrially by abrasion, high-pressure steam, treatment with sodium hydroxide solution, or mechanically. Abrasion is effected by rough, moving surfaces which remove the outer surface of the product, but it has the drawback of considerable loss of raw material. Steam peeling consists of heating the product to

a temperature of up to 80°C and subjecting it to pressures of 392–686 kPa for between 30 s and 3 min. In sodium hydroxide peeling, the product is preheated then immersed in a 10–20% solution at a temperature of 60–90°C for between 1 and 5 min depending on the type of product. The drawback of all these methods is the substantial loss of raw material involved (8–20% in potatoes, depending on their shape and age). Using sodium hydroxide with infrared heating can cut down sodium hydroxide solution consumption by 80%, reduce raw material loss by one third, and reduce water consumption by up to 95% (Canet and Alvarez, 2006).

7.3.1.2 Cutting

After washing and peeling, the product may be subjected to any of a number of procedures (e.g., sorting, paring, trimming, cutting, and pulping) depending on the type and variety of the final product. There is a wide range of equipment for high-yield performance of these operations (Urschel Laboratories, 2006).

Figure 7.2 depicts the operation of a DiversaCut 2110® Dicer by Urschel Laboratories, Inc. This equipment uniformly dices, strip cuts, and slices at high production capacities and is specially adapted for potatoes. The full range of slice thicknesses can be achieved by removing the crosscut spindle and the circular knife spindle: Flat & Crinkle Slices (1.6 to 25.4 mm). Strip Cuts: Flat and crinkle strips in a wide variety of widths can be made (by removing either the crosscut knife spindle or the circular knife spindle; also minimum flat strips such as 3.2 × 3.2 mm × length of product; crinkle strips such as 7.1 × 7.1 mm x length of product, or maximum flat strips, for instance 25.4 × 25.4 mm × length of product). Dices: a slicing knife, circular knife spindle, and crosscut knife spindle are used for dicing (the dice size is changed by using the required cutting spindles and adjusting the slice thickness). Circular knife cuts: 3.2–76.2 mm. Crosscut knife cuts: 3.2–38.1 mm. Crosscut knife crinkle cuts: 7.1–15.9 mm.

There is also an Urschel model CC® slicer, able to produce flat potato slices, standard Crinkle, 'V' slices, oval shreds, crescent shred, 'V' shred, and strip cuts. Then there is the Urschel® model GRL strip cutter, designed specifically for cutting plain and crinkle cut potato strips with slice thicknesses up to 14 mm and crosscut knife cuts from 7.1 to 22.2 mm.

All these operations must be performed with the utmost care and under the most stringent hygiene conditions to prevent contamination of the product and mechanical damage. The varying degrees of complexity and automation of the process according to product type require a thorough understanding of the mechanical properties of each individual final product. Further progress is needed in this field to improve automation and to optimize procedures (Canet, 1980).

After the preparatory procedures and before blanching, or immediately after blanching and after cooling and draining depending on the product, thorough inspections are essential to eliminate unwanted material from the line. If done manually, such inspections either reduce the line output or require a lot of manpower. There are now computerized inspection systems

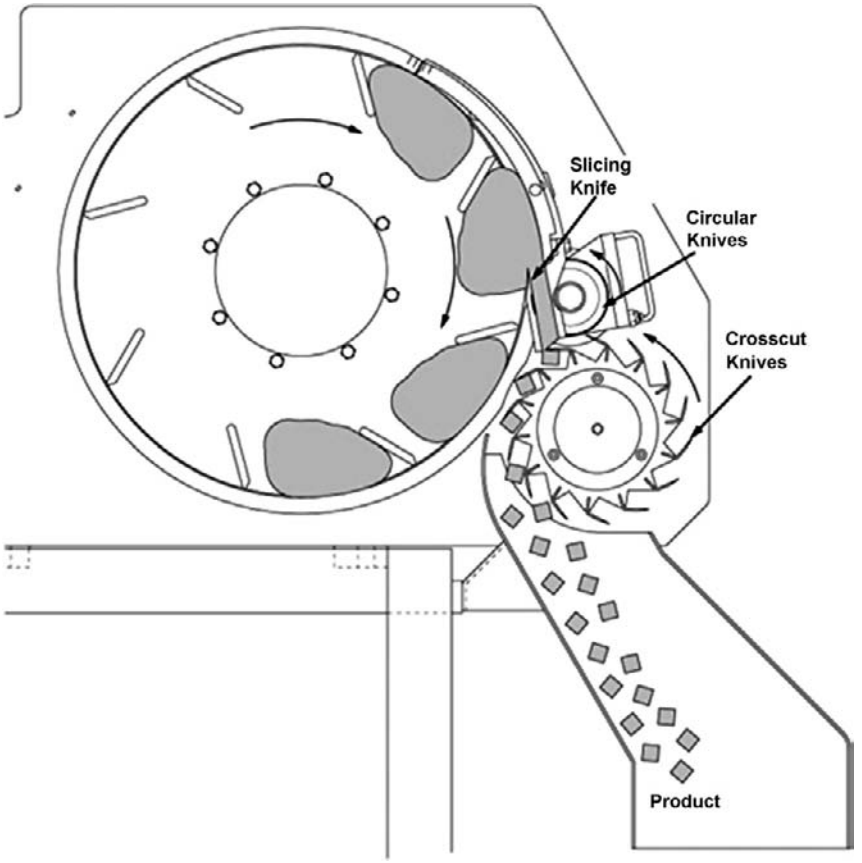

Figure 7.2: Diagram showing the operation schema of the DiversaCut 2110® Dicer by Urschel Laboratories, Inc. This equipment uniformly dices, strip cuts, and slices a wide variety of vegetables. (From Anonymous. How to cut fruits and vegetable products. Urschel laboratories incorporated. Valparaiso, Indiana, USA, with permission.)

using visible spectrum, infrared or X-ray detectors, or TV images, which help to raise output. Nowadays, inspection is faster, more precise and more economical, and only a very small part of the inspection process is manual (Canet and Alvarez, 2006).

7.3.2 Thermal treatments effects

As mentioned above, there are different stages in the production of frozen potatoes during which there may be significant loss of product quality: initial processing and preparation prior to freezing, the freezing step itself, and the frozen storage which follows freezing (Reid, 1990). For example, it is well known that blanching leads to a loss of nutrients and other product quality characteristics such as texture, flavor, and color. Recrystallization and surface drying are accelerated

by temperature fluctuations during frozen storage of vegetable products, causing more mechanical damage when the temperature fluctuation is large and/or the storage temperature is high (Alvarez and Canet, 1998a, 2000b). In addition, the effects of freezing depend on whether the tissue has been blanched. Rigorous blanching accentuates the damage caused by freezing, producing structural changes which are detectable even after cooking (Canet, 1980; Alvarez, 1996). The freezing process itself causes damage to cell structures, but more appropriate methods can be used in order to optimize quality. Alvarez et al. (1997) showed that when potato tissue was cooled at a low temperature (3°C) for a long time (30 min) prior to freezing, the mechanical strength of the tissue increased at the different freezing rates tested. Lasztity et al. (1992) reported that when blanched then frozen, potato tissue was no longer capable of maintaining differences in concentration and pressure in its cells owing to the damage to the cell walls. Since most frozen potato tissues are usually heated before consumption and the mechanical properties resulting from freezing can be considerably altered by the heat treatment applied, further studies to investigate the properties of thawed products after heat treatments (cooking and/or frying) are needed in order to obtain more information for optimization of potato-tissue processing.

All those processes that can compromise final quality during freezing should be considered separately, but it has to be realized that there are significant interactions between them. Understanding and minimizing the effects of each stage in the production of frozen vegetables, particularly blanching and frozen storage, can optimize their quality (Reid, 1990).

For example, low-temperature long-time (LTLT) blanching of potatoes (cv. *Kennebec*), both without further processing and prior to cooking or freezing and cooking, significantly increased firmness retention as measured from compression parameters (Canet et al., 2005c). Figure 7.3 shows the rheological behavior of potato samples subjected to the different blanching treatments without further cooking, cooked samples, and frozen and cooked blanched samples. The engineering stress value of potato tissue blanched at 60°C increased only by about 6% of its original value after 60 min (Figure 7.3a). As expected, when blanched tissue was cooked and frozen-and-cooked, firmness relative to that of the blanched tissue decreased. However, there was a significantly more notable increase in firmness as a function of blanching time at 60°C.

When tissue blanched at 60°C was cooked, engineering stress (Figure 7.3b) increased after 60 min by about 270% of its original value (corresponding to the unblanched cooked control). When tissue blanched at 60°C was frozen and cooked (Figure 7.3c), engineering stress increased after 60 min by about 85% of its original value (corresponding to the unblanched–frozen–cooked control). It certainly appears that a strong 'gel' was formed in the potatoes blanched at 60°C; this was especially apparent after further processing. The difference between blanched-and-cooked samples and blanched-frozen-and-cooked samples can only be ascribed to the freezing process. It is well known that freezing produces an irreversible negative effect on vegetable

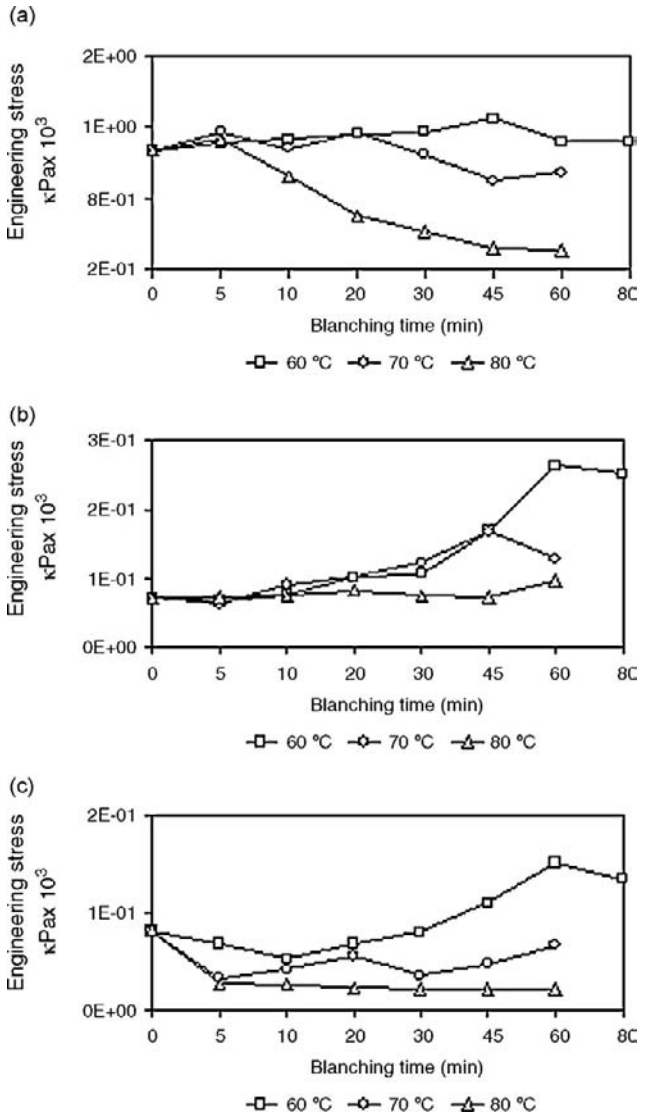

Figure 7.3a–c: Effect of LTB on the engineering stress in potato samples. (a) Engineering stress in blanched potato samples. (b) Engineering stress in blanched-and-cooked potato samples. (c) Engineering stress in blanched-frozen-and-cooked potato samples.

textural quality due to crystallization. Some cell wall materials may be lost upon cooking due to freezing damage, which would result in lower firmness than could be expected in potato tissue that has only been blanched and cooked. This result suggests that it is important to consider all the different stages involved in the frozen potato production process so as to determine the true effect of LTLT on their texture.

7.3.2.1 Blanching and pre-frying

Blanching is a thermal treatment commonly applied in a variety of vegetable preservation treatments and is particularly important in freezing because it impacts quality very strongly (Canet and Alvarez, 2006). The product is heated, typically by brief immersion in water at 85–100°C or by steaming at 100°C. The primary objective is to inactivate enzymes responsible for alterations in sensory quality attributes (off flavors and odors) and in nutritional value (loss of vitamins) during storage. Blanching also affords a series of secondary benefits, in that it destroys vegetative cells of microorganisms present on the surface, thus enhancing the effect of washing. It eliminates any remaining insecticide residues, enhances the color of green vegetables and eliminates off-flavors produced by gases and other volatile substances that may have formed in the time between harvesting and processing.

It is well-known that blanching leads to a loss of nutrients and other product quality characteristics such as texture, flavor, and color. Rigorous blanching accentuates the damage caused by freezing, producing structural changes which are detectable even after cooking (Canet, 1980; Alvarez, 1996). The duration of blanching varies according to the method employed, the variety of potato, the tuber size, and the degree of ripening; however, the chief factor affecting processing time is blanching temperature. Oxidases, peroxidases, catalases, and lipoxygenases are destroyed by the heat of blanching, and blanching effectiveness is usually monitored by measuring peroxidase activity in view of its high heat resistance (Canet, 1989).

Some authors have investigated the possibility of improving the textural quality (in this case firmness) of whole new potatoes, applying low temperature blanching at 60, 65, 70, 75, 80, 90, and 100°C for times up to 1 h (Abu-Ghannam and Crowley, 2006). The activity of pectin methyl esterase (PME) was determined for whole new potatoes with an optimum activity of 2.92 μmol/min/g at 65°C for 15 min. Processing was by immersion in a thermostatically controlled water bath at 90 or 100°C for times up to 25 min and with and without blanching at 65°C or 75°C for 15 min. Firmness was significantly higher ($P < 0.05$) in processed potatoes blanched at 65°C than in those cooked at 95 or 100°C without blanching.

Low-temperature long-time (LTLT) blanching has been used as a thermal pretreatment prior to different processing operations such as freezing, dehydration, canning, sterilizing, and frying. The literature gives different optimum blanching conditions for different potato-processing operations. Brown and Morales (1970) recommended 80°C for 15 min for LTB followed by 95°C for 1 min for blanching of potatoes prior to frying. By blanching the potato strips with different chemicals under varying conditions of temperature and pH, Jaswal (1970) found that the overall texture of the samples improved after blanching for 15 min at 70°C in 0.5% $CaCl_2$ solution; this is because the calcium ions strengthen the protopectin by supplying extra bonds to the pectinic acid chains. French fry texture has also been found to improve when low-temperature blanching was carried out before frying at 200°C for 4 min, indicating stronger intercellular bonds in response to

changes produced in the pectin substances by pectinesterase (PME) (Aguilera, 1997). Frying has also been tried out on fresh and blanched potato strips (cv. *Monalisa*) following a conventional high-temperature short-time (HTST) water treatment (Alvarez et al., 2000). The independent variables in the process were frying temperature (between 170 and 200°C) and frying time (between 4 and 6 min). Frying of fresh strips at 185°C for 4.5 min resulted in minimum optimal points for hardness 1 and hardness 2, Volodkevich parameters and oil content, and saddle points for color parameters in approximately the same combination. Blanching before frying resulted in loss of firmness, evidencing the loss of cell wall integrity through excessive breakdown of protopectin during water treatment followed by frying. Blanching also produced discoloring of strips and increased the amount of oil absorbed by them during frying, thus contributing to the loss of overall sensory quality. The authors posed the need to consider how the complete freezing process can be optimized to take full advantage of enzyme inactivation and/or activation for minimum loss in texture, color modification, and oil retention.

Kaur and Singh (2007) studied the effects of blanching and coating (guar gum 0.5%) on moisture content, oil uptake, and textural properties of fried strips of six potato cultivars. Untreated fried strips contained less moisture, whereas blanched, guar gum-coated strips (GGCS) presented higher moisture retention after frying. Oil uptake was lowest during frying in blanched-GGCS as compared to untreated-and-blanched strips. The blanched-GGCS scored highest for rupture force, core force, and springiness followed by blanched and untreated strips of all varieties.

The potential utilization of sweet potatoes in the production of fries has also been examined by Taiwo et al. (2007), who investigated the influence of various pretreatments (blanching, freezing, osmotic dehydration, and air-drying) on moisture loss, color development, and temperature rise in the product. Pre-treated sweet potato disks were fried in pure canola oil at 170°C for 0.5–5 min. Air-drying, freezing, and osmotic dehydration in 3% NaCl solution accelerated moisture loss during frying. Both the frying time and the pretreatment influenced the moisture content of the core, and final values ranged between 66.2% and 78.7%. Pretreated samples presented higher L, a^*, and b^* values, which became significant with longer frying periods and resulted in improved product color. Air-drying and osmotic dehydration are valid pretreatments in the production of sweet potato fries.

In current frozen French fry manufacturing practice, achieving the target texture in potato strips from the blancher relies on 'to-bite' assessment by line operators on a trial-and-error basis. Liu and Scanlon (2007) recently presented a quantitative description of the texture changes in terms of mechanical properties of the strips as affected by the blanching conditions (temperature and time). Strips from the central planes of each sorted tuber were excised and blanched in a steam-heated kettle at temperatures ranging from 62.8°C to 90.6°C for 2–20 min. The texture of the blanched strips was evaluated using an indentation method. The authors found that at lower temperatures (<74°C), blanching time had little effect on the texture of blanched strips. However,

at higher temperatures (≥74°C) the texture, as expected, softened with increasing temperature and time. A model was developed to describe the change of texture with blanching temperature and time. The model, validated using tubers from the next year's crop, offers guidelines for line operators to manipulate blancher conditions so that they can consistently achieve the target texture of blanched strips.

In response to recent food quality and safety requirements, the SAFES (systematic approach to food engineering systems) methodology has been developed to precisely describe and quantify changes taking place throughout the operations involved in food processing (Barrera et al., 2007). The application of SAFES methodology to French fry manufacture has proven to be a useful tool for describing some textural attributes produced by quantitative changes in water and oil content and in the volume and state of aggregation of the starch. The study also shows the need for additional information in certain areas of knowledge, such as the inactivation rate of the enzymes that catalyze oxidation and the extraction rate of reducing carbohydrates during the blanching step. Several hypotheses have been formulated as regards the amount of water retained by starch granules after the blanching process, and crust formation and development during the deep frying step.

Due to increasing demand from health-conscious consumers, more emphasis has been placed on investigating alternative techniques to replace conventional deep-fat frying in order to produce health-friendly snack products, including potato chips. Low-pressure superheated steam drying (LPSSD) has recently been proven to have potential for producing fat-free potato chips if performed in combination with appropriate pre-drying treatments (Pimpaporn et al., 2007). LPSSD at 90°C with combined blanching and freezing pretreatments has been proposed as the most favorable combination for drying potato chips in terms of drying behavior and dried product quality.

The announcement by Swedish researchers in April 2002 that they had detected acrylamide in many different foods has generated a large number of scientific publications from different parts of the world. The wide spectra of subjects studied includes: the kinetics of acrylamide formation and degradation, the mechanisms proposed for its reduction; the analytical methods and techniques used for its determination; and the experimental results obtained either with model systems or by industrial processing in the case of potato crisps and French fries. Masson et al. (2007) have reviewed the state of the art as regards the occurrence of acrylamide in foods subjected to heat treatments in industrial operations such as frying, baking, toasting, and extrusion, considering the application of potato pretreatments such as washing, blanching, and immersion in acid solutions. Special emphasis is placed on the differences between atmospheric frying and vacuum frying and on the health risks associated with acrylamide intake through common foods. Color formation in pre-dried potato slices during frying and acrylamide formation in the final potato chips have also been addressed (Pedreschi et al., 2007).

A study has recently been published summarizing the current state of knowledge regarding acrylamide formation in foods during thermal processing, with the focus on frying of potatoes (Gökmen and Palazoğlu, 2008). Current knowledge indicates that acrylamide formation is significantly influenced by the composition in terms of concentrations of reducing sugars and asparagines, and by frying conditions in terms of temperature and duration. The authors show how a frying model, which is based on processing variables and kinetic data regarding acrylamide formation and degradation, may potentially save considerable time, money, and effort during the process design and optimization stages. Novozymes has developed Acrylaway®, a commercial asparaginase for food applications that effectively reduces acrylamide in baked and fried food products without influencing product taste or appearance.

7.3.2.2 Stepwise blanching

One of the procedures used to palliate the negative effects of blanching on texture is stepwise blanching. Previous research involving stepwise blanching was conducted to study the effects of temperature and time variables on the pectinesterase activity (PME) and texture modifications in potatoes (Bartolome and Hoff, 1972; Canet et al., 1982b; Alvarez et al., 1999; Alvarez and Canet, 1999b). The literature gives different optimum blanching conditions for different potato-processing operations and for different potato varieties. Brown and Morales (1970) recommended 80°C, 15 min for the first step and 95°C, 1 min for the second for blanching of potatoes prior to frying. Canet et al. (1982b) compared blanching of cylindrical potato specimens at boiling point (97°C, 2 min) with both one-step blanching (80°C, 6 min) and stepwise blanching at 50°C, 60°C, and 70°C, followed by cooling and a second step at boiling point (97°C, 2 min); they concluded that stepwise blanching consisting of low-temperature long-time (LTLT) pretreatment (70°C, 10–15 min) followed by cooling and high-temperature short-time (HTST) blanching (97°C) reduced damage to the tissue structure. This stepwise blanching has produced substantial improvements in final product textures of potatoes cv *Jaerla* (Anonymous, 2004; Canet et al., 1982b), cv *Monalisa* (Alvarez, 1996; Alvarez and Canet, 1999b; Alvarez et al., 1999), and cv. *Kennebec* (Canet et al., 2005c), including after freezing and final preparation (Canet et al., 1982a, 2005c; Canet and Espinosa, 1984; Alvarez and Canet, 1999b; Alvarez et al., 1999).

Several theories have been put forward in the literature reviewed (Andersson et al., 1994) to explain this firming effect in potato: retrogradation of starch; leaching of amylose; stabilization of the middle lamellae and cell walls by activation of the pectin methyl esterase (PME) enzyme. What the enzyme does is to demethylate the carboxymethyl groups of pectic polysaccharide chains. The reduction in the degree of methylation may in turn trigger different processes related to texture and firmness, such as crosslinking by Ca^{2+} ions, increased hydration at the demethylated sites, reduced susceptibility to heat induced β-degradation of pectins and enhanced shielding and repulsion forces by the electric charges within the biopolymer matrix of the cell walls. As such, PME has a long record of confusing effects on observed firmness. Further

experimentation is required to elucidate the role of each mechanism, and especially to determine the main contributor to the process of firming in different species and varieties. As indicated by Canet (1980) and Andersson et al. (1994), most probably no one theory alone can explain the firming effect, and more than one mechanism seems to be involved.

The increase in firmness with respect to that of unblanched potato samples has been found to diminish in the order: blanched at 60°C for 60 min and cooked > blanched at 60°C for 60 min frozen and cooked > blanched at 60°C for 60 min (Canet et al., 2005c). The blanching temperature, which resulted in increased firmness, is also in the region of 60°C for sweet potatoes (Truong et al., 1998). The authors found that in samples blanched at 60°C without further processing, this firming effect intensified with blanching time, especially from 45 to 150 min. Compared to the unblanched cooked sweet potatoes, the steam-cooked sweet potato samples blanched at 60 and 70°C for 45–150 min were respectively about 4.2–13.6 times and 3.8–6.2 times firmer. It has been stated that the effect of de-esterification of pectin by endogenous PME on firmness becomes evident only after heating at high temperatures, as in sterilization and cooking, and there are various results that confirm previous findings. De-esterified pectin is less susceptible to subsequent β-eliminative degradation and is therefore more heat-stable. Post-processing results in greater pectin insolubility, which is generally thought to increase cell–cell adhesion (Stolle-Smits et al., 1998).

It has been stated that the differences in optimum conditions for stepwise blanching proposed by various researchers are due to the levels of PME enzyme activity occurring in the different potato varieties used, and that this is affected by the maturity of the potato and the season. Canet et al. (2005c) kept potato tubers (cv. *Kennebec*) in refrigerated storage, and firmness and PME activity were periodically sampled over a period of 80 days. The PME activity of potato tissues blanched at 60, 70, and 80°C for varying periods of time was measured (Figure 7.4a).

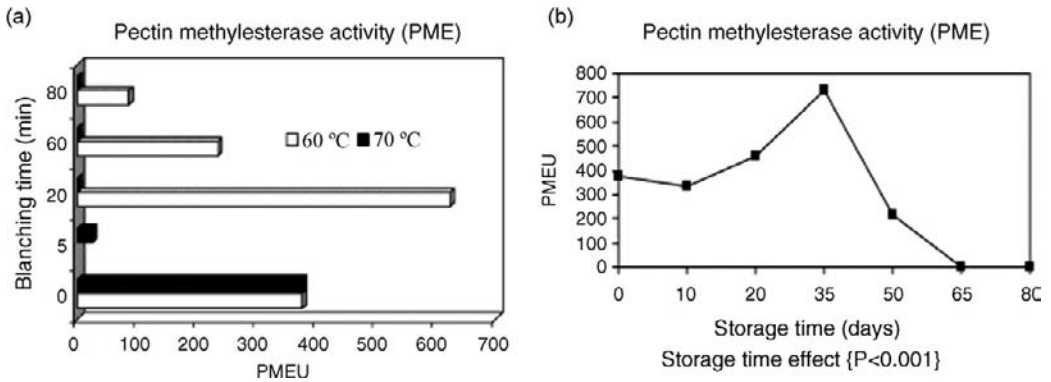

Figure 7.4a,b: PME activity. (a) Effect of LTB on PME activity. (b) Effect of storage time on PME activity.

PME activity increased by about 65% in the fresh tubers after 20 min at 60°C, but after 60 min it declined to about 40% of its original value. At 60°C, PME in potato tissue (cv. *Kennebec*) appears to have a relatively short inactivation time, or possibly its optimum activation temperature is <60°C. In this experiment also, PME activity declined to less than 10% of its original value after 5 min at 70°C, and no activity at all was detected when the time at this temperature was prolonged. Similarly, no PME activity was detected in samples blanched at 80°C. In potato tubers (cvs. *Nicola* and *Irene*), Van Dijk et al. (2002) found that the PME activity tended to remain constant during preheating at 60°C for 60 min.

The effect of storage time on the PME activity of the fresh tissue is shown in Figure 7.4b. PME activity was about 95% greater than its original activity (at 0 days) after 35 days in storage, but after 50 days it had declined to about 42% of the original activity. Moreover, after 65 and 80 days in storage, no PME activity was detected in the fresh potato tissues. As Tijskens et al. (1997) pointed out, the relationship between measured PME activity and observed firmness is inherently complex. Certainly, the total exerted effect of a measured level of PME activity can never be established on the basis of a single time measurement. Substantial compositional changes have been observed in the pectic polymers of fresh potatoes during storage (Van Dijk et al., 2002). It seems reasonable to assume that the increase in PME activity after 35 days would cause changes in the pectic polymers, leading to a firmer texture.

7.3.2.3 Freezing, freezing/thawing cycles and thawing/cooking

The freezing process as such consists of lowering the product temperature to −18°C at the thermal center, resulting in crystallization of most of the water and some solutes. Ice crystallization occurs only after a degree of supercooling – i.e. reduction of the temperature to between −5°C and −9°C in a matter of seconds. In the freezing stage, most of the water in the product undergoes a phase change to ice; this change is not complete until the final temperature at the thermal center is at least as low as the storage temperature.

The duration of the freezing process depends on the freezing rate (°C/h). This is defined by the International Institute of Refrigeration (2006) as the difference between initial temperature and final temperature divided by freezing time, freezing time being defined as the time elapsing from the start of the pre-freezing stage until the final temperature has been attained. This will be affected by product size (particularly thickness) and shape, as well as by the parameters of the heat transfer process and the temperature of the cooling medium.

The beneficial effect of rapid freezing rates on structure and texture has been reflected in the results of texture analysis by various methods (histological, sensory, imitative, and objective) in studies of potatoes (Canet, 1980; Canet et al., 1982a; Alvarez and Canet, 1995; Alvarez 1996). Histological examinations of the beneficial effect of rapid freezing rates have shown that blanching and cooking have adverse effects in that they mask the different structural alterations

caused by rapid or slow freezing (Manzini et al., 1965). In contrast, other studies using objective methods of texture analysis have detected beneficial effects of rapid freezing rates and stepwise blanching on final texture of potatoes, even after cooking (Canet, 1980; Canet et al., 1982a; Alvarez, 1996).

The aspect of freezing that really produces irreversible negative effects on textural quality is crystallization (Canet, 1989); therefore, the freezing rate is particularly important in the phase of maximum ice crystal formation. Freezing rates can now be modulated in the various processing stages using programmed freezing. This is a possibility that the food industry ought to consider seriously as it would make it possible to optimize quality while achieving lower costs than those associated with conventional freezing.

Although in theory the temperature fluctuations to which vegetables are subjected rarely go as far as total thawing and subsequent re-freezing, the effect of such fluctuations at different rates can usefully be examined in order to assess the mechanical damage inflicted on tissue structure and the cumulative effect.

Alvarez et al. (1997) studied the effect of the freezing rate and programmed freezing on the rheological parameters and tissue structure of potato (cv. *Monalisa*). In particular they analyzed the effect on potato tissues of three different freezing rates (0.5, 1.25, and 2°C min^{-1} down to −8°C), thawing up to +20°C at the same rates, and one, two, three, or four successive freeze/thaw cycles. The effect of the freezing rate on the zone of maximum crystallization was also examined, along with different combinations of programmed freezing, and the effect of prior cooling was assessed. The alteration of rheological behavior of slow-thawed potato tissues was minimized by pre-cooling (3°C for 30 min), slow cooling (0.5°C min^{-1}) before and after the maximum ice crystallization phase, and quick freezing (2°C min^{-1}). This is thought to occur because pre-cooling cuts down the time lapse between freezing at the surface and freezing at the product's thermal center, so that freezing-induced expansion takes place before too rigid a crust forms on the surface. The structure is thus better able to withstand the internal stresses and the result is a product with higher mechanical strength. Another study was conducted on the effect of different freezing rates (0.5, 1.25, and 2°C min^{-1}) and pre-cooling (3°C for 30 min) on the mechanical strength of potato tissues (cv. *Monalisa*) at temperatures ranging from −3 to −18°C (Alvarez and Canet, 1997). SEM examination of the tissues showed differing degrees of mechanical damage to tissue structure and a linear increase in the tissue's mechanical strength caused by pre-cooling. These effects are best studied using the shear test.

Figure 7.5 shows examples of the time course of the potato specimen center and the air temperatures during one, two, and four freeze/thaw cycles performed at a high freezing rate (2°C min^{-1}) and a low thawing rate (0.5°C min^{-1}). The structural rigidity of samples subjected to four freeze/thaw cycles was less than half that of samples subjected to only one cycle (Alvarez and

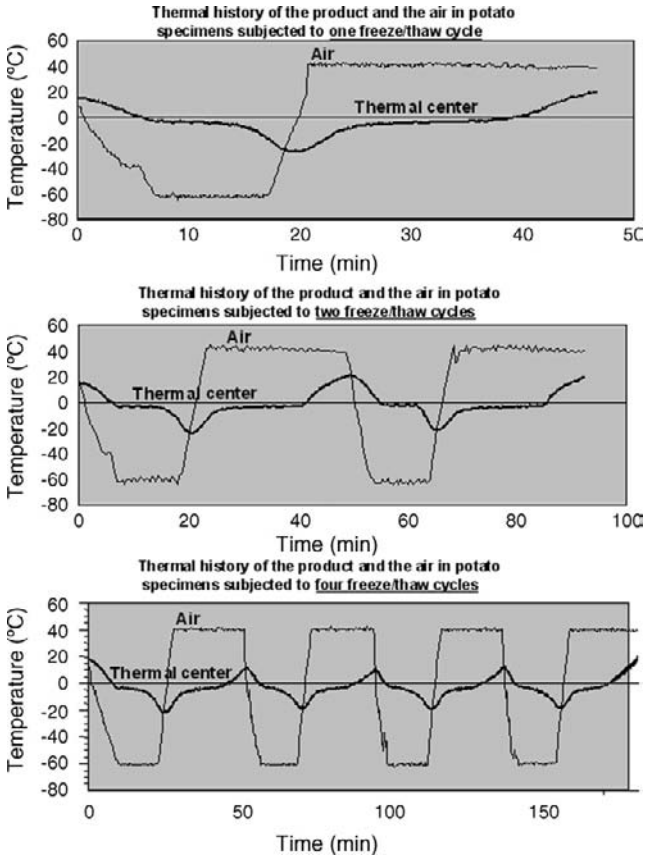

Figure 7.5: Thermal history of the product and of the air in potato specimens subjected to one, two, and four freeze/thaw cycles.

Canet, 1995). It was found that, after two or three cycles, softness ceased to be much affected by subsequent cycles (Figure 7.6). The authors concluded the following: (1) that pre-cooling and a high freezing rate during the phase of maximum ice crystal formation has a positive effect on potato texture and tissue structure, (2) that slow thawing has a positive effect; and (3) that it is essential not to subject potatoes to more than one freeze/thaw cycle.

The texture of edible potato tissue is conferred by the cell wall and its middle lamella and is affected by changes in cell adhesion and pectin degradation (Shomer et al., 1993). On the other hand the texture of cell suspensions is affected by factors such as intercellular friction, density, cell wall elasticity, surface properties, intracellular solutes, protein coagulation, and starch gelatinization (Pitt, 1992). According to Faulks and Griffiths (1983), the primary factors controlling the texture of mashed potatoes are the solids content and the amount of free starch. Although inside each cell the starch remains encapsulated by the cell wall, the rheology of

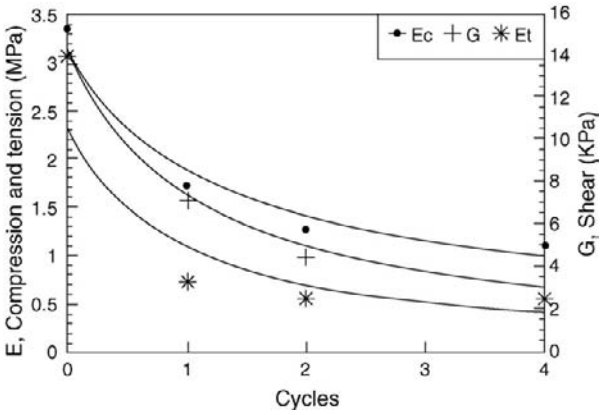

Figure 7.6: Models fitted for the effect of the number of freeze/thaw cycles on the apparent moduli of elasticity in the compression (E_c) and tensile tests (E_t), and on the modulus of rigidity (G_s) in the shear test.

mashed potatoes and the elasticity, plasticity, and friction of potato cells are affected by the ability of the swollen starch to swell the cells and by starch leakage from the cells (Shomer et al., 1993; Shomer, 1995).

Alvarez and Canet (1999a) froze mashed potatoes made from dehydrated potato flakes in order to determine the changes that occurred in the cell structure. Breakdown of the cell wall by freezing reduced the oscillatory rheological properties, and it was found that the frozen/thawed mashed potatoes consisted of dilute dispersions of swollen and disrupted intracellular starch granules. In addition, the cells became less elastic, reducing the friction between them. The viscoelastic behavior of starch pastes is likely to be related to the volume fraction, number, and size distribution of the swollen particles they contain, and such factors increase with increasing flake concentration. Wong and Lelievre (1981) found that decreases in dynamic viscosity and rigidity were associated with a reduction in the swelling capacities of wheat starch pastes. In mashed potato, Shomer et al. (1993) attributed the nature of the rheological parameters to swelling relationships between the cell wall and the starch by considering the viscosity of the extracellular solution.

During freezing, the cell walls are ruptured by the crystallization of water, and a redistribution of amylose and amylopectin in the swollen starch granules by growth and dissolution of ice crystals leads to local differences in amylose and amylopectin concentrations. The ice crystals impose a shear stress on swollen starch granules, thus contracting and disrupting them. The locally concentrated amylopectin is prone to molecular aggregation, causing shrinkage of the swollen starch granules. From a structural point of view, the different frequency behavior observed may be attributed to the fact that after thawing the samples are composed of larger swollen starch

aggregates. In order to explain the results, it is necessary to describe the changes in the granular and molecular structure of starch in mashed potatoes after freezing.

Freezing of mashed potatoes involves initial processing, freezing, frozen storage, and thawing. Freezing of foods can have a detrimental effect on their sensory and water-holding properties as a result of physical disruption of cells or cell components (Jul, 1984) or of changes in the structure of certain macromolecules (Suzuki et al., 1994). Technological solutions adopted to minimize the effects of freezing include the use of rapid freezing methods. The effect of freezing conditions on the quality of freeze-chilled reconstituted mashed potato has been examined by O'Leary et al. (2000) and Redmond et al. (2002). Freeze-chilling led to a significantly darker, firmer product (higher probe penetration force) and increased centrifugal drip loss compared with chilled product. The length of time in frozen storage had no effect on drip loss, firmness/adhesiveness, vitamin C content, total viable count, or the sensory score in freeze-chilled and frozen mashed potato from three potato cultivars (Redmond et al., 2003).

The structure and quality of commercial mash made from flakes is also detrimentally affected by freezing (Alvarez et al., 2005; Canet et al., 2005b), and therefore the authors do not recommend either freezing or frozen storage of this type of mashed potato. However, the effects of freezing temperature (−80, −40, or −24°C) and thawing mode (microwave or overnight at 4°C) on quality parameters of mashed potatoes made from tubers (cv. *Kennebec*) were also examined in the cited study. Mashed potatoes were tested for texture profile analysis (TPA) and cone penetration, oscillatory and steady rheometry, color, dry matter, Brix, and sensory attributes. In natural mashed potatoes, TPA hardness and oscillatory parameters showed that processing resulted in a softer product than the fresh control. The parameters were lower in the samples thawed at 4°C than in those thawed by microwaving at all the freezing temperatures used, which may be ascribed to gelatinization of the starch released from damaged cells. It was concluded that natural mash made from *Kennebec* potatoes should be frozen quickly and thawed by microwave in the conditions described to obtain a product more like freshly made mashed potato. Another strategy to minimize damage from freezing and thawing is to incorporate compounds that interact with water and offer protection against the deleterious effects of thawing in particular, for example cryoprotectants (Sych et al., 1990; Downey, 2003). Exactly how these substances work is as yet not clearly defined, although they have been reported to slow down the rate of ice crystal growth and alteration of crystal shapes (Bolliger et al., 2000). Hydrocolloids and proteins, the two kinds of biopolymers used in food by technologists to control structure, texture, and stability (Dickinson, 1998), both possess cryoprotectant properties (Downey, 2003). In addition, hydrocolloids are known specifically for their water-holding characteristics and are used in starch-based products to influence the gelatinization and rheological properties of starches.

It is generally accepted that each hydrocolloid affects the pasting and rheological properties of starch-based systems, like mashed potato, in a different way (Chaisawang and Suphantharika,

2006). There are many possible factors involved in this, the most important being the molecular structure of the hydrocolloids and/or the ionic charges of both starches and hydrocolloids (Shi and BeMiller, 2002; Chaisawang and Suphantharika, 2005). Starch is also widely used in the food industry as a thickening, stabilizing, and gelling agent (Morikawa and Nishinari, 2000; Mandala et al., 2004), but uses of native starch are limited since pastes present problems including retrogradation, syneresis, and slow resistance to shear treatment (Korus et al., 2004). To improve the physical and chemical properties of these pastes, starches have been chemically modified, for example by acid hydrolysis, oxidation, etherification, and crosslinking; in this connection, the authors have investigated the influence of the addition of modified cornstarch on the quality of frozen/thawed mashed potatoes (Alvarez et al., 2007a). The authors examined the effects of modified cornstarch concentration, freezing rate, and thawing mode on quality properties of mashed potatoes. Oscillatory parameters showed that increasing starch concentration resulted in a softer product in which gel strength decreased in direct proportion to concentration in quick-frozen and microwave-thawed product. Quick freezing made for firmer mashed potatoes, whereas oscillatory, ITPA, and penetration parameters were lower in the samples thawed at 4°C.

Downey (2002) studied the effect of addition of hydrocolloids (xanthan gum, guar gum, pectin, carrageenan) and dairy proteins (sodium caseinate, whey protein concentrate) on centrifugal drip loss and maximum resistance to penetration force in frozen and thawed, cooked puréed vegetables (potatoes [cv. *Rooster*], carrots and turnips). The author showed that depending on the vegetable, quality after thawing may be maintained or improved through selection of an appropriate cryoprotectant. The textural and sensory properties of this group of products were strongly influenced by the type and concentration of cryoprotectant and their crossed interactions. However, mixtures of cryoprotectants may offer specific advantages with regard to alterations in the physical properties of food ingredients. These can arise from either synergistic interactions or phase separation phenomena (Doublier, 1997). Downey (2003) also studied the effects of cryoprotectant mixtures on physical properties of frozen and thawed puréed cooked potatoes (cv. *Rooster*). Ingredients were incorporated as mixtures of: (1) guar, pectin, and whey protein concentrate; and (2) xanthan, carrageenan, and sodium caseinate. Experimental design techniques were used to design the experiments and evaluate the results. Both ingredient mixtures significantly reduced maximum resistance to penetration and centrifugal drip loss. While modeling of resistance to penetration was quite successful, drip loss proved more difficult, mainly due to the skewed distribution of the values of this response variable for both ingredient mixtures. The experimental approach that was designed has potential for tailoring these physical properties to predetermined levels in order to meet specific consumer expectations in a range of food products. However, in both studies, the same author noted that the cryoprotectants were added to the finished final products at ambient temperature, so that it is improbable firstly that the right solution and mix of cryoprotectants would be achieved in the purées, and secondly that the temperature required for the added ingredients to exhibit their potential thickening and/or

stabilizing properties would be reached. At the same time, in both experiments the author thawed the product at ambient temperature, so that again the suspicion arises that the purées were not exposed to the temperatures necessary, for example to enable those polysaccharides possessing gel-forming properties to exhibit that ability, and with it their parallel effect on sensory quality, and in particular on the texture of these vegetables.

It is also the case that consumers at home normally use microwave appliances to thaw and heat frozen foods. In view of the importance of temperature history for product functional and sensory properties, it is clearly essential to obtain information on the effects of such thermal treatment (Downey, 2003). Cryoprotectants such as hydrocolloids (amidated low-methoxyl (ALM) and high methoxyl (HM) pectins), kappa- and iota-carrageenans (k-C and i-C), xanthan gum (XG)) and dairy proteins [whey protein (WP), sodium caseinate (SC)] have been added to mashed potatoes to investigate ways of improving the effects of freezing and thawing (Alvarez et al., 2007b; Fernández et al., 2007). In these studies, thawing was carried out using a domestic microwave oven. Alvarez et al. (2007b) found that each hydrocolloid and protein, depending on concentration, affected the mechanical properties [instrumental textural profile analysis (ITPA), cone penetration (CP) test], the total color difference (ΔE^*) with respect to fresh control (FC) and the sensory attributes of fresh (F) and frozen/thawed (F/T) mashed potatoes in a different way. In the F/T samples, adding 5 and 8 g kg^{-1} ALM, 3, 5, and 8 g kg^{-1} k-C, 1.5, 3, 5, and 8 g kg^{-1} i-C and 1.5, 5, and 15 g kg^{-1} WP significantly increased ITPA consistency. Also, adding 2.5 and 5 g kg^{-1} XG significantly enhanced ITPA consistency of the F/T product. In both F and F/T samples, k-C produced the highest ITPA consistency and also a high CP average force; this indicated a stronger synergistic effect in k-C/denatured milk protein systems, although the excessive thickening and stickiness it conferred was judged undesirable by the panelists. Adding 8 g kg^{-1} HM pectin had a disruptive effect on the mashed potatoes and reduced both ITPA consistency and CP average force. In all cases, freezing and thawing reinforced the gel structure of the products as compared to F and FC counterparts. The ΔE^* values were higher in F samples containing ALM and HM pectins. Dairy proteins affected the taste and odor of the mashed potatoes, which were judged unacceptable in the sensory analysis. Samples containing 0.5 and 1.5 g kg^{-1} added XG were preferred organoleptically for the creamy mouthfeel they produced. Taking the data overall, the results show that amidated ALM pectin, k- and i-C and XG are suitable for improving the mechanical properties of mashed potatoes, although the amounts of carrageenans that can be used are limited by their effects on the stickiness of the product. In the given experimental conditions, HM pectin, WP, and SC were not suitable for use in mashed potatoes.

Fernández et al. (2007) have characterized the rheological behavior of the mashed potatoes with added biopolymers using steady shear measurements. Fresh and frozen/thawed mashed potatoes present shear thinning with yield stress (Canet et al., 2005a), and dynamic shear data reveal weak gel-like behavior in potato purées (Alvarez et al., 2004). The effects are strongly

dependent on the type and level of the biopolymer added. Adding higher levels of ALM and HM pectins (3–8 g kg^{-1}) enhanced the rheological properties of frozen/thawed mashed potatoes as compared to their fresh counterparts, suggesting that they could be useful as texturizers. Fresh and frozen/thawed samples with added k-C and i-C were more structured. Freezing and thawing did not significantly affect the steady data of the product with added carrageenans and xanthan, highlighting the potential of these gums to stabilize the texture of frozen and microwave-thawed mashed potatoes as compared with fresh counterparts.

Frozen purées made from mixtures of vegetables (containing potato) are a relatively new kind of high-quality product with a good potential market in Europe. Starch-based systems have been reported to display synergistic properties in the presence of other polysaccharides or of proteins (Doublier, 1997). In the manufacture of these frozen vegetable purées, potato is added before freezing. These products may therefore be considered starchy foods, and as such may present quality problems such as syneresis and organoleptic and textural changes, especially if they are subjected to freeze–thaw cycles. These problems have been ascribed in hydroxypropyl potato starch pastes to phase separation caused by retrogradation of the starch (Eliasson and Kim, 1992; Kim and Eliasson, 1993; Kim et al., 1993).

Alvarez and Canet (2001c) analyzed the influence of freeze–thawing cycles (up to six) on the viscoelastic properties of three commercial frozen vegetables purées made from mixtures of potato with other vegetables (broccoli/potato, carrot/potato and celery/potato). The purpose of the study was to ascertain the changes occurring in dynamic properties of frozen purées during heating (20–90°C) and cooling to 20°C. The effect on the rheological properties of freeze–thaw cycles prior to cooking was analyzed. In all three purées the onset temperature of complex modulus (G*) increase upon heating seemed to be related to the onset temperature of gelatinization of the starch grains. The most substantial effects on viscoelastic properties were observed on cooling. The increase in G* and the decrease in the phase angle (δ) of the purées on cooling appear to be due to either water loss or retrogradation of the starch grains. Repeated cycles led to concentration of dissolved starch components and changes in the cell and tissue structure of the vegetable components, although the effect of cycling on rheological behavior depends on the measurement temperature.

When the number of freeze–thaw cycles was increased, δ peaks were reached in shorter times (∼1000 s at 80°C), possibly indicating slower relaxation processes in these systems with successive freezing and thawing. Similar behavior has been reported for amorphous amylopectin in this dynamic property (Kalichevsky et al., 1992). Upon cooling, irrespective of the number of freeze–thaw cycles, structural rearrangements can involve local aggregation phenomena that lead to an increased degree of phase separation and/or a more coarsely aggregated system, which is accompanied by an increase in the phase angle as the gel becomes more non-homogenous (Hermansson, 1997). Clearly, the effect of the successive freeze–thaw cycles on rheological

behavior of the purées is dependent on either the measurement temperature or the type of product, so that the effect conferred by the cycles at room temperature is altered if the product is subjected to heating and/or cooling. At room temperature, the increase of G* values was linear with the number of freeze–thaw cycles for broccoli and carrot purée, indicating that successive cycles progressively lead to the formation of a coarsely aggregated structure.

A key factor to consider when using starch in food or industrial applications is that the starch must be properly cooked at an adequate concentration, because the rheological properties of starch pastes are highly dependent on the concentration and cooking conditions (Kim et al., 1993). Whenever frozen vegetable purées have to be thawed (i.e. heated) before consumption, their physical properties may be considerably altered by the final thawing methods and conditions. Few studies have compared the effects of cooking and/or thawing methods and conditions on rheological properties of either solid vegetable tissues or semisolid products.

Alvarez and Canet (2001b) also examined the combined effect of the number of successive freeze–thaw cycles and different final thawing conditions on rheological properties in the same frozen vegetable purées. The number of freeze–thaw cycles ranged from zero (that is, thawing only) to four and were applied to three types of commercial product containing potato (broccoli–potato, carrot–potato, and celery–potato). After freeze–thaw cycles, purées were thawed at room temperature by microwaving at three different settings, and in a saucepan. In fact, rheological properties were affected more by the thawing conditions than by the number of cycles applied. Saucepan thawing raised the values of these properties, apparently as a result of considerable water loss during heating. After one or two cycles (depending on thawing conditions), broccoli–potato presented much greater elasticity and apparent freeze–thaw stability than the others, and celery–potato purée presented the highest fluidity. Loss modulus values in the latter were possibly more significantly affected by thawing conditions because of the higher initial water content. The results showed a complex dependent relationship between the dynamic properties on the one hand and structural factors of the purées and processing parameters on the other. The behavior of G' in response to these effects was different in the celery–potato purée samples. In this product, with the exception of the samples thawed without heating (i.e. at room temperature), there was a consistently significant reduction of G' values after even one freeze–thaw cycle; the G' values then increased with successive cycles, so that after four cycles the values of G' in this product were slightly lower than in the uncycled samples only when thawing was carried out in the microwave at the two slowest rates. Also Hegedusic et al. (1993) reported that the most significant changes in the phase transition temperatures (freezing and thawing) of apple purée-like systems occurred after the first freezing, while after the second and the third cycles these changes were minor. In celery–potato purée the occurrence of changes in water-binding properties of the particles seemed to be most intense during the first recrystallization. Also, native potato starch has been found to turn into sponge-like material after only one freeze–thaw cycle (Kim and Eliasson, 1993).

The frozen vegetable purees studied present the characteristic rheological behavior of gel-like products. The elasticity of these starchy foods clearly decreased after four successive freeze–thaw cycles, and this decrease was most pronounced when final thawing was performed by heating in a saucepan. Broccoli–potato purée presented greater gel strength and improved stability to freeze–thaw treatments, which may be attributed partially to a higher starch concentration from two different sources (broccoli and potato). The final thawing plays a significant role in determining the dynamic properties of the products. Given the complexity and the differences in the rheological behavior of these products, more researchers should be directed to study the relationships between physical, chemical, structural, and rheological changes in such frozen purées caused by freezing, freeze–thaw, and thawing treatments, and also to determine how far the rheology and structure of the potato starch influence their rheological behavior.

7.3.2.4 Storage and temperature oscillation

Frozen vegetable tissue is unstable during storage and the quality deteriorates to a variable extent depending on the type of product and the storage temperature (Canet, 1989). Loss of quality is caused solely by physical and chemical alterations taking place within the product itself, as a result of recrystallization and sublimation of water. Jul (1984) reported that the effect of recrystallization during storage and distribution of frozen products cancels out the beneficial effects of fast freezing. The number, size, shape, and orientation of the ice crystals change during storage, resulting in successive melting on the surface of smaller crystals and recrystallization on larger ones. Long periods of frozen storage are not necessarily harmful if a constant low temperature is maintained. For example, Canet (1980) reported that a number of mechanical properties of blanched and frozen potatoes were unaffected by storage at -24°C.

Alvarez and Canet (1998a, 2000a, b) have also studied the effect of temperature fluctuations during frozen storage on the quality of potato tissue (cv. *Monalisa*). The authors examined the effect of different ranges of temperature fluctuation (-24 to -18°C, -18 to -12°C, -12 to -6°C, -24 to -12°C and -18 to -6°C) on the compression, shear and tension parameters of packaged and unpackaged frozen potato tissue. The initial temperature, duration, and number (0, 2, 4, 8, 16, 24, and 32) of fluctuations were varied. Figure 7.7 shows the thermal history of the product and fluctuations in the air temperature in two series performed between -18 and -6°C on unpackaged potatoes. The highest parameter values occurred in samples subjected to fluctuations between -24°C and -18°C, and the lowest values in the range -18 to -6°C. The mechanical strength of the frozen tissue decreased as the number of fluctuations increased and in most cases was lower in the packaged samples. At -12 to -6°C and -18 to -6°C, the tissues attained temperatures very close to the zone of maximum ice crystal formation. Fluctuations of up to -6°C accelerated the melting of small ice crystals, thus increasing the amount of available water; this re-froze immediately, causing an increase in the size but a decrease in the

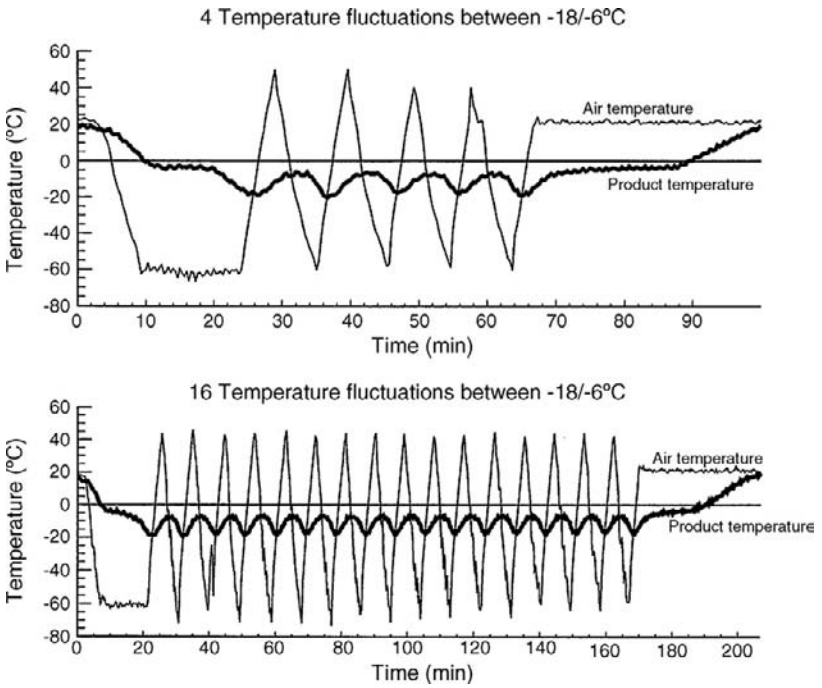

Figure 7.7: Thermal history of the product and of air temperature in unpackaged samples subjected to 4 and 16 fluctuations in the range −18 to −6 °C.

number of ice crystals. The high degree of structural deterioration resulting from temperature fluctuations in packaged samples was due to sublimation of ice on the sample surface, causing greater drying of the tissue. Moreover, the damage caused by recrystallization and sublimation was cumulative.

On the other hand, plots of log (rheological parameters and moisture content) versus number of temperature fluctuations in the ranges −24 to −18°C and −18 to −6°C showed two distinct regions; the first was a rectilinear plot with a steep negative slope up to four fluctuations. The second was also a rectilinear plot with a shallow negative slope beyond four fluctuations (Alvarez and Canet, 2000a). Figure 7.8 shows the softening curves obtained for unpackaged and pre-packaged samples subjected to temperature fluctuations in both ranges by plotting log shear force. These two-stage softening rate curves were consistent with the biphasic model and qualitatively similar to those for thermal softening of the vegetables. The authors found that two substrates, S_a and S_b, may be involved in lending firmness to potato tissue in freezing and frozen storage conditions. By analogy with earlier works (Huang and Bourne, 1983; Bourne, 1987, 1995), the term 'frozen storage firmness' was proposed to describe the amount of firmness that is resistant to degradation by freezing with temperature fluctuations during frozen storage and final thawing of the product.

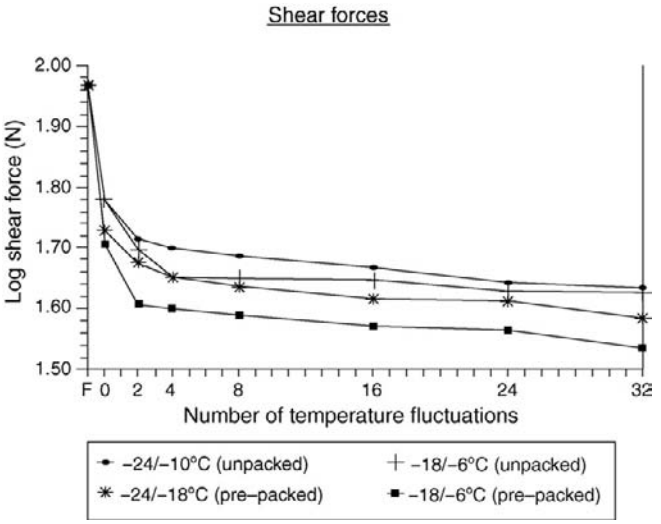

Figure 7.8: Shear force (log scale) versus number of temperature fluctuations applied in the ranges −24 to −18 °C and −18 to −6 °C for unpackaged and pre-packaged frozen potato tissue.

Also, principal component analysis (PCA) clearly separated samples subjected to −18/−6°C from those subjected to −24/−18°C (Alvarez and Canet, 2000b). Frozen samples undergoing up to four fluctuations formed a separate cluster from those undergoing a higher number. Analysis also clearly separated unpackaged from pre-packaged samples in response to slower freezing rates reached in the latter.

It is a fact of practical interest that after four fluctuations, an increase in the number of fluctuations during simulated frozen storage caused little further deterioration of potato firmness. These findings provide new insights into the operating parameters of storage of frozen potato and post processing.

7.4 Softening kinetics

Optimal thermal process design relies on relevant and accurate kinetic data for quality evolution (Van Loey et al., 1995). The different thermal processes involved in the production of frozen potatoes affect overall textural quality in different ways. Tissue softening occurs at different rates and is governed by different physicochemical mechanisms. It is therefore necessary to establish softening kinetics and derive kinetic parameters relating to the softening that takes place in the tissue in each of the stages comprising the full production process. As a result of technological advances, the various operations entailed in the process of potato freezing nowadays take place in different media and/or under different processing conditions. Tissue softening induced by thermal treatments in different media depends on the temperature reached at the thermal center

of the product in the heating medium, and on the heating rate attained. These two parameters determine the shape of the tissue softening curves and hence the associated kinetic parameters.

It has been found that applying the theories of chemical kinetics to the rate of thermal softening of vegetable tissue could provide useful insights into softening mechanisms, and could point the way to developing technologies that produce firmer-textured processed products, even though the progress of the reaction is measured by a physical test (firmness) rather than a chemical test (Huang and Bourne, 1983; Bourne, 1987, 1989).

In most studies quantifying loss of firmness, the thermal softening of vegetable tissues is described by one or two first-order kinetic rate processes (Bourne, 1987, 1989; Kozempel; 1988; Rahardjo and Sastry, 1993). Different authors have used different mechanical tests and generally only one rheological parameter as an indicator of product texture. Huang and Bourne (1983), Bourne (1987) and Kozempel (1988) used a back-extrusion test cell, taking maximum force readings as a texture measurement. Harada et al. (1985a, b) and Harada and Paulus (1986) used maximum shear force to characterize the behavior of potatoes and three other low-starch tubers during cooking. Verlinden et al. (1995) used a uniaxial compression test in which the rupture force was taken as a measure of texture. By estimating kinetic parameters from different rheological parameters, it is possible to establish what rheological parameters are best suited to predict tissue softening and the relationships between the apparent rate constants of the rheological parameters if different treatment methods are used.

Only a few kinetic studies have been accompanied by histological analyses and chemical and biochemical studies with which to ascertain the true role of the main structural components in thermal softening. Changes in texture that occur during processing are the result of changes in the chemistry of cell wall and middle lamella hydrophilic polymer material that affect the physical properties (Van Buren, 1979; Canet, 1980; Bourne, 1989). However, in potato tissue, where starch is the major component of the dry matter, it can be assumed that the phenomena associated with gelatinization are involved in the texture changes that occur during cooking (Pravisani et al., 1985; Verlinden et al., 1995).

Alvarez et al. (2001) determined the kinetic parameters for characterization of the softening of potato tissue (cv. *Monalisa*) by heating in water. They used compression, shear, tension, and stress–relaxation rheological parameters to represent tissue firmness and to determine what structural components and changes in such components could be contributing to potato tissue firmness during water heating. Kinetics of thermal softening of potato tissue have also been studied in relation to different heating and cooking methods (Alvarez and Canet, 2001a, 2002), providing a complete characterization of potato tissue (cv. *Monalisa*) softening with the various different methods used, and ultimately enabling a comparison of the estimated kinetic parameters in terms of factors related to tissue softening in each one.

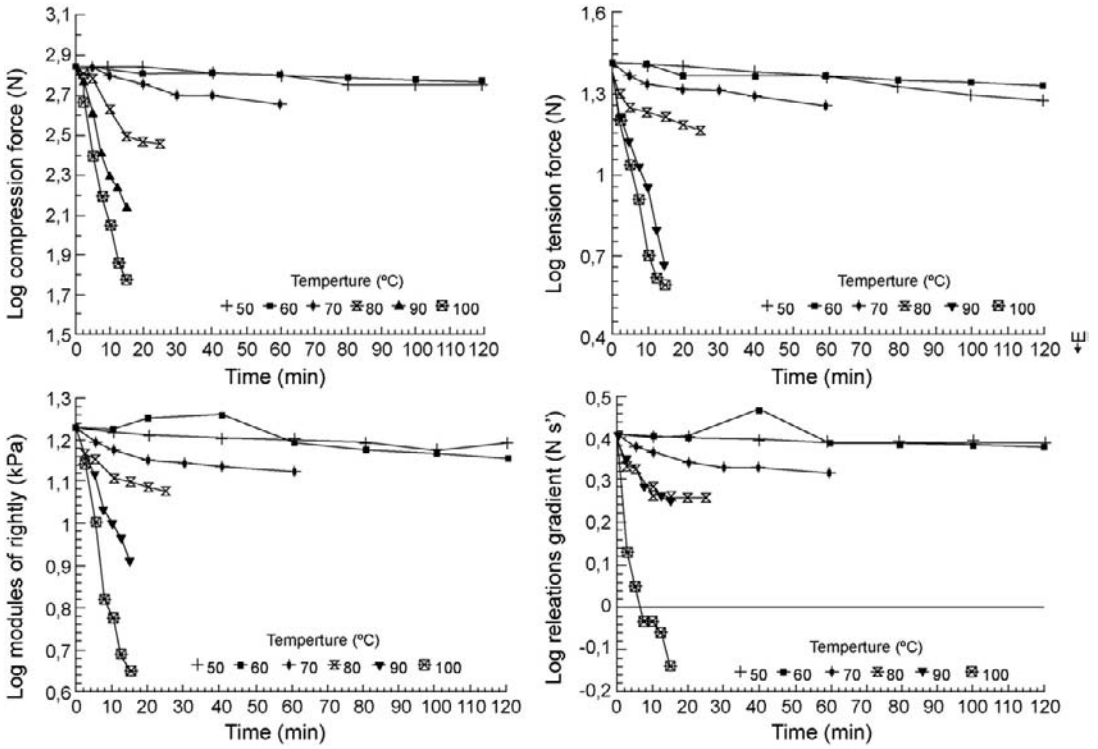

Figure 7.9: Softening curves for rheological parameters in water heating.

The rate of thermal softening of potato tissue by water treatment at 50, 90, and 100°C was consistent with one pseudo first-order kinetic mechanism, while at 70 and 80°C the rate of softening was consistent with two simultaneous pseudo first-order kinetic mechanisms. Kinetic theory was successfully used to detect an increase in firmness caused by heating at 60°C, mainly between 20 and 40 min, presumably by pectin methyl esterase (PME) activation (Alvarez et al., 2001). Figure 7.9 shows the softening curves, obtained for a rheological parameter derived from each of the mechanical tests performed by plotting log Fc (maximum compression force), log Gs (modulus of rigidity), log Ft (maximum tension force), and log Sr (relaxation gradient) vs. process time in the range of temperature studied. A comparison of the plots indicated that two substrates Sa and Sb may be involved in lending firmness to potato tissue at 70 and 80°C. At these temperatures, mechanism 1 is more probably due to gelatinization and light cooking, whereas mechanism 2 is more likely to represent the changes in the pectic substances in the cell wall and interlamellar region. At 90 and 100°C the gelatinization process was fast and therefore the simple mechanism that was fitted presumably reflects the degree of solubilization of the pectic substances. At 50 and 60°C there was practically no gelatinization, so that the simple mechanism that was fitted presumably represented incipient solubilization of pectic material.

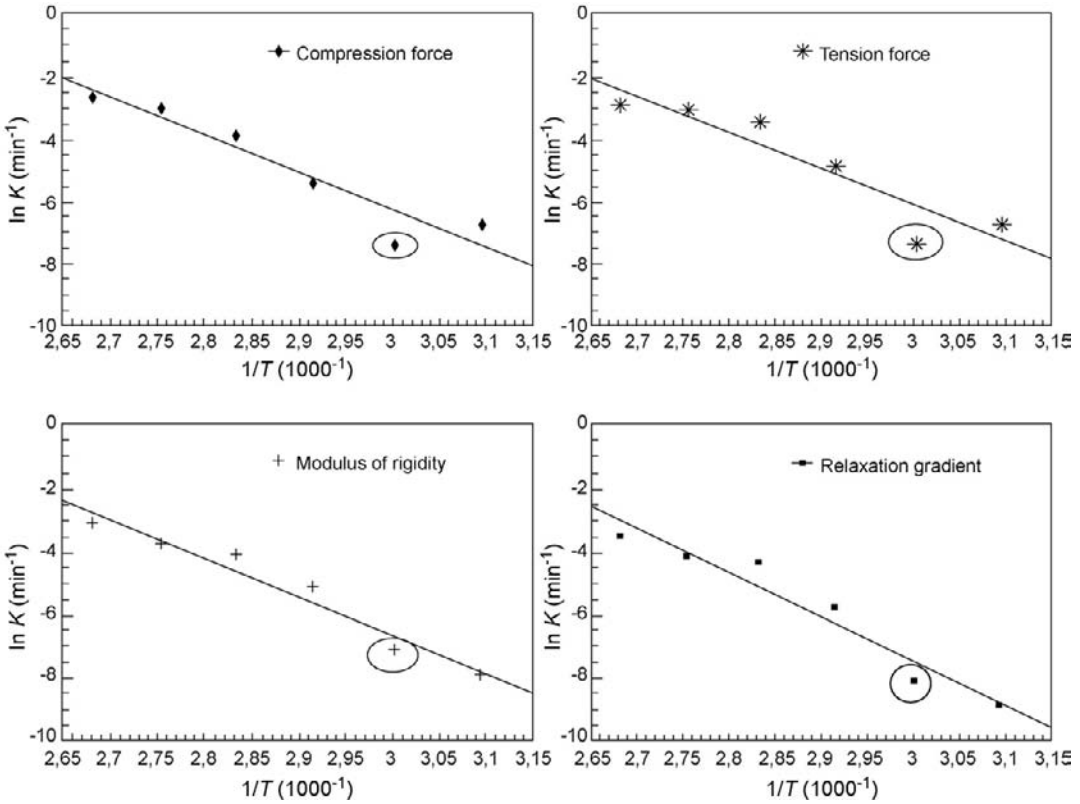

Figure 7.10: Arrhenius plots of ln apparent rate constants vs. reciprocal absolute temperature for potato tissue treated in water.

Figure 7.10 shows typical Arrhenius plots for rheological parameters from each mechanical test. The rate constants of the first mechanisms at 60°C ($3{\cdot}10^{-3}\,K^{-1}$) were the least linear; this effect is mainly apparent in plots of maximum compression and tension forces (in plots, rate constants at 60°C are circled). This could be another consequence and evidence of the fact that in the experimental potato variety the optimum temperature for activation of the PME enzyme is close to 60°C.

These results were consistent with the findings of other studies. Optimum conditions for stepwise blanching of frozen/thawed potato tissues (cv. *Monalisa*) were within temperature and time ranges of 60–65°C and 25–35 min using different rheological parameters to detect the firming effect of PME enzyme (Alvarez and Canet, 1999b). Stationary points for the first step of stepwise blanching with maximum PME activity exhibited critical temperature and time values at 64.39°C and 28.02 min after freezing and steaming of potato tissue (Alvarez et al., 1999). PME activity retention was 50% of fresh tissue activity in water heating at 60°C for 60 min (Bartolome and

Hoff, 1972). Also, Moledina et al. (1981) reported 83% retained PME activity in water blanching at 60°C for 30 min and none at 75°C for 10 min. The highest PME activity was found in potato samples (cv. *Kennebec*) blanched in water at 55°C for 80 min; however, the levels of activity at 60°C for 60 min and in the samples blanched at 55°C for 40 min were also very high (Canet et al., 2005c).

The cooking degree depends on the internal temperature reached at the thermal center, the heating method and the heating rate attained in the treatment (Collison et al., 1980). Only a few studies compare the effects of blanching techniques and cooking methods on the rate of thermal softening of vegetable tissue. In this connection, another study was carried out to compare the effect of three different heating methods (Alvarez and Canet, 2001a) on the rate of softening of potato tissue (cv. *Monalisa*). On the basis of chemical kinetics, the kinetics of thermal softening of potato tissue heated by steaming, steaming and hot air, and microwave exposure were evaluated using the rheological properties from four objective methods as firmness indicators. In the three heat treatments, the rate of thermal softening of tissue could be described by two simultaneous first-order kinetic mechanisms. A comparison of kinetic parameters showed that steaming produced a greater degree of softening than the other two heating methods used. The firmness ratios for shear force showed that approximately 16% of the firmness of fresh potato is retained after steaming treatments as compared to 46% and 36%, respectively, for steaming and hot air, and microwave. With these heating methods, gelatinization contributes less than cell wall structure to potato tissue softening as determined either by kinetic parameters or by microscopic observations. Bourne (1989) proposed that the amount of firmness that is resistant to heat degradation (F_{02}), called 'thermal firmness,' be used rather than rate constants as a guideline to study the effects of processing on the firmness of vegetables. The ratio of texture to the original texture (F_{02}/F_{01}) has also been proposed as a measure for the amount or degree of cooking in potatoes. Kozempel (1988) showed that the rate of change of this dimensionless quantity is not affected by the potato variety or batch. Comparison of the firmness ratios of the rheological properties showed that the highest percentage of total firmness retained in the tissue was given by the unrelaxed force F_{unr}, indicating that this property is less sensitive in detecting tissue softening; on the other hand firmness ratios for shear rheological properties were all very low and also very similar, so that this assay may be considered the most suitable for characterizing steaming-induced softening of potato. For their part, Harada et al. (1985a) found that the shear force measurement was suitable for describing changes in firmness of three potato varieties treated in water at 90, 100, and 110°C.

Color, as a quality attribute of cooked and fried potatoes, is affected by the extent and nature of the heat during thermal processing. As well as by studies dealing with establishment of the kinetics of softening in potato, the improvement of color parameters has been made possible by increasing knowledge of the kinetics of color change. Analysis of kinetic data allows processors to minimize undesirable changes and optimize color retention. The kinetics of color change during cooking

and frying of potatoes have been evaluated by Nourian and Ramaswamy (2003a). Potatoes were cut into cylinders and cooked in a temperature-controlled water bath at 80–100°C or fried in a commercial fryer at 160–190°C for selected times. Color changes associated with cooked and fried potatoes were evaluated using a tristimulus colorimeter in the *L, a, b* mode. In the case of cooked potatoes, *L* and *b* values decreased while ΔE and *a* values increased with time at each cooking temperature. In fried potatoes, *L* decreased while *a*, *b* and ΔE increased as frying time increased. A modified first-order model was used to characterize color change kinetics of both cooked and fried potatoes based on changes occurring between the initial and a maximum or minimum value. Temperature sensitivity of rate constants was adequately described by the Arrhenius and z-value models. In addition, test samples were subjected to a compression test and three textural properties (hardness, stiffness, and firmness) were derived from the resulting force–deformation curves (Nourian and Ramaswamy, 2003b). Texture parameters of cooked potatoes declined as cooking time progressed, and the rate of texture change associated with each temperature was found to be consistent with two pseudo first-order kinetic mechanisms, one more rapid than the other. Textural values of fried potatoes were found to increase with frying time and also followed a first-order kinetic model. Temperature sensitivity of rate constants was also adequately described by Arrhenius and z-value models.

A kinetic model based on two irreversible serial chemical reactions was recently proposed to fit experimental data of texture changes during thermal processing of potato products (Moyano et al., 2007). The model links dimensionless maximum force with processing time. Experimental texture changes were recorded during frying of French fries and potato chips at different temperatures, and the literature data for blanching/cooking of potato cubes were taken into consideration. In the case of blanching/cooking, the proposed model gave root mean square values (RMSs) in the range of 1.2 to 17.6%, much better than the 6.2 to 44.0% obtained with the traditional first-order kinetics. The model is likewise able to predict the transition from softening to hardening of the tissue during frying.

Cooking kinetics of potato tubers determined by vibration techniques have been reported by Blahovec et al. (2007). Non-destructive determinations of characteristic tuber resonant frequencies (modes M-1 and M-2) displayed in the amplitude–frequency plots (AFP) before, during, and after steam cooking of the whole tubers were used to establish cooking kinetic coefficients for four different potato varieties. The results suggested that this method is suitable only for smaller tubers; the sloughing and cracking of larger tubers induced by longer cooking times confound the measurements.

7.5 Texture optimization

Therefore, because of the different variables involved in the freezing process and the above-mentioned interactions, a statistical technique which takes this into account should be used to determine optimum freezing conditions. One such technique particularly suited to this

application is response surface methodology (RSM), which combines the methods of planned and efficient experimental designs with least squares modeling to identify optimum conditions for the process response. RSM is a popular and effective method for solving multivariate problems and optimizing several responses in many types of experimentation (Lah et al., 1980; Montgomery, 1991; Garrote et al., 1994; Myers and Montgomery, 1995), because it can simultaneously consider several factors at many different levels and corresponding interactions among these factors on the basis of a small number of observations.

7.5.1 Frozen potatoes

Garrote et al. (1993) optimized processing conditions for chemical peeling of potatoes by response surface methodology. RSM was used to determine the effects of NaOH concentration (4–20%), process temperature (55–95°C), and time (1–7 min) on the yield, peeling quality, unpeeled skin, and total usage of NaOH. Also evaluated were titratable NaOH in the potato tissue, NaOH penetration and 'heat ring' depths during one-stage chemical peeling of potatoes (cv. *Huincul*). The best peeling quality, maximum yield and minimum total usage of NaOH was obtained for the following ranges: concentration 11–13%, time 5–5.70 min, and temperature 90–95°C. The maximum temperature for which the 'heat ring' and NaOH penetration depths were equal was 72 °C, at that temperature and with 20% NaOH and 7 min, peeling quality was very good and there was no 'heat ring.'

It is obvious that the optimization of vegetable blanching alone requires a 'just sufficient' heat treatment to inactivate enzymes responsible for deleterious changes during freezing and frozen storage (Reid, 1990; Alvarez and Canet, 1999b). Once a tighter protocol is devised for blanching, other aspects such as the application of programmed freezing to suit the characteristics of each product may be investigated in order to reduce tension build-up in the product during this process.

For optimization of stepwise blanching of frozen–thawed potato tissues (cv. *Monalisa*), Alvarez and Canet (1999b) used RSM to compare the effect of temperature and time of the first step of blanching (LTLT) on compression, shear, tension, and stress–relaxation parameters of frozen–thawed potato tissues. The authors found that the optimum temperature range for all mechanical properties was 60–65°C and the optimum time range 25–35 min. In order to optimize stepwise blanching with respect to all the dependent variables considered, the contour plots of apparent modulus of elasticity in compression (Ec), maximum tension force (Ft) and first-cycle relaxed force (Fr1) were overlaid (Figure 7.11). These mechanical properties were chosen to narrow down the optimum zone by means of the significance of the second-order models fitted for these parameters. E_c and F_r set the limits on the optimum zone because of their importance for the study, and F_t could be optimized within the band demarcated by the other two parameters. The shaded area defines the optimum ranges of blanching temperature (60–65°C) and time (25–35 min). The relaxed force in the first cycle derived from stress–relaxation param-

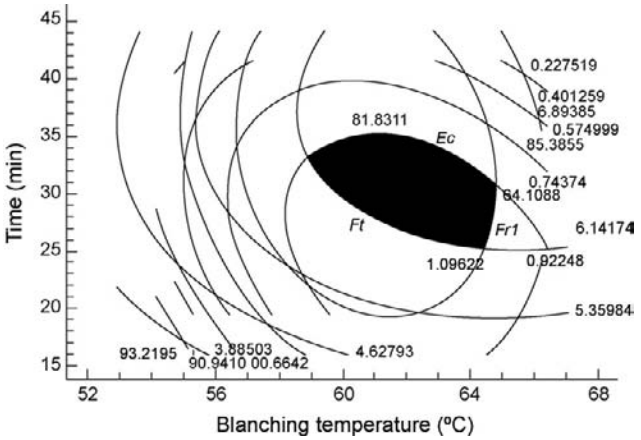

Figure 7.11: Overlay of contour plots for apparent modulus of elasticity in compression (E_c), maximum tension force (F_t) and first-cycle relaxed force (F_{r1}). The shaded area represents optimum conditions.

eters was the most appropriate parameter for detecting the firming effect presumably exerted by PME on frozen potato tissues as a consequence of stepwise blanching with the first step (LTLT) at 65°C and 30 min.

RSM was also used to determine optimum freezing conditions for pectin methyl esterase (PME) activity, rheological parameters, and textural properties in potato tissues (cv. *Monalisa*) (Alvarez et al., 1999). In the frozen potato production process the second step in stepwise blanching prior to freezing was treated as a fixed factor and performed at 97°C for 2 min; and likewise, the freezing rate in the freezing step, which was carried out at -2°C min^{-1}. The effects of variation in levels of temperature (57.93–72.07°C) and time (15.86–44.14 min) in the first blanching step on PME activity were studied using a central composite rotatable design. Then, a Box-Behnken factorial design was used to investigate the effects on rheological parameters and textural properties of simultaneous variation of temperature (60–70°C) and time (20–40 min) in the first blanching step, and of steaming temperature (112–122°C) and time (1–3 min). Blanching temperature was the independent variable that most influenced either enzymatic activity or rheological parameters. PME activity was expressed as specific activity (units of PE activity per mg protein) and was determined at two different stages: after cooling following the first blanching step (PME_1) and after freezing of the previously thawed samples (PME_2). Enzymatic activity after sample freezing (PME_2) was less than determined after the first blanching step (PME_1). The highest levels of activity before and after freezing were detected in the samples treated at 65°C for 30min. Comparison of activities in the control samples (97°C, 2 min) before and after freezing showed that the freezing process caused the loss of almost 55% PME activity, the rest of the loss being caused by the second blanching step. Figure 7.12 shows the retained

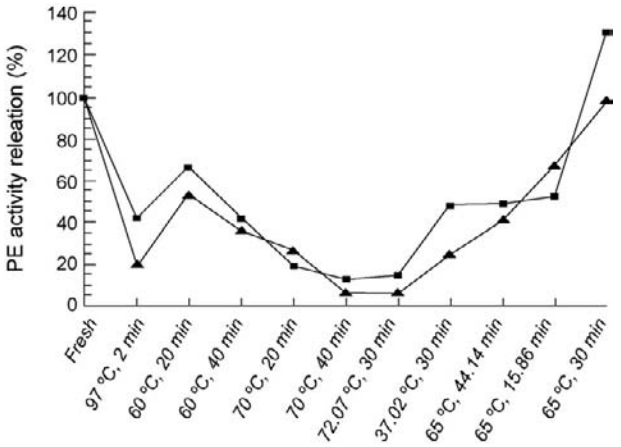

Figure 7.12: PME activity retention (%) in relation to fresh tissue activity. Squares (■) represent percentages calculated with PME activity values determined after the first blanching step (PME_1). Triangles (▲) represent percentages calculated with PME activity values determined after freezing (PME_2).

enzymatic activity with respect to the value of activity determined in the fresh potato tissue, which was 15.33 units of activity per mg protein. In the control samples subjected to conventional blanching (this treatment involved only the second blanching step at 97°C for 2 min), the values of PME_1 and PME_2 were 6.41 and 2.96 units of activity per mg protein respectively. As Figure 7.12 shows, the correlations among PME_1 and PME_2 activity values were very high (0.96), indicating that the effect of the first blanching step on enzymatic activity is detected after freezing and thus confirming previous findings (Canet, 1980; Canet et al., 1982b). Activity after treatment at 65°C, 30 min was 30% higher than in fresh tissue and was very close to the latter after freezing. These results are comparable with the findings of Bartolome and Hoff (1972), who reported 50% retained activity at 60°C, 60 min and total inactivation of the enzyme at 70°C, 60 min. Moledina et al. (1981) reported 83% retained PME activity at 60°C, 30 min and none at 75°C, 10 min.

Stationary points showing maximum PME activity (Alvarez et al., 1999) had critical temperature and time values of 64.22°C and 29.37 min before freezing and 64.39°C and 28.02 min after freezing and steaming of the tissues (Figure 7.13), and these were very close to the values obtained for some mechanical and textural properties (Figure 7.14), confirming analogous earlier studies (Alvarez and Canet, 1999b). For example, stationary points showing maximum apparent modulus of elasticity in compression (Figure 7.14a) also had critical values which were within the region delimited by the ranges studied (62.04°C, 30.57 min) and were close to the critical values of PME activities (Figure 7.13). This rheological parameter has been identified as the mechanical response of cell turgor pressure in potato tissues (Canet, 1980) and may be suitable

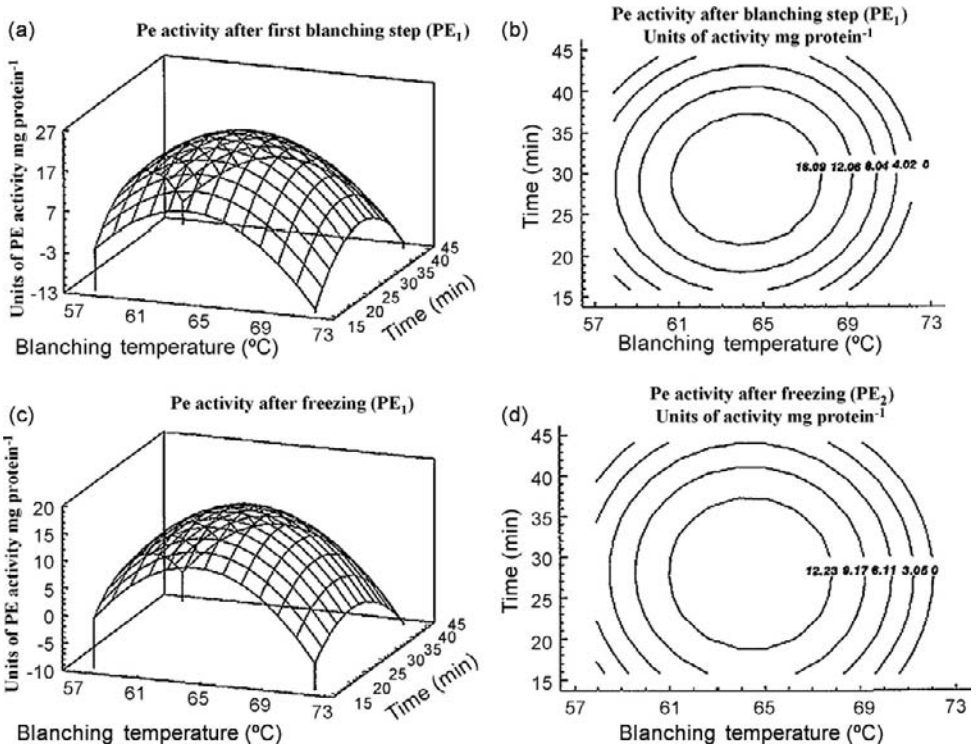

Figure 7.13: PME activity response surfaces and contour plots as functions of blanching temperature and time.

for detecting an increase in cohesion forces between adjacent cells caused by the enzymatic activity. Results showed a high correlation between increases in PME activity and potato tissue firmness under optimum experimental freezing conditions, demonstrating that the enzyme is one of the main contributors to that firmness which determines the textural quality of frozen potato tissues (cv. *Monalisa*).

The optimization of LTLT blanching prior to freezing and cooking was also studied using RSM, to find out how the complete process affected the texture of the potatoes (Canet et al., 2005c). A central composite rotatable design was used to study the effects of variation in levels of LTLT temperature (52.93–67.07°C) and time (31.72–88.28 min) on compression parameters and PME activity. The highest compression values were recorded at the replicated center points (60°C, 60 min) and in blanched samples at the axial point (60°C, 88.28 min), whereas the highest PME activity was found in samples blanched at 55°C for 80 min; however, the levels of activity at the replicated center points (60°C, 60 min) and in the samples blanched at 55°C for 40 min were also very high. Stationary points showing maximum mechanical resistance had critical temperatures and times in the ranges (58–60°C and 66–75 min) used for each independent variable, whereas a

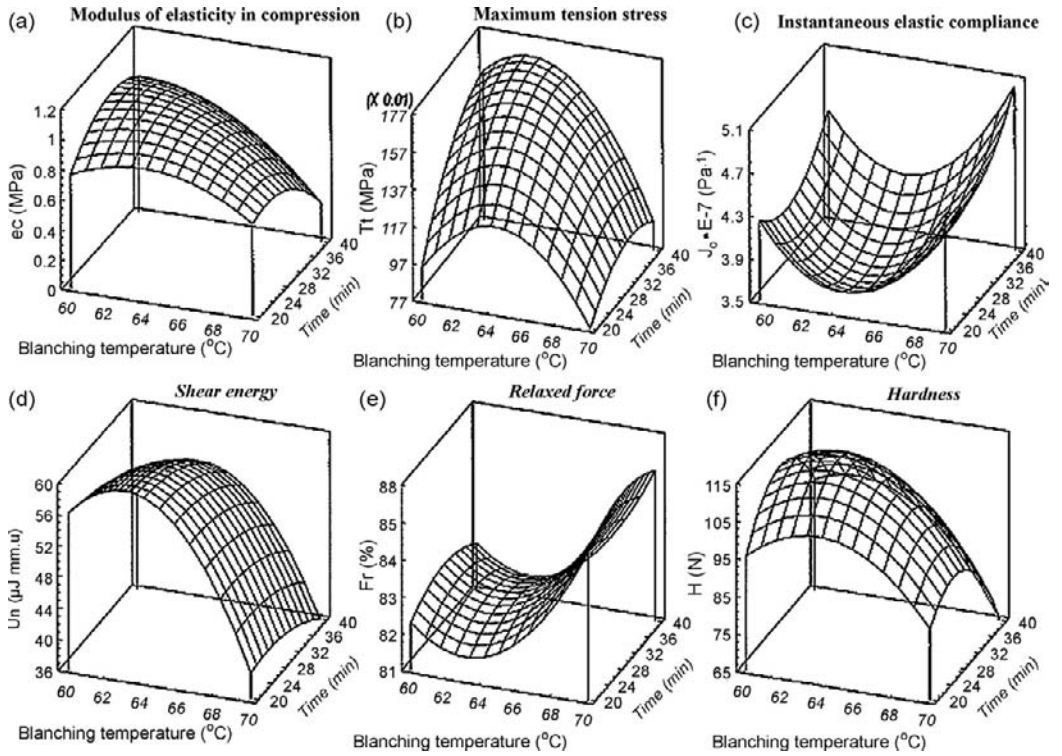

Figure 7.14a–f: Mechanical and textural property response surfaces as functions of blanching temperature and time. Steaming level: temperature 117 °C, time 2 min.

stationary point for PME activity was not calculated because of the low percentage of explained variability of the model. However, from the shape of the plots it seems reasonable to assume the existence of a relationship between the increase in PME activity and firmer texture (Figure 7.15), suggesting that the changes in the composition of the cell wall caused by the PME activity contribute to the firmness of frozen cooked potatoes (cv. *Kennebec*).

Severini et al. (2004a) used RSM to investigate variables affecting the firmness of blanched potato slices such as type of blanching, time of treatment, NaCl or $CaCl_2$ concentrations, and lactic acid concentration. The results showed that the mathematical models provided a good estimate of the effects of individual and interactive factors on the retention of firmness in processed potato. The best results were obtained with MW blanching of slices dipped in a solution containing calcium chloride. RSM was used to investigate the way in which variables such as treatment time, sodium or calcium chloride concentrations, and lactic acid concentration affect microwave blanching of potato slices dipped in these solutions (Severini et al., 2004b). Two three-factor five-level second-order central composite designs were developed to analyze the target variables. Results showed that blanching in calcium chloride–lactic acid

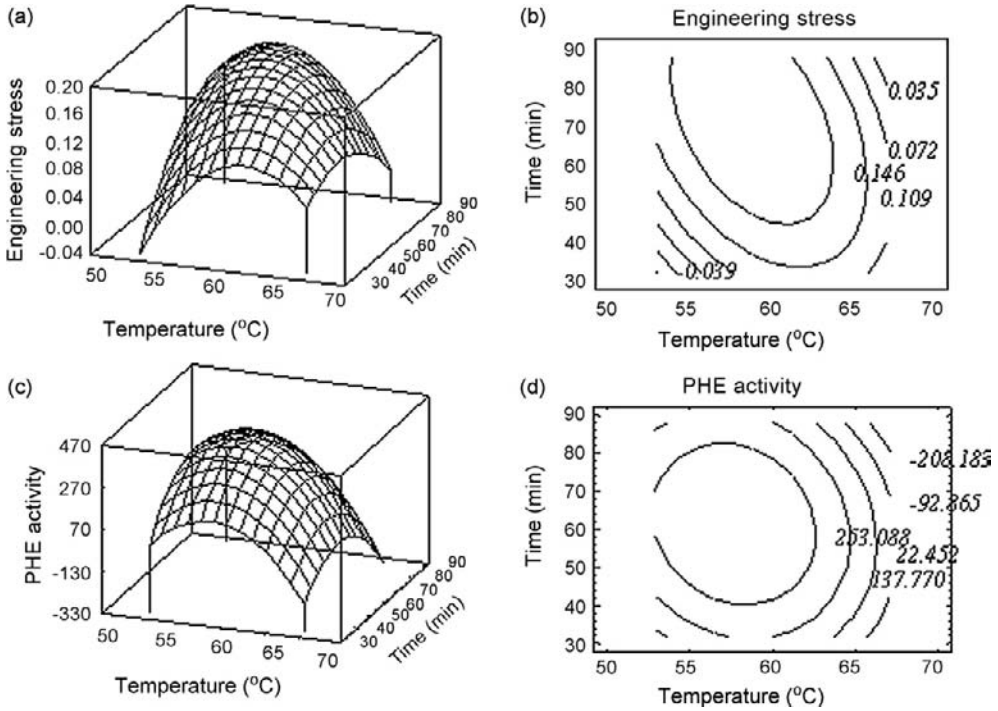

Figure 7.15a–d: Engineering stress and PME activity response surfaces and contour plots as functions of blanching temperature and time. (a) Engineering stress response surface. (b) Engineering stress contour plot. (c) PME activity response surface. (d) PME activity contour plot.

solution was more effective than blanching in sodium chloride–lactic acid solution with regard to polyphenoloxidase inactivation. Color measurements showed that, in the given operational conditions, the best results were achieved by short blanching in sodium chloride–lactic acid solution at a high lactic acid concentration and a low NaCl concentration and by short blanching in calcium chloride–lactic acid at a low lactic acid concentration or a low calcium chloride concentration.

7.5.2 Frozen mashed potatoes

Pretreatments can have a considerable effect, particularly on the texture of the final potato product. The Add-Back process used in the manufacture of dehydrated instant mashed potato granules includes an essential pre-preparation stage consisting of precooking the potato at 70°C for 20 min and cooling in water prior to steam cooking (Moledina et al., 1978). This serves to render the cell wall less degradable by cooking (Bartolome and Hoff, 1972), thereby enabling the potato cells to withstand the forces generated by compression, mixing and rubbing during the continuous mash-mixing stage. Another technique, a low-temperature blanching (LTB) process,

has been reported by numerous authors, offering a promising approach to increasing firmness retention in processed potato.

Fernández et al. (2006) studied the effect of low-temperature long-time (LTLT) blanching prior to cooking on color, textural, firmness and oscillatory parameters, sensory attributes and overall acceptability of either fresh or frozen/thawed mashed potatoes using RSM to establish the optimum temperature and time for blanching in both types of mashed potatoes. A central composite rotatable design was used to determine the effects of variation in levels of blanching temperature (57.93–72.07°C) and time (15.86–44.14 min) on quality parameters. LTLT prior to cooking produced a lighter-colored fresh mashed potato (higher L*/b* ratio) than cooking alone; this was to be expected given that LTLT causes sizeable losses of color (Pala, 1983). In both fresh and frozen/thawed-mashed potatoes, blanching at 65°C for 30 min had the effect of thickening the mashed potatoes, which could be the result of PME enzyme activation rendering the cell binding less degradable. Scores for overall acceptability were higher in the frozen/thawed blanched mashed potatoes than in their fresh counterparts, highlighting the potential of blanching to improve the quality of mashed potatoes that are subjected to freezing and thawing. Stationary points for instrumental parameters showing maximum thickening had critical temperatures (approximately 67–69°C) and times (approximately 26–30 min) within the ranges used for each independent variable in both fresh and frozen/thawed mashed potatoes. The results showed a very high correlation between structural reinforcement and overall acceptability in optimum experimental blanching conditions. For fresh and frozen/thawed mashed potatoes the panelists scored the samples blanched at 65°C, 30 min significantly lower for overall acceptability than the unblanched controls, possibly because of excessive thickening. Therefore, it would be a quality gain and a desirable goal to make mashed potatoes which scored as well as fresh control for overall acceptability after freezing and thawing and even after a long time in frozen storage. This approach (LTLT prior to cooking) has potential for the design of mashed potatoes with a specified color and texture depending on the subsequent treatment.

7.6 Conclusions and Outlook for the Future

Recent trends in consumer preferences have stimulated the development of novel concept-driven technologies that provide the required processing through non- or mildly thermal means (Welti-Chanes et al., 2005). Accordingly, much of the recent scientific research for the food industry has focused on non-thermal processing techniques, of which high-pressure processing (HPP) is one of the few that are considered to have real potential in commercial settings (Sun, 2005). High-pressure processing is a technology that potentially addresses many, if not all, of the most recent challenges faced by the food industry. It can facilitate the preparation of food products that have the quality of fresh foods but the convenience and profitability associated with prolonged shelf life (McClements et al., 2001). Many aspects associated with the use of

high pressure as a processing method in the food industry have been reviewed by Norton and Sun (2008).

Eshtiaghi et al. (1994) dried high-pressure pretreated potato cubes in a fluidized bed and compared them to untreated, pressure-treated or water-blanched dried samples. Drying rates varied with pretreatments. Freezing produced the highest drying rates. Pressure-treated and water-blanched samples retained highly acceptable colors. Freezing or hot-water blanching or high-pressure pretreatment, followed by freezing, gave good rehydration. High-pressure treatment resulted in incomplete rehydration, but when combined with freezing, the water uptake was between 2.1 and 4.8 mL/g. Retention of cell wall structures of frozen samples during drying was presumed to be responsible for more efficient mass transfer. Texture measurements revealed significant effects of pretreatments. Pressure-treated samples had the nearest texture to that of the raw material. No major differences in color were observed.

Luscher et al. (2005) published studies of pressure-supported freezing and subzero cooling. They calculated the true compressive stress and strain in treated potatoes from force–deformation curves, and their results showed that freezing to ice III enhanced the texture of the potato. They also showed that although pressure-shift freezing (PSF) preserved the skeletal cell structure of the tissue, it promoted cell permeability. Volume changes during freeze–thaw cycles at a pressure of 200 MPa were found to have a detrimental effect on membrane permeability. During subzero cooling, membrane permeability was also adversely influenced by phase transition within the membrane itself, which gave rise to cell lysis.

Demonstration of the existence of liquid and solid metastable phases within the phase diagram of water and water-containing food products has opened up a new range of possibilities in pressure-shift freezing (PSF) and pressure-induced thawing (PIT) processes for potatoes (Urrutia-Benet et al., 2007). A reduction of processing time was obtained in a metastable region (for pressures above 200 MPa and temperatures below -20°C) thanks to the incrementing of temperature gradients (in food material, before and after pressure release; in the case of PSF, and between sample and heating medium for PIT). The enzymatic activity of polyphenoloxidase (PPO) was chosen to evaluate the effectiveness of PSF and PIT processes for food preservation, since it is dependent on the cell disruption caused by ice nucleation during freezing, and the crystal growth that occurs during storing and thawing. The results of laboratory and pilot-scale experiments showed that the activity of PPO was no greater after freezing and thawing processes when pressure was applied, and was even slightly reduced in the metastable region. Additionally, pilot-scale evaluations of key quality-related parameters (color, drip loss, texture, and microstructure) showed better responses for PSF and PIT than for atmospheric freezing and thawing. However, using relative fail stress and strain values, the authors showed that supercooling in the metastable zone (i.e. around 12°C below the triple point) produced no additional benefits as regards texture or drip loss in the samples when compared to traditional PSF – i.e. 200 MPa and 20°C, followed

by PIT treatment at 200 MPa. In fact, with this traditional pressure–temperature combination, which supports the formation of ice I, overall sample texture was better than in samples which underwent PSF-PIT treatments with pressure–temperature combinations of 240 MPa/−28°C, 280 MPa/−20°C, and 280 MPa/−28°C. Other than better color retention and slight enzyme inactivation, the only obvious benefit presented by supercooling in the metastable zone was that the swelling of cells in the potato tissue was reduced thanks to the development of smaller ice crystals.

Also, freezing time increased when freezing to the metastable zone of the phase diagram; the increase in applied pressure only promotes efficiency of PIT, as shown in their previous study (Urrutia-Benet et al., 2006). Therefore, more work is needed to conclusively prove the benefits of PSF applications which supercool within the metastable zone of ice III. For the moment, as suggested by Schluter et al. (2004), some considerations must be taken into account before freezing to other ice modifications, or cooling to the ice III metastable zone, such as volume changes, overall freezing time and phase transition time. As regards process efficiency, due to the protracted precooling time, cooling to the ice III zone does not benefit PSF (pressure shift freezing: a sample is frozen by means of pressure release, causing instantaneous crystallization of ice, homogeneously distributed throughout the sample); with PIT (pressure-induced thawing: a frozen sample can be forced to a phase transition from ice to liquid water by applying pressure along the melting curve of ice I), on the other hand, efficiency is increased.

Also, Salengke and Sastry (2007) recently investigated the effect of ohmic pretreatment on the oil uptake of potato slices during frying and subsequent cooling. The treated potato slices were heated either by directly sandwiching the slices between two electrodes or by heating them in a 0.11% salt solution. The results of this study indicate that oil uptake during frying and subsequent cooling of potato slices was reduced by ohmic pretreatment without a liquid medium (direct sandwiching). However, when the ohmic pretreatment was carried out in a liquid medium, there was no apparent effect. This might be due to an increase in the initial moisture content and porosity of the samples due to water absorption during treatment.

Trends in frozen potato products include the development of improved microwaveable products, more flexible manufacturing techniques, improved defect removal and reduction in fat content of fast-serve products.

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CHAPTER 8

Advanced Analytical Techniques to Evaluate the Quality of Potato and Potato Starch

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8.1 Introduction

The potato is an important vegetable and can be consumed in different forms, either fresh as table stock or processed into various products. Potato tubers contain 13–37% dry matter; 13–30% carbohydrates; 0.7–4.6% proteins; 0.02–0.96% lipids; and about 0.44% ash. In addition, ascorbic acid and other vitamins, phenolic substances, minerals, and nucleic acids are present (Kadam et al., 1991). Chemical composition and structure of components, such as starch, non-starch polysaccharides, sugars and other carbohydrates, organic and inorganic compounds, and proteins, influence the quality of potatoes and potato products. Dry matter content is one of the most important factors with regard to the texture of potatoes (Thygesen et al., 2001). For example, the yield of potato chips and French fries, and the texture of French fries and canned and reconstituted dehydrated potatoes are directly related to the dry matter content of the potatoes (O'Donoghue et al., 1996; Rodriguez-Saona et al., 1997). Thus, specific gravity or dry matter content, and specific component analysis (sugar, reducing sugars, minor minerals, and organic acid content) are usually used to evaluate the quality of potatoes. The quality of potato tubers for table and processing use depends on genetic and environmental factors, and storage temperature, duration, and subsequent conditioning (Dhumal et al., 1991). Sensory qualities, such as appearance, texture, taste, off-flavors and odors, are influenced by the potato variety, fertilizer type, environmental conditions, and other factors.

Starch is the major carbohydrate in potato and is an agriculturally important commodity with many food and non-food uses (Ellis et al., 1998). It is the basic source of energy for a majority

of the world's population. Since starch is the major component of the dry matter of potato, its molecular organization and interactions with non-starch polysaccharides and sugars are important factors influencing sensory attributes and shelf life of potato products, such as mashed potatoes, French fries, and potato chips (Lisinska and Leszczynski, 1989). In human nutrition, starch plays a major part in supplying the metabolic energy that enables the body to perform its different functions. Starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), according to the rate of glucose release and its absorption in the gastrointestinal tract (Englyst et al., 1992). RDS is the starch fraction that causes a sudden increase in blood glucose level after ingestion, while SDS is digested completely in the small intestine at a lower rate. RS is the starch portion that cannot be digested in the small intestine, but is fermented in the large intestine. Studies suggest that slowly digested starch and enzyme-resistant starch have significant implications for human health (Englyst et al., 1992; Lehmann & Robin, 2007).

An understanding of physicochemical and structural properties of starch will offer the possibility to control the quality of potato and potato products (e.g. digestibility), and to produce new potato starches with added value. As a result, it is critical to understand and apply advanced analytical techniques to characterize and evaluate the quality of potatoes and potato starch. In this chapter, the advanced analytical techniques to evaluate the quality of potatoes and potato starch are introduced and discussed in detail.

8.2 Potato Analysis and Quality Evaluation

8.2.1 Dry matter and specific gravity

The dry matter of a potato is the major determinant of texture of the raw or cooked product (Thygesen et al., 2001) and texture, in turn, is the most important of the sensory attributes that contribute to favorable mouthfeel and consumer acceptance (Tarn et al., 1992).

The characteristic most commonly used as an estimator of potato dry matter is specific gravity (Storey and Davies, 1992). Specific gravity of potato tubers can be determined using the formula:

$$\text{Specific gravity} = \text{Weight in air}/(\text{Weight in air} - \text{Weight in water})$$

The weight in air/weight in water method is one of the traditional methods of specific gravity determination. Selected sample units are first weighed in air and then the same unit is re-weighed suspended in water. Typically a computerized apparatus is used, such as that described by Tai et al. (1985). A potato hydrometer which is used with a fixed weight of potatoes may also be used for this purpose.

Specific gravity, and therefore dry matter content, varies among tubers of the same plant and across different parts of the tuber; it is highest in the outer cortex area and lowest in the inner

pith area. The degree of variation varies with cultivar and with environment. For some uses of potato where a uniform product quality is required, such as some forms of processing, a minimum amount of variation is desirable.

The total solid (dry matter) content of potato can be obtained by freeze-drying. Dry matter content is determined from the difference in the weight of potato samples before and after freeze-drying. The potato dry matter can also be estimated from the specific gravity measurements. In recent years, near infrared (NIR) spectroscopy has been used to measure specific gravity of potatoes. The NIR spectra (700–1100 nm) of potato samples are acquired, and partial least squares (PLS) regression analysis can be used to develop a predictive model for specific gravity (Scanlon et al., 1999).

8.3 Chemical Composition

8.3.1 Sugars

Sucrose, fructose, and glucose are the three major sugars in potato tubers. The sugar content of the tuber varies with potato variety and storage temperatures. In general, analysis of sugars (i.e. composition and content) is carried out using high-performance liquid chromatography (HPLC), high-performance anion exchange chromatography (HPAEC), and gas chromatography. Gas chromatographic analysis of reducing sugars requires conversion of sugars into suitably volatile derivatives such as alditol acetates and trimethylsilyl ethers (Davison and Young, 1964; Shaw and Moss, 1964; Blakeney et al., 1983).

The level of reducing sugars in potatoes is important in processing, since frying at high temperatures results in a Maillard reaction between these sugars and amino acids, yielding dark-colored, bitter-tasting products. The acceptable upper limit of reducing sugar content to obtain acceptable processing color is 0.25–0.5% of fresh weight (Gottschalk and Ezekiel, 2006). Another value of low sugar levels in potatoes is that during high-temperature processing, sugars interact with asparagines to produce acrylamides (Amrein et al., 2003) which are potential human carcinogens. Lastly, if free sugar content exceeds 5% of fresh weight of tubers, the potato has a sweet taste and is considered unacceptable for fresh and processing purposes.

To determine the free sugar content, a homogenized potato sample (e.g. dry matter) (1 g) is mixed with hot water (e.g. 80°C, 100 mL) for 60 minutes to allow dissolution of sugars. Glucose, fructose, sucrose, lactose, and maltose can be quantified by extraction into hot water, followed by HPLC analysis. Samples are analyzed using a Dionex Bio-LC with a Carbopac PA1, 4 × 250 mm column with 50 mM NaOH as the mobile phase. Calibration standards are made from dilutions of pure sugars, in concentrations ranging from 1 μg/mL to 50 μg/mL. Free sugars are calculated as mg/g of supernatant based on the peak area for potato samples and calibration standards.

8.3.2 Total starch content

Total starch content can be determined from fresh potato tubers or dry matter using enzymatic hydrolysis. In general, potato starches can be completely dissolved by cooking the sample in the presence of thermostable alpha-amylase followed by amyloglucosidase hydrolysis to glucose. The glucose content is subsequently measured by either a colorimetric procedure or biochemical analyzer. Starch content is calculated as glucose (g/100 g) × 0.9. Analysis of a single sample can be completed within 70 min (total starch assay procedure – megazyme amyloglucosidase/alpha-amylase method, AACC method 76–13). Grinding of samples must be sufficient to disrupt the cell walls and/or protein network surrounding the starch granules in order for the starch to be fully hydrolyzed to glucose.

8.3.2.1 Fresh tuber

Peeled potatoes are cut into thin slices and ground to powder with liquid nitrogen in a mortar. Ethanol (2 mL, 80%, v/v) is added to 500 ± 20 mg of ground sample in a plastic tube (40 mL) which is kept at 70°C for 20 minutes in a water bath. The sample is then centrifuged for 10 min at a rate of 20 000 ***g***. The pellet is resuspended in 5 mL of 80% ethanol and recentrifuged twice. The three supernatants are discarded while the remaining ethanol is evaporated from the pellet at 80°C in a water bath for about 20 minutes. The pellet is then dissolved in 20 mL of 0.02 N NaOH solution at 100°C in a water bath for 30 minutes or until the formation of a clear solution. An aliquot (0.8 mL) of the starch solution is mixed with 200 μL amyloglucosidase solution. The mixture is incubated at 55°C for 12 hours. The degree of hydrolysis is then verified. Dextrose and sucrose are measured using a 2700 Select Biochemistry Analyzer (YSI Incorporated, Yellow Springs, OH, USA). The principle of the reaction is that when a sample is injected into the sample chamber of YSI 2700, glucose diffuses into the membrane, which contains glucose oxidase. The glucose is immediately oxidized to hydrogen peroxide and D-glucono-δ-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The current flow at the electrode is directly proportional to the hydrogen peroxide concentration, and hence to glucose concentration. When the sucrose level is not zero, the digestion is considered incomplete and further incubation (4 hours) is performed. Total starch content is calculated from the dextrose content of the starch hydrolyzate.

$$\text{Total starch (mg/g fresh)} = \frac{\text{Dextrose (mg/L)} \times (1000(\mu\text{L})/800(\mu\text{L})) \times \text{V(L)}}{\text{Weight of fresh potato (g)}}$$

where V is the volume of the pellet–NaOH solution, 1000 μL is the test solution volume, and 800 μL is the potato sample volume (Liu, 1997).

8.3.2.2 Potato dry matter

Potato dry matter is ground to a powder using a mortar and pestle. To 100 mg potato dry matter, 100 μL (300 U) of α-amylase (e.g. from *Bacillus* species, Sigma A-6380, St Louis,

MO) solution, and 2.9 mL of 45 mM MOPS buffer (pH 7.0) are added. The sample is heated in a boiling water bath for 6 minutes with constant stirring, and is then cooled below 50°C. One hundred μL (20 U) of amyloglucosidase (e.g. from *Rhizopus* mold, Sigma A-7255, St Louis, MO) solution and 3.9 mL of 200 mM sodium acetate buffer (pH 4.5) are added to the sample. The sample is mixed and incubated at 50°C for 30 minutes with constant stirring. The sample is then diluted by adding 10 mL of distilled water, mixed thoroughly and centrifuged at 9600 ***g*** for 10 min. The glucose content of supernatants is measured with a YSI 2700 Select Biochemistry Analyzer (Yellow Springs, OH), or using glucose/oxidase peroxidase reagent and a UV/visible spectrophotometer. Total starch content of potato dry matter can also be determined based on AACC method 76-13 (AACC, 2000). Control samples, which are not treated with enzymes, are also included in the procedure to determine free glucose content. Regular maize starch is used as a standard in the experiment to ensure enzyme activity.

Starch content of potato dry matter is calculated as follows:

$$\text{starch content} = 0.9 \times (\text{glucose content after incubation} - \text{glucose content of the blank})$$

In recent years, a new research method was developed to determine total starch content of potato dry matter by comparing the gelatinization enthalpy of potato dry matter and standard starch using differential scanning calorimetry (DSC) (Liu et al., 2005).

8.3.3 Dietary fiber content

Total dietary fiber (TDF) content of potato dry matter is determined according to the AACC (2000) method 32-05 following the total dietary fiber assay procedure (Megazyme k-TDFR 01/05). This is a gravimetric method that is simpler and faster than other analysis methods. In addition to total dietary fiber content, both soluble and insoluble dietary fiber content can be determined by this method.

The principle of the method is outlined as follows. Samples of potato dry matter are cooked at ~100°C with heat-stable α-amylase to gelatinize, hydrolyze, and depolymerize the starch; incubated at 60°C with protease (to solubilize and depolymerize proteins) and amyloglucosidase (to hydrolyze starch fragments to glucose); and treated with four volumes of ethanol to precipitate soluble fiber and remove depolymerized protein and glucose. The residue is filtered; washed with 78% ethanol, 95% ethanol, and acetone; dried; and weighed. One duplicate is analyzed for protein content and the other is heated at 525°C to determine ash content. The TDF is the weight of the dried residue less the weight of the protein and ash.

Exact details of the method can be found in the total dietary fibre determination procedure AOAC Method 991.43 and AACC Method 32-07 (AOAC, 1995; AACC, 2000).

8.3.4 Protein content

Protein content of dry matter can be determined using a nitrogen analyzer such as the Thermoquest CE Instrument (NA 2100 Protein, ThermoQuest Italia S.P.A., Ann Arbor, MI, USA). Potato dry matter (30 mg) first undergoes a controlled combustion then is energetically oxidized and reduced, producing a gas mixture that is passed through a chromatographic column. Pure eluted combustion gas passes through a thermoconductivity detector, which generates an electrical output signal that is proportional to the amount of eluted gas. The nitrogen content is determined based on the peak area. Atropine, DL-methionine, acetanilide, and nicotinamide are used to produce a standard curve. Protein content is calculated by multiplying the nitrogen content by a factor of 6.25 (Liu et al., 2007a).

8.3.5 Amino acid content

The most abundant amino acids found in potato are asparagine and glutamine (Millard, 1986; Brierley et al., 1996; Eppendorfer and Bille, 1996). Free amino acids can be extracted from freeze-dried tuber extracts, and their concentration determined using reverse-phase HPLC (Brierley et al., 1996). Freeze-dried extracts are dissolved in 50 mM HCl, diluted with water, and derivatized with ortho-phthaldialdehyde-2-mercaptoethanol reagent. Chromatographic separation of individual amino acids is performed using a Beckman Ultrasphere ODS column (4.6×250 mm, 5 μm particle diameter, 800 μm pore size), with gradient elution. Mobile phases used are: A – 200 mL/L of 0.35 M sodium propionate buffer, pH 6.5, and 80 mL/L of acetonitrile; B – 300 mL/L of acetonitrile, 250 mL/L of methanol, and 30 mL/L of dimethyl sulfoxide. Amino acids are detected and quantified using a fluorescence detector (λ_{ex} 340 nm, λ_{em} 455 nm).

8.3.6 Lipid content

Potatoes contain a small amount of lipid, comprising approximately 0.1% of the fresh weight of the tuber (Galliard, 1973). Despite the minute quantity, lipids play an important role in the stability of processed potato products, since lipid degradation can lead to off-flavors and rancidity.

Lipids can be identified and quantified using thin-layer chromatography (TLC) and gas chromatography (GC) (Galliard, 1968). Extraction of lipids is achieved by homogenizing potato tubers with isopropanol in a blender, followed by a series of filtrations and extractions with chloroform-methanol (2:1). Chloroform is removed by rotary evaporation and the residue is redissolved in benzene-ethanol (4:1). This extract is passed through a DEAE-cellulose column, and the fractions collected are subjected to TLC on 250 μm layers of silica gel G, using three solvent systems. Fatty acid methyl esters for GC analysis are prepared by transmethylation of the parent lipids, or by diazomethane treatment of the free fatty acids released by acid

hydrolysis. The gas chromatograph is equipped with a flame ionization detector and a polyethylene glycol adipate (PEGA) column.

A newer approach for lipid analysis is electrospray ionization tandem mass spectrometry (ESI-MS/MS) (Welti et al., 2002). This method requires limited sample preparation and sample size to identify and quantify lipids. Fauconnier et al. (2003) used ESI-MS/MS to analyze phospholipid and galactolipid levels during aging of potato tubers.

8.4 Quality Evaluation

8.4.1 Texture

Texture is an important and complex quality characteristic. It is determined by variety and by structural and biochemical properties of tuber tissue, with some environmental influence. A considerable amount of research has been undertaken to replace subjective sensory evaluation with objective instrumental measurements (Van Dijk et al., 2002).

Texture has a number of component attributes, and some of them can be assessed by mechanical means. The texture or firmness of cooked potatoes is evaluated by subjecting each sample to a compression test using a universal testing machine equipped with a load cell. Cooked potato cylinders are compressed in a single-cycle compression–decompression test. Uniaxial compression is measured with an Instron machine with a 100 N load cell. Measurements are performed on hot potato cylinders (depth: 12 mm, height: 10 mm) from 15 potatoes immediately after cooking, at a deformation rate of 20 mm/min. Stress and strain at fracture are calculated by the Instron series IX version 7.40 software and means of 15 repetitions are calculated.

The texture of the processed potato can also be measured by shearing using a single blade, 1 mm thick, attached to the crosshead of the Instron Testing Machine. The processed whole potato with its skin intact is placed on the steel platform of the Instron and sheared longitudinally to a depth of 25 mm at a cross-head speed of 50 mm/min. Maximum shear force (kN) is calculated from the plot of force against displacement.

Sloughing or disintegration of potatoes during cooking is a major attribute of texture that can be measured directly. In these tests the potato sample is cooked and sieved, and the mass of the remaining cooked potato tissue on the sieve is recorded (see for example Hejlová et al., 2006).

Martens and Thybo (2000) used digital image analysis of scanning electron microscope images of potato tissue and of starch to successfully relate microstructure characteristics to textural properties.

A good (70%) prediction of sensory texture attributes of cooked potatoes has been obtained with nuclear magnetic resonance imaging, however, not all sensory attributes of texture are

predicted with equal reliability (Thybo et al., 2000, 2004). In addition, NMR imaging of raw potatoes provides structural and anatomical information related to sensory attributes of the cooked sample. Near-infrared spectra obtained from homogenates of potato samples have been related to sensory assessments of texture and to dry matter (Thybo et al., 2000; Van Dijk et al., 2002).

8.4.2 Sensory

Sensory evaluation of cooked potato quality is based on appearance and mouthfeel scored by trained or untrained evaluators using some form of hedonic scale on which the assessor records the perceived value of the attribute, typically on a nine-point scale, from extreme like to extreme dislike.

A commonly used classification for the evaluation of cooked potatoes is the four types: A (firm, non-mealy), B (firm, slightly mealy), C (rather loose, mealy) and D (loose, very mealy) (Van Marle et al., 1997). The literature contains many variations in terminology for the component attributes of texture. However, a small number of attributes is generally considered to account for most of the variation. These attributes include reflection (appearance), mealiness, graininess, firmness, hardness, springiness, chewiness, and moistness (Thybo and Martens, 1998). The relative weights of these components determine the suitability of a particular variety for a specific purpose such as boiling, baking, salads, and processing.

Preparation of boiled potatoes for sensory analysis involves peeling and cooking the samples in boiling water for 25–30 minutes or until cooked through as determined by a sharp probe. Hot samples are presented to the panel of assessors whole or prepared as required according to the particular study. The number of assessors, design of the assessments, replication and analysis are determined by the objectives of each experiment.

8.4.3 Color

Color is important in all forms of potato products, boiled, baked, fried, and chipped. Where a limited number of broad classifications are used, as in some commercial processing situations, use is made of visual reference charts showing a limited range of colors. Such charts are available for French fries (Munsell USDA Frozen French Fry Standard, X-Rite Right On Colour) and for potato chips (Colour Standards Reference Chart for Potato Chips, Snack Food Association).

For most other purposes, both before and after processing, color is measured using a colorimeter such as a Minolta Chroma Meter (Minolta Corp., Ramsey, NJ). The instrument is calibrated against a standard-white (Minolta) reference plate. Hunter *L* (whiteness), a (greenness) and b (yellowness) values were determined for each sample and made directly from the potato surface (Nourian et al., 2003).

8.4.4 Glycemic index

Glycemic index (GI) is a measure of the effect of the consumption of a carbohydrate food on blood glucose levels. The glycemic index, introduced by Jenkins et al. (1981), provides a ranking of carbohydrates on a scale from 0 to 100 according to the postprandial (after meal) impact on blood sugar levels. It is defined as the incremental area under the blood glucose response curve associated with a 50 g carbohydrate portion of a test food expressed as a percent of the response to the amount of carbohydrate from a standard food taken by the same subject.

The World Health Organization also provides guidelines for GI methodology (Anon., 1998). The principles and recommendations are as follows. The portion of food tested should contain 50 g of glycemic (available) carbohydrate and the glucose response should be measured in capillary whole blood at 0, 15, 30, 45, 60, 90, and 120 minutes after the commencement of the meal. The area under the curve (AUC) is calculated geometrically using the trapezoid rule. Either white bread or glucose can be used as the standard food. GI values using white bread are approximately 1.4 times those recorded when glucose is used as the standard food. Since blood glucose responses vary considerably from day to day within subjects, to determine a food's GI rating, it is recommended that the standard food response be measured at least three times per subject and that at least six subjects should be included in the test. The GI rating (%) is calculated by dividing the AUC for the test food by the AUC for the reference food (same amount of glucose) and multiplying by 100.

8.5 Potato Starch Analysis

8.5.1 Isolation

In order to understand the structure and functional properties of potato starch, starch has to be extracted from potato. Potato starch can be easily isolated from fresh tubers or potato dry matter. The main extraction processes include tuber soaking, disintegrating, and centrifugation or filtration. Soaking is carried out using an aqueous solution of sodium bisulfite at controlled pH to prevent biochemical reactions. Disintegrating and centrifugation are used to separate starch from other components. In general, starch granules can be liberated from the tuber by disruption of the cell walls. This is done during tuber disintegration by a cylindrical drum containing rotary saw blades on its circumference, or a juice extractor for extraction on a small scale.

The detailed procedure of starch isolation from potato tubers is as follows. Potato tubers (10 kg) are washed, peeled, sliced into 2–3 cm cubes, and soaked in distilled water containing 20 mM sodium bisulfite and 10 mM citric acid for 2 hours to prevent darkening. The cubes are then disintegrated using a centrifugal juice extractor, the pulp is suspended in 6 L of distilled water and passed through the extractor again, and starch milk is collected. The milk is allowed to sediment for 30 minutes; the supernatant and the suspended solids are removed by decantation, and the

starch sediment is resuspended in 10 L of distilled water. The starch granules are recovered by filtration, repeated washing and filtration, and finally by ambient air-drying (Liu et al., 2002).

8.5.2 Chemical composition and molecular structure

Most potato starches are composed of a mixture of two polysaccharides, a linear fraction, amylose, and a highly branched fraction, amylopectin. The content of amylose is between 15 and 25% for most starches. The ratio of amylose to amylopectin varies from one starch to another. The two polysaccharides are homoglucans with only two types of chain linkage, α-(1 $\rightarrow$ 4) in the main chain and α-(1 $\rightarrow$ 6)-linked branch chains. Physicochemical properties of potato and its starch are believed to be influenced by amylose and amylopectin content, molecular weight, and molecular weight distribution, chain length and its distribution, and phosphorus content (Jane and Chen, 1992).

8.5.3 Amylose

To determine the amylose content of starch, the iodine reaction has been most commonly used because amylose and amylopectin have different abilities to bind iodine. The methods such as blue value (absorbance at 680 nm for starch–iodine complex using amylose and amylopectin standards), and potentiometric and amperometric titration have been used for more than 50 years. These procedures are based on the capacity of amylose to form helical inclusion complexes with iodine, which display a blue color characterized by a maximum absorption wavelength (λ_{max}) above 620 nm. During the titration of starch with iodine solution, the amount (mg) of iodine bound to 100 mg of starch is determined. The value is defined as iodine-binding capacity or iodine affinity (IA). The amylose content is based on the iodine affinity of starch vs. purified linear fraction from the standard: 100 mg pure linear amylose fraction has an iodine affinity of 19.5–21.0 mg depending on amylose source. Amylopectin binds 0–1.2 mg iodine per 100 mg (Banks and Greenwood, 1975). The amylose content determined by potentiometric titration is considered an absolute amylose content if the sample is defatted before analysis.

In addition to the above methods, the Concanavalin A method (Con A) has been employed for amylose determination (Gibson et al., 1997). Con A is used to precipitate amylopectin from a starch solution. Amylose content of starch may also be determined by measuring the melting enthalpy of the amylase–lipid complex using differential scanning calorimetry (DSC) (Kugimiya and Donovan, 1981). In this technique, lipid is added to starch during gelatinization, and a starch–lipid complex is formed during cooling and storage. The complex is then melted by heating near or slightly above 100°C. Based on the value of the melting enthalpy, amylose content can be calculated. Amylose content of starch can be determined using size exclusion chromatography based on elution times for different molecular sizes of amylose and amylopectin (Colonna and Mercier, 1984).

8.5.4 Amylopectin

Amylopectin is a branched polysaccharide. It consists of α-D-glucopyranose residues linked mainly by α-(1 → 4) linkages (as in amylose) but with a greater proportion of non-random α-(1 → 6) linkages, which gives a highly branched structure. Amylopectin is one of the largest biological molecules. It can be enzymatically debranched using debranching enzymes, isoamylase and pullulanase, which specifically hydrolyze the branch linkages and produce linear chains. Size exclusion chromatography (SEC) and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) are the two basic techniques used to estimate the chain length and chain length distribution of amylopectin (Hizukuri, 1986; Wong and Jane, 1997) after enzymatic debranching. A multi-angle laser light scattering detector used with size exclusion chromatography (SEC/LALLS) has been applied for the determination of molecular weight, molecular weight distribution, and degree of branching of amylopectin (Yu and Rollangs, 1987).

The detailed procedure to analyze chain length and chain length distribution of amylopectin is as follows. Fractionated potato amylopectin is dispersed in 2 mL of 90% DMSO (5 mg/mL) by stirring in a boiling water bath for 20 minutes. After cooling, methanol (6 mL) is added with vortexing, and the tube placed in an ice bath for 30 minutes. The pellet, which was recovered by centrifugation (1000 ***g*** for 12 min), is dispersed in 2 mL of 50 mM sodium acetate buffer (pH 3.5) by stirring in a boiling water bath for 20 minutes. Following equilibration of the tube at 37°C, isoamylase (5 μL) is added. The sample is incubated at 37°C with slow stirring for 22 hours. The enzyme is inactivated by boiling for 10 minutes. An aliquot (200 μL) of the cooled debranched sample is diluted with 2 mL of 150 mM NaOH. The sample is filtered (0.45 μm nylon syringe filter) and injected into the HPAEC-PAD system (50 μL sample loop).

The HPAEC-PAD system consists of a Dionex DX 600 equipped with an ED50 electrochemical detector with a gold working electrode, GP50 gradient pump, LC30 chromatography oven, and AS40 automated sampler. The standard triple potential waveform is employed, with the following periods and pulse potentials: T1 = 0.40 s, with 0.20 s sampling time, E1 = 0.05 V; T2 = 0.20 s, E2 = 0.75 V; T3 = 0.40 s, E3 = –0.15 V. Eluents are prepared in distilled deionized water with helium sparging; eluent A is 500 mM sodium acetate in 150 mM NaOH, and eluent B is 150 mM NaOH. Linear components are separated on a Dionex CarboPac™ PA1 column with gradient elution (−5 min to 0 min, 40% A; 5 min, 60% A; 45 min, 80% A) at a column temperature of 26°C and a flow rate of 1 mL/min. A CarboPac™ PA1 guard column is installed in front of the analytical column. Data are collected and the weight fractions of DP 6–12, 13–24, 25–36 and ≥37 are calculated based on the area of peaks (Figure 8.1) (Liu et al., 2007b).

Structural analysis of isolated amylose and amylopectin components has been carried out by standard methods based on methylation, periodate oxidation, and partial acid hydrolysis studies. Methylation and periodate oxidation studies established the linkage types and frequency of

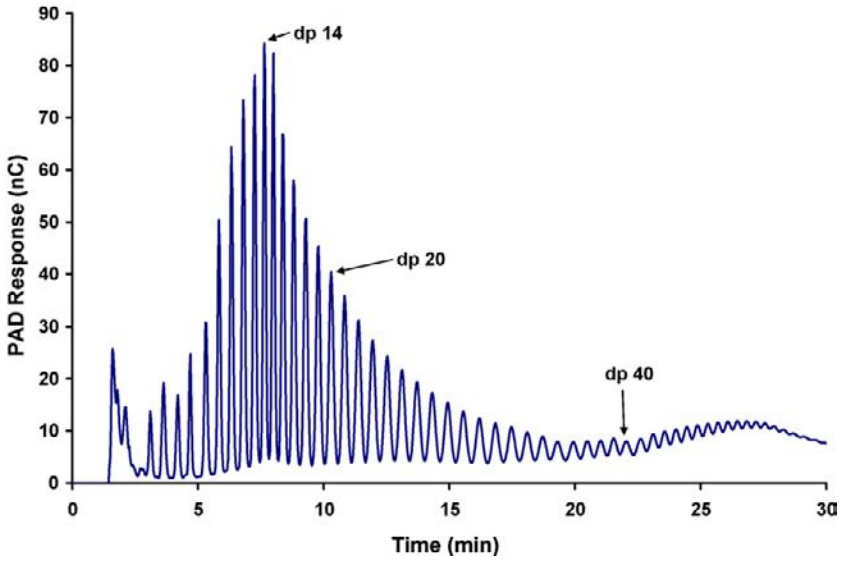

Figure 8.1: The profile of chain length and its distribution in potato starch by high-performance anion exchange chromatography (HPAEC).

branching and, together with the characterization of oligosaccharides from partial hydrolysis with acid and/or with enzymes, provided evidence that the α-D-glucopyranose residues are joined mainly by (1 → 4) bonds with 4–5% joined by (1 → 6) bonds (Guilbot and Mercier, 1985; Morrison and Karkalas, 1990). In order to conduct structural analysis, amylose and amylopectin should be isolated from starch.

8.5.5 Separation of amylose and amylopectin

The following steps can be used to fractionate potato starch into amylose and amylopectin, based on the method of Jane and Chen (1992).

(a) Weigh 5 g starch into 500 mL flask; add 375 mL of distilled water (1.33% w/v) and a stir bar. Stir for 30 minutes in a boiling water bath to gelatinize the sample.

(b) Check the pH after the sample has cooled enough to handle. Add phosphate buffer (40 g/L NaH_2PO_4, 10 g/L Na_2HPO_4) until the pH is between 5.9 and 6.3.

(c) Split the sample into two 500 mL flasks and autoclave at 121°C for 180 minutes on a fluid cycle.

(d) Boil the sample with stirring for 2 hours. Transfer to 1000 mL round-bottom (RB) flask, add 80 mL butanol (∼20% of starch solution volume), and reflux for 1 hour.

(e) Allow flask to cool enough to handle, then transfer flask to styrofoam cooler for slow cooling overnight.

(f) Transfer sample to three 250 mL centrifuge bottles and centrifuge for 30 minutes at 8700 ***g***. Decant supernatant into a clean 1000 mL RB flask (amylopectin – see step i). Transfer amylose precipitate to a 1000 mL RB flask, add 400 mL of distilled water, 80 mL of butanol, and reflux for 1 hour. Store flask overnight in the styrofoam cooler.

(g) Repeat centrifugation and refluxing twice more.

(h) Centrifuge, decant, and discard supernatant, and transfer amylose to a glass petri dish. Cover dish with aluminum foil and pierce foil in many places to provide ventilation. Dry in a vacuum oven at 50°C.

(i) Use a rotary evaporator (40°C, ~75 mbar, reduce pressure to 50 mbar as evaporation proceeds) to concentrate the supernatant from step f. Add butanol (20% of the volume of concentrated supernatant), shake by hand, transfer to two 250 mL centrifuge bottles and centrifuge for 30 minutes. Decant supernatant into clean bottles, add another volume of butanol, shake and centrifuge again. Decant supernatant into a 1000 mL RB flask and concentrate on a rotary evaporator.

(j) Add excess methanol to the concentrated amylopectin solution (1 part amylopectin solution: 2 parts methanol) to precipitate the amylopectin. Transfer to two 250 mL centrifuge bottles, centrifuge for 15 minutes. Transfer amylopectin to a glass petri dish and dry in a vacuum oven (step h).

(k) Weigh the dried amylose and amylopectin to determine yield of each, then grind using a lab mill.

(l) Check the purity of each sample by gel permeation chromatography: 2 mg of sample prepared in 90% DMSO to give a sample concentration of 1 mg/mL. Boil with stirring for 1 hour, and continue to stir until filtration and injection (>2 hours). Filter sample with a 0.45 μm nylon syringe filter, and inject 100 μL into the GPC system (1.0 mL/min DMSO containing 5 mM $NaNO_3$; RI detector 40°C, columns 80°C; columns = 2 x PLgel 20 μm Mixed A, PLgel 20 μm guard column).

8.5.6 Phosphorus and phosphate esters

Potato starch usually contains 0.01–0.6% (w/w) phosphorus. Although phosphorus is present at very low levels, it has a significant effect on the physicochemical properties of starch. Phosphorus is in part responsible for the high swelling power, paste stability, and resistance to enzyme hydrolysis of potato starch. Potato starches contain significant amounts of monophosphate esters

covalently bound to starch (Lim et al., 1994; Kasemsuwan and Jane, 1995). The distribution of the phosphate monoesters on the C_2, C_3, and C_6 hydroxyls of the glucose units in potato starch has been reported to be 1, 38, and 61%, respectively (Hizukuri et al., 1970; Tabata and Hizukuri, 1971). The above authors showed by isoamylase debranching and β-amylase treatment that phosphate groups are present in the long branch chains (B chains with an average degree of polymerization $\sim$ 41).

Because of the charged nature of phosphate monoesters, electrostatic repulsion between molecules increases and the properties of starch gelatinization and pasting change (Galliard and Bowler, 1987). Different cultivars and fertilization regimens resulted in different phosphorus contents in potato starch (Liu, 1997). By controlling the enzymes responsible for the incorporation of phosphate groups into starch, it is possible to obtain potato starch that has various phosphorus contents and desired functionality for different applications.

The phosphorus content can be determined by wet oxidation of the starch with sulfuric (or nitric) acid and hydrogen peroxide followed by colorimetric estimation of the phosphomolybdate complex. A detailed procedure for gravimetric determination can be found in AACC method 40–57 (AACC, 2000). However, large sample sizes (2–5 g) are required in this method.

8.5.7 Granular structure

Starch occurs naturally as water-insoluble granules. The organization of the starch granule is complicated and depends on botanical origin. Water is an integral component of granule structure and participates in the important hydration process that takes place during gelatinization and subsequent granule swelling and dissolution. The structure of the starch granule also depends on the way amylose and amylopectin are associated with intermolecular hydrogen bonds. The presence of α-(1 $\rightarrow$ 6) bonds in amylopectin is responsible for the alternation between amorphous and crystalline zones (Imberty et al., 1991). The molecules of amylose and amylopectin are distributed throughout the granule. The degree of mutual binding by hydrogen bonds between amylose and amylopectin and between multiple amylopectin molecules is responsible for structural heterogeneity of starch granules. When these bonds are strong, numerous, and regular, the chains associate as crystalline networks. In contrast, in the amorphous areas, hydrogen bonding is weaker, and this portion of the macromolecules is more independent due to steric reasons (the presence of α-(1 $\rightarrow$ 6)-linkages). Many physical techniques have been used to probe granular and crystalline starch structure; microscopy (light and electron), wide-angle X-ray scattering and diffraction, small angle X-ray scattering, solid state ^{13}C-NMR, and Fourier transform infrared spectroscopy (FTIR) are the most widely used.

8.5.8 Microscopy

Potato starch granules are generally large, voluminous, and oval-shaped with an eccentric hilum. When viewed under polarized light, the granules are birefringent, indicating a high degree of

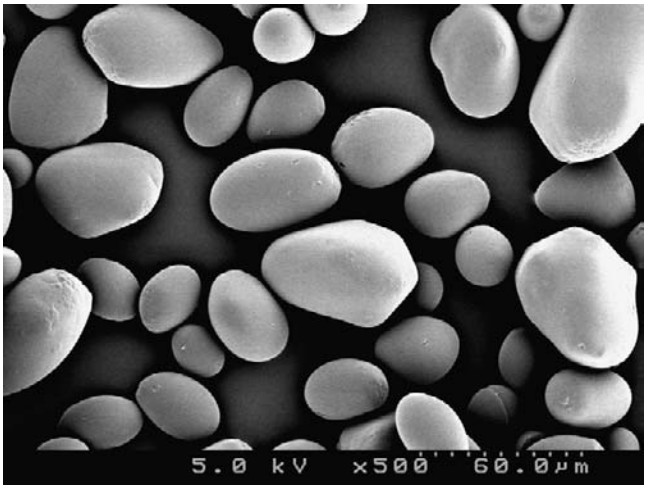

Figure 8.2: Scanning electron micrograph (SEM) of potato starch.

molecular order within the granule. Various methods are used to study starch structure such as morphology and internal arrangement. Microscopy shows the distribution of various structures in the granule and some of their details. The complementary methods of scanning electron microscopy (SEM) (Figure 8.2), atomic force microscopy (AFM), and transmission electron microscopy (TEM) have been widely used in recent years.

Each of the above techniques has specific advantages. Light microscopy is used to identify the type of starch from the size and shape of the granules and the hilum position. It involves shining a light source at a sample and observing the transmitted or reflected image via a series of lenses which allow for magnification and focusing. Using polarized light microscopy, all potato starches exhibit a dark 'Maltese cross' birefringent pattern. Using a light microscope equipped with a polarizer and a heated stage, the change of birefringence during heating (e.g. starch gelatinization) can be characterized. SEM uses a beam of electrons to illuminate a sample, rather than the traditional beam of light (i.e. photons). It allows the shape and surface features of starch granules to be viewed in three dimensions. The granules are applied to double-sided tape on a stub, coated with a thin layer of a reflective metal (e.g. gold:palladium 60:40) with a sputter coater, and irradiated with a beam of electrons. AFM has the ability to look at samples with little or no preparation and does not require ultra-high vacuum or dry samples (which SEM requires). One significant advantage for biological systems is the ability to image samples under wet conditions. AFM offers the advantage of highly localized ($\sim$nm) topographic imaging, as well as qualitative material property imaging for non-topographic insights into granular structure. TEM is a powerful technique for looking at very small-scale objects, smaller than those seen with SEM and AFM. However, it involves complex preparation techniques that include either metal shadowing or staining. In addition to light microscopy, SEM, and AFM, TEM has provided further detail about the internal structure of the granule.

8.5.9 Crystalline structure

Starch granules consist of amorphous and crystalline regions. The crystalline regions, or crystallites, are formed from the short branch chains of amylopectin molecules arranged in a cluster. The areas of branching points are believed to be amorphous. The semi-crystalline starch can be analyzed by various techniques such as wide angle X-ray scattering and diffraction, small angle X-ray scattering, solid-state ^{13}C-NMR, and Fourier transform infrared spectroscopy (FTIR). More recently, the organization of the semi-crystalline shells in the 9–10 nm lamellae has been described as a chiral side-chain liquid crystalline polymer (SCLCP) by Donald and co-workers (Waigh et al., 2000).

8.5.10 X-ray analysis

When a crystal is irradiated with X-rays, the X-rays split to form a pattern distinctive to the crystal structure. The X-ray radiation interacts with the starch structure on the nanometer scale. With X-rays, the scattering from a sample is from the electrons in the system, i.e. those around the atomic nuclei. The scattering is detected at a certain distance from the sample known as the camera length. Changing the camera length allows the scale being studied to be changed. After reduction with a suitable software package, a scattering profile is obtained. This will give details of the crystalline and amorphous regions. X-ray diffraction analysis (i.e. small-angle X-ray scattering and wide-angle X-ray diffraction) is the traditional method of crystallographic structure determination, and standard techniques and analytical procedures can be used to study the crystalline structure of starch. The most straightforward application is in the determination of crystal lattice spacing, but with refinements, complete structural analyses can be achieved, including the positions of pendant atoms or groups. Wide-angle X-ray diffraction is sensitive to crystalline order over distances from about 0.3 to 2 nm, such as crystallinity in starch granules. Small-angle X-ray scattering (SAXS) is usually used for the determination of longer-range information (l–100 nm) about the alternating crystalline and amorphous lamellae in the granules. Other information that can be obtained by X-ray analysis includes crystal size and perfection, the periodic length of lamellar polymers, the degree of preferred orientation in polycrystalline samples, and the conformation of chains in amorphous polymers (Campbell and White, 1989).

Native starch granules exhibit three main types of X-ray diffractogram. The A type is characteristic of most starches of cereal origin; the B type of potato (Figure 8.3), other root and tuber starches, and amylomaize starches and retrograded starch; the C type of smooth pea and various bean starches.

The differences between the A- and B-type crystallites relate to the packing of double helices in the crystal unit cell and the number of water molecules stabilizing these double helices. In the B-type crystal, double helices are packed in a hexagonal unit cell, an arrangement generating a central channel containing 36 water molecules per unit cell. In the A-type crystal, double

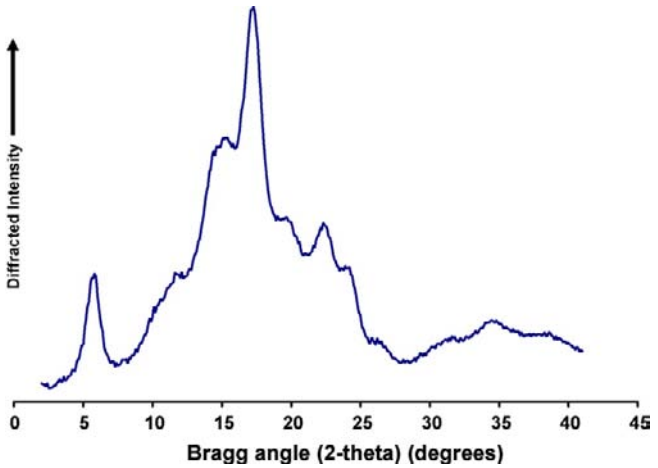

Figure 8.3: Wide angle X-ray diffraction profile of potato starch.

helices are packed in a monoclinic unit cell, an arrangement corresponding to a densely packed structure with only four water molecules per unit cell (Imberty et al., 1991).

The X-ray diagram from wide-angle diffraction is used to determine the areas of amorphous and crystalline phases of each sample. To separate crystalline and amorphous regions, a smooth line is fitted to the points of minimum intensity and the region above the resulting curve (A_c) is taken to represent the crystalline region. A straight line is fitted to connect the peak at 2.5–40° 2θ. The area between this line and the crystalline region (A_a) is considered to represent the amorphous region. Crystallinity can be calculated from the crystalline and amorphous areas by means of this division of the overall pattern.

$$\text{Crystallinity (\%)} = (A_c)/(A_c + A_a) \times 100$$

The range of 15–45% crystallinity has been reported for various starches (Zobel, 1988). However, the value of crystallinity varies with the method of determination, moisture content of starch granules, and starch source.

8.5.11 Nuclear magnetic resonance (NMR)

Based on the magnetic properties of some nuclei, NMR spectroscopy can be used for the quantification of structural features in starch. As a spectroscopic technique, NMR is considered a short-distance range probe to measure order at the level of individual helices. The most useful nuclei in starch research are ^{1}H and ^{13}C. There is increasing application of NMR studies in starch by technical advances in equipment and computing capability. The technique of solid state ^{13}C-NMR together with cross-polarization magic angle sample spinning (CP-MAS) and signal decoupling has been widely applied in starch granular structure elucidation. The determination

of relaxation times (T_1 and T_2) allows solid to be differentiated from liquid, and thus the mobility and micro-environment of water molecules in the granule can be determined. Based on the information of chemical shifts (δ value) and peak area of starch samples, quantitative estimates of double helix content of starch can be determined. Double helical content of starch was found to be in the range of 40–50%, greater than starch crystallinity (15–45%), indicating granular starch contains a significant amount of non-crystalline double helices (Gidley, 1992). Using this analytical technique, potato starch was found to have 50% double helix content (Gidley, 1992). In addition, the degrees of branching of starches and amylopectins can be determined using ^{l}H and ^{13}C-NMR spectroscopy (Gidley, 1985). A 2-dimensional ^{1}H–^{13}C wideline separation (WISE) solid-state NMR technique has been used to probe water–starch interactions on the molecular distance scale (Kulik et al., 1994).

8.5.12 Infrared spectroscopy

Infrared spectroscopy, considering interactions at a local range order, has already been used to describe the organization and structure of starch at various water contents. The FT-IR spectrum of starch has been shown to be sensitive to changes in structure on a molecular level (short-range order). In the 1300–800 cm^{-1} region, the CC, CO, CH stretching and COH bending modes are observed. The absorbance bands at 1022 cm^{-1} and 1047 cm^{-1} are characteristic of amorphous and crystalline structures in starch, respectively (Van Soest et al., 1995). Thus, the ratio of 1047 cm^{-1}/1022 cm^{-1} has been used to express the amount of ordered crystalline domains to amorphous domains in starches (Van Soest et al., 1995; Xie et al., 2006).

IR spectra of starch can be obtained with an IR spectrometer such as a Digilab FTS 7000 spectrometer, Digilab USA, Randolph, MA, equipped with a thermoelectrically cooled deuterated tri-glycine sulfate (DTGS) detector using an attenuated total reflectance (ATR) accessory at a resolution of 4 cm^{-1} by 128 scans. Spectra are baseline-corrected, and then deconvoluted between wavenumbers 1200 to 800 cm^{-1}. A half-band width of 15 cm^{-1} and a resolution enhancement factor of 1.5 with Bessel apodization are employed. Intensity measurements are performed on the deconvoluted spectra by recording the height of the absorbance bands from the baseline.

8.5.13 Fine structure of amylopectin

Amylopectin chains can be classified into three types according to their length and branching points. The shortest A chains carry no branch points. The B chains are branched by A chains or other B chains. The C chain carries other B chains and contains the sole reducing terminal residue. The amylopectin chain profile can be obtained by size exclusion chromatography or ion-exchange chromatography of enzymatically debranched amylopectin. The A:B chain ratio in amylopectin can be determined using debranching enzymes. The debranching enzymes, isoamylase and pullulanase, specifically hydrolyze the branch linkages to produce linear chains.

To debranch starch, starch is dispersed in 2 mL of 90% DMSO (5 mg/mL) by stirring in a boiling water bath for 20 minutes. After cooling, methanol (6 mL) is added with vortexing, and the tube placed in an ice bath for 30 minutes. The pellet, which was recovered by centrifugation (1000 ***g*** for 12 minutes), is dispersed in 2 mL of 50 mM sodium acetate buffer (pH 3.5) by stirring in a boiling water bath for 20 minutes. Following equilibration of the tube at 37°C, isoamylase (5 μL) is added (EN102, 68,000 U/mg protein, Hayashibara Biochemical Laboratories, Inc., Okayama, Japan). The sample is incubated at 37°C with slow stirring for 22 hours. The enzyme is inactivated by boiling for 10 minutes. An aliquot (200 μL) of the cooled debranched sample is diluted with 2 mL of 150 mM NaOH. The sample is filtered (0.45 μm nylon syringe filter) before injection to the chromatography system (Liu et al., 2007b).

8.5.14 Size exclusion chromatography

An HPLC system, equipped with a Waters solvent delivery system (M-45), two PLgel 20 μm Mixed-A columns (300 × 7.5 mm) with 20 μm guard column (50 × 7.5 mm) (Polymer Laboratories, Amherst, MA) and a refractive index detector (model 2410) (Waters, Milford, MA), can be used to study the molecular size and size distribution (e.g. molecular weight and weight distribution) of starch.

8.5.15 Anion exchange chromatography

The HPAEC-PAD system consists of a Dionex DX 600 equipped with an ED50 electrochemical detector with a gold working electrode, GP50 gradient pump, LC30 chromatography oven, and AS40 automated sampler (Dionex Corporation, Sunnyvale, CA). The standard triple potential waveform can be employed, with the following periods and pulse potentials: T1 = 0.40 s, with 0.20 s sampling time, E1 = 0.05 V; T2 = 0.20 s, E2 = 0.75 V; T3 = 0.40 s, E3 = –0.15 V. Data are collected using Chromeleon software, version 6.50 (Dionex Corporation, Sunnyvale, CA). The weight fractions of DP 6–12, 13–24, 25–36 and ≥37 are measured based on the area of peaks. The average chain length is also calculated. Eluents are prepared in distilled deionized water with helium sparging; eluent A is 500 mM sodium acetate in 150 mM NaOH, and eluent B is 150 mM NaOH. Linear components are separated on a Dionex CarboPac^{TM} PA1 column with gradient elution: 0 min, 40% eluent A; 5 min, 60% eluent A; 45 min, 80% eluent A at a column temperature of 26°C and a flow rate of 1 mL/min. A CarboPac^{TM} PA1 guard column is installed in front of the analytical column.

8.6 Starch Functionality

8.6.1 Swelling

When starch is heated in excess water, the crystalline structure is disrupted (due to breakage of hydrogen bonds) and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin. This causes an increase in granule swelling

and solubility. Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction is influenced by the amylose/amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching, and conformation.

Swelling degree can be determined as swelling power or swelling factor. Starch suspensions (1%, w/w) are heated at certain temperatures for 30 minutes. The starch sample is centrifuged and the supernatants removed. The sediments are weighed to determine swelling power (Liu et al., 2003).

8.6.2 Amylose leaching

Starch (20 mg, dry basis) in water (10 mL) is heated at certain temperatures in sealed tubes for 30 minutes. The tubes are then cooled to room temperature and centrifuged. Supernatant is withdrawn and its amylose content is determined according to the method of Williams et al. (1970). The value of amylose leaching reflects the association of amylose, and interactions between amylose and amylopectin in the starch.

8.6.3 Gelatinization

Starch gelatinization is the disruption of molecular orderliness within the starch granule. It results in granular swelling, crystallite melting, loss of birefringence, viscosity development, and solubilization.

A variety of analytical techniques have been employed to probe starch gelatinization. These include: viscometry, optical microscopy, electron microscopy, differential scanning calorimetry (DSC), X-ray diffraction, nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and most recently simultaneous X-ray scattering. Microscopic examination of granules undergoing gelatinization allows the observation of the degree and duration of swelling, as well as the integrity and size of the swollen granules. Thermal analytical techniques (e.g. DSC) are able to detect the heat flow changes associated with both first-order and second-order transitions of starch polymers. DSC has been widely employed to study gelatinization (the procedure will be described below). X-ray diffraction can be used to study the crystallinity change and to characterize the transition of crystal structure during starch gelatinization. The molecular structure information of starch during gelatinization can also be obtained using FTIR and NMR techniques. FTIR detects the absorption of different bond vibrations in starch molecules during gelatinization (Liu et al., 2002). It also provides the information of structure change (e.g. crystalline vs. amorphous) during the process. NMR provides information on the loss of detectable structural order within granules during gelatinization. Using ^{13}C-CPMAS NMR, Cooke and Gidley (1992) suggested

that double helix melting, rather than loss of crystallinity, could be primarily responsible for gelatinization enthalpy. X-ray scattering is used to study the structure of semi-crystalline carbohydrate polymers. Using the high intensity of a synchrotron of small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS), real-time experiments of starch gelatinization can be carried out. The technique provides new insights into the organization of structure within the granule at length scales characteristic of the lamellar structure (Jenkins and Donald, 1998).

However, some of these methods are experimentally limited by certain parameters, such as starch/water ratio and the temperature range over which gelatinization can be studied. For example, DSC is particularly well suited to investigate the phase transition of starch–water systems because it permits the study of starch transitions over a wide range of moisture content, the determination of transition temperatures over 100°C, and the enthalpy changes during transitions.

In DSC, the measuring principle is to compare the rate of heat flow to the sample and to an inert material which are heated or cooled at the same rate. To study gelatinization, samples of potato starch are weighed into high-volume pans. Distilled water is added using a micropipette to make suspensions with 70% moisture content. Pans are sealed and equilibrated for 2–4 hours at room temperature before heating in the differential scanning calorimeter equipped with a refrigerated cooling system. The measurements are carried out at a fixed heating rate (e.g. 10°C/min) from 5 to 180°C. The instrument is calibrated using indium and an empty pan as a reference. The enthalpy (ΔH) of phase transitions is measured from the endotherm of DSC thermograms based on the mass of dry solid. Gelatinization temperatures such as onset, peak, and completion temperature are also measured from DSC thermograms (Figure 8.4).

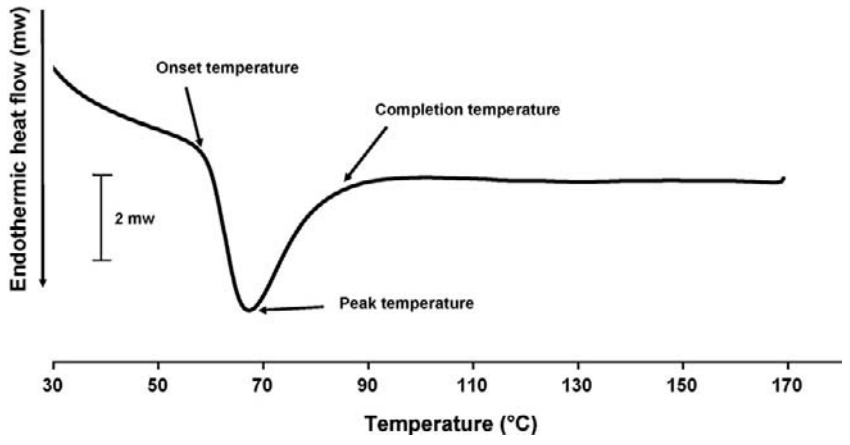

Figure 8.4: The gelatinization of native potato starch at 30% solid content by DSC.

8.6.4 Retrogradation

Starch retrogradation has been used to describe changes in physical behavior following gelatinization. It is the process that occurs when starch molecules reassociate and form an ordered structure. Under favorable conditions, a crystalline order appears and physical phase separation occurs.

Retrogradation properties of starch can be analyzed by differential scanning calorimetry (DSC), X-ray diffraction, nuclear magnetic resonance (NMR), rheological analysis, Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, and microscopy. The helices and variably ordered semi-crystalline arrays of those helices in retrograded starch can be determined by X-ray diffraction for the crystal pattern and crystallinity, NMR for the double helix content, or differential scanning calorimetry (DSC) to observe enthalpy changes when retrograded starch structures are lost on heating.

Mita (1992) examined changes in storage modulus (G'), loss modulus (G'') and loss tangent ($\tan\theta$) with storage time at 22°C for a 12.5% (w/w) potato starch paste by measurement of dynamic viscoelasticity. The authors observed a rapid increase in G' at the early stages of aging, and a slow increase in G'' during the latter stages. This was attributed to entanglement of solubilized amylose and to an increase in rod-like growth of crystals, respectively.

Bulkin et al. (1987) analyzed the retrogradation of potato starch (52% starch in water) gelatinized at 90°C and then cooled to room temperature by rapid Raman spectroscopy. The authors observed a narrowing of the half band width of the 480 cm^{-1} band with storage time. After 6 hours, the spectrum was very similar to that of the initial sample, and by 50 hours there was no visible change in the Raman spectrum. A plot of half band width of the 480 cm^{-1} band vs. storage time revealed four stages (I–IV) in the retrogradation process: (i) an initial rapid phase (representing conformational ordering involving the formation of double helices in amylopectin branches within a single polymer molecule); (ii) a plateau (representing the induction time for onset of amylopectin helix aggregation and crystal growth); (iii) a slow process (representing primary amylopectin aggregation and crystallization); and (iv) a very slow process (representing crystalline phase propagation and perfection).

DSC can also be used to determine starch retrogradation. After heating potato starch (30% (w/w)) to 180°C, samples are cooled to 5°C. Once the temperature reaches 5°C, the sample is immediately removed from the DSC and stored at low temperature. After a certain number of days, the sample pan is placed into the sample holder of the DSC, and heated from 5 to 180°C at 10°C /min. The instrument is calibrated using indium and an empty pan as a reference. The enthalpy (ΔH) of phase transitions is measured from the endotherm of DSC thermograms based on the mass of dry solid. Transition temperatures such as onset, peak, and completion temperature are also measured.

8.6.5 Starch rheology

Rheology is the study of stress–deformation relationships. During processing, a starch dispersion is subjected to a combination of high heating and shear rates that affect their rheological properties as well as the final characteristics of the product. Starch gelatinization, especially granular swelling, changes the rheological properties of starch. The subsequent retrogradation will further modify the rheological properties. Depending on the starch concentration, the final structure of starchy products will be a thickened solution or a gelled structure. When starch is cooked, the flow behavior of a granule slurry changes markedly as the suspension becomes a dispersion of swollen granules, partially disintegrated granules, dissolved amylose, and a number of intermediate species. The cooked product is called a starch paste. In general, a starch paste can be described as a two-phase system composed of a dispersed phase of swollen granules and a continuous phase of leached amylose. If the amylose phase is continuous, aggregation with linear segments of amylopectin on cooling will result in the formation of a strong gel.

Most rheological studies are conducted at temperatures lower than 95°C and in a range of shear rates sometimes irrelevant to processing conditions. The dynamic rheometer allows the continuous assessment of dynamic moduli during temperature and frequency sweep testing of the starch suspensions. The storage moduli (G') (elastic response) is a measure of the energy stored in the material. The loss modulus (G'') (viscous response) is a measure of the energy dissipated or lost per cycle of sinusoidal deformation. The ratio of the energy lost to the energy stored for each cycle can be defined by tan δ, which is used to indicate the degree of elasticity of a system. The G', G'', and tan δ are used to evaluate the rheological properties of starch and starch products. The G' values depend on the swelling power of starch (Eerlingen et al., 1997).

Rheological properties of potato starch have also been widely investigated using a Brabender Visco Amylograph (BVA) and Rapid Visco Analyzer (RVA). The RVA provides information on starch characteristics similar to the BVA with additional versatility of testing parameters. It has the advantages of using a small sample size, short testing time, and the ability to modify testing conditions.

In the RVA profile (Figure 8.5), native starch granules are generally insoluble in water below 50 °C. Thus, the viscosity is low. When starch granules are heated, the granules absorb a large amount of water and swell to many times their original size. The viscosity increases on shearing when these swollen granules have to squeeze past each other. The temperature at the onset of this rise in viscosity is known as the pasting temperature. The pasting temperature provides an indication of the minimum temperature required to cook a given sample. When a sufficient number of granules become swollen, a rapid increase in viscosity occurs. Granules swell over a range of temperatures, indicating their heterogeneity of behavior. The peak viscosity occurs at the equilibrium point between swelling and polymer leaching. Peak viscosity and temperature indicate the water-binding capacity of the starch. As the temperature increases further and

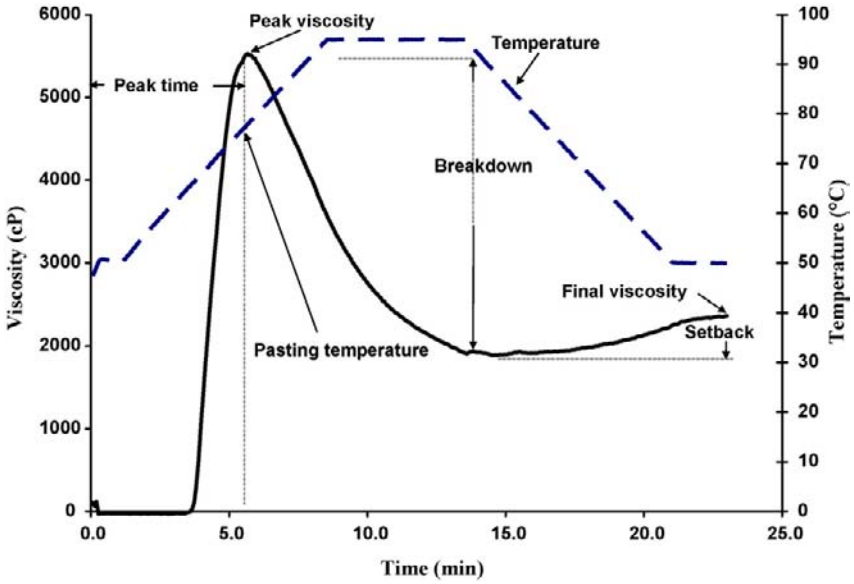

Figure 8.5: RVA pasting profile of potato starch.

is maintained at a high temperature for a period of time, the granules rupture and the more soluble amylose leaches out into solution and undergoes alignment. Due to mechanical shear (stirring), granules rupture and subsequent polymer alignment occurs, which decreases the apparent viscosity of the paste. This process is defined as breakdown. The viscosity at this stage gives an indication of paste stability. It is important to stress that only intact swollen granules can give paste viscosity, and not fragmented granules or solubilized starch. As the system is subsequently cooled, re-association between starch molecules, especially amylose, occurs to various degrees. In sufficient concentration this usually causes the formation of a gel, and the viscosity will increase to a final viscosity. The final viscosity gives an indication of the stability of the cooled, cooked paste.

Potato starch shows the highest peak viscosity and the lowest pasting temperature with moderate final viscosity and lower setback (Liu et al., 2003), compared to other commercial starches. This indicates that potato starch gelatinizes rather easily compared to other starches, producing more viscous pastes that easily break.

8.7 Final Remarks

A better understanding of the molecular and structural changes that occur in starch and potatoes would enable effective control of their functional behavior during processing and consumption, as well as in the development of modified starch products. Modern techniques have been developed and applied to study starch structure, phase transitions, and interactions of starch

with other potato constituents, functionality, and applications in food and non-food products. Our understanding of the role of starch in the potato has greatly increased over the past few decades. This is due in part to greater knowledge of the structure and functionality, aided by the application of advanced analytical techniques. We believe that more advanced analytical techniques will be applied in the evaluation of potato food quality and characterization of potato starch. More cost-effective, simple, rapid, and non-destructive methods will be developed to meet the requirements of industry, academia, and governments in the potato and starch sectors.

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CHAPTER 9

Textural and Rheological Characteristics of Raw and Cooked Potatoes

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9.1 Introduction

The potato (*Solanum tuberosum* L.) is one of the staples in human diet, and it is an important raw material for the starch industry as well. The color, size, and texture of potatoes are the main quality attributes assessed by the consumer for acceptability. Texture is related to the potato tuber's resistance to an applied force (Kaur et al., 2007; Nourian et al., 2003) and this essential quality attribute of potatoes (raw and processed) is a function of potato structure (Abu-Ghannam and Crowley, 2006). When a force is applied to potato structure, accepted to be a network of interconnected cells, a failure or rupture occurs at the point of minimum resistance. In this system, either the cells can separate through rupture of the middle lamella or the cells can burst, depending upon the strength of the middle lamella. Usually, raw potatoes undergo cell rupture, while cooked ones undergo cell separation as a result of thermal destabilization of pectic materials in the middle lamella (Aguilera and Stanley, 1990). During industrial processing, the potatoes are subjected to a variety of thermal treatments. The optimization of these industrial processes requires knowledge of potato structure and starch gelatinization. The textural changes occurring during thermal processing and cooking of potato tubers have been associated with the gelatinization and retrogradation behavior of starch (Linehan and Hughes 1969; Alvarez et al., 2001; Kaur et al., 2002; Shomer 1995; Shomer et al., 1995; Ormerod et al., 2002) and cell wall and middle lamellae structural components (Ormerod et al., 2002; Alvarez and Canet, 1998; Van Marle et al., 1997). Post-harvest storage also affects the textural quality of potatoes (Herrman et al., 1996; Cottrell et al., 1995; Spychalla and Desborough, 1990). Low-temperature storage results in a change in starch to sugar ratio of potatoes. Interrelationships between physical and biochemical quality characteristics of potato tubers have been reported between texture, starch, and reducing sugars during storage (Barichello et al., 1990; Kaur et al., 2002). Various methods of measuring texture-related and rheological properties can provide information on the behavior of foods when deformed; the best method to use depends on the type of food and the purpose

of the measurement. On the basis of relaxation and creep experiments, several researchers have shown that fruits and vegetables exhibit visco-elastic behavior (Canet, 1980; Alvarez and Canet, 1998; Kaur et al., 2002).

9.2 Cooking and Sensory Characteristics

Cooking and sensory characteristics are important both at domestic and industrial processing of potatoes. Cooking characteristics of potatoes such as cooking time, cooked potato weight, and cooking loss have been used to select the suitable potato cultivars with desired processing characteristics. The cooking time of raw tubers can be determined approximately by cooking unpeeled tubers in boiling water until a kitchen fork could easily penetrate the tubers. The estimation of the cooking time of a potato by using a texture analyzer or Instron Universal Testing Machine can be an accurate and precise method. Sample preparation plays an important role during estimation of cooking time by texture analysis. The tubers are washed and the periderm is peeled with a knife. Each tuber is cut into two equal halves and cylindrical pieces are taken from each half, excluding the core region, with the help of a cork borer and then trimmed to a small height. The cooking time is determined by cooking several cylindrical pieces of a tuber in boiling water. After every 2–3 minutes of cooking, each cylindrical piece is tested for compression on a texture analyzer using a flat probe and a suitable load cell. The cooking time is determined as the time required to reach the lowest compression force during 50% compression of the sample. The cooking time of potatoes in water may vary from 20–30 minutes while the compression force of cooked potatoes normally ranges between 0.15 N to 0.25 N at their optimum cooking times (Kaur et al., 2002; Singh et al., 2008). The effect of cooking time on compressive force of cooked potatoes has been shown in Figure 9.1.

The cooking time of the tubers has been observed to depend mainly on dry solids, starch content, and specific gravity. Though these three properties are inter-related, starch characteristics of the tuber have a major effect on the cooking time. Shorter cooking times have been reported for the tubers having starches with lower gelatinization temperature. The tubers of different potato cultivars differ in tuber dry solids, starch content, and specific gravity (Table 9.1). The dry solids and starch contents of potato tubers are known to vary considerably (6–18%) between cultivars (Bu-Contreras and Rao, 2001; Kaur et al., 2002). Factors that can affect dry solids content and specific gravity of potatoes are date of planting, soil type, fertilizer use, harvesting period, and post-harvest storage conditions (Toolangi, 1995). Singh et al. (2008) reported a shorter cooking time for the tubers of cultivars with high dry matter content. Also a high degree of tuber mealiness character is associated with a lower cooking time. For the determination of water uptake and total solids loss during cooking, the pre-weighed raw cylindrical pieces were cooked in a small quantity of boiling water for approximately 30 minutes. After draining and rinsing, the cooked cylindrical pieces are weighed for the determination of water uptake (%). The rinse water is collected and dried to estimate total solids loss (% wt. of the cylindrical piece

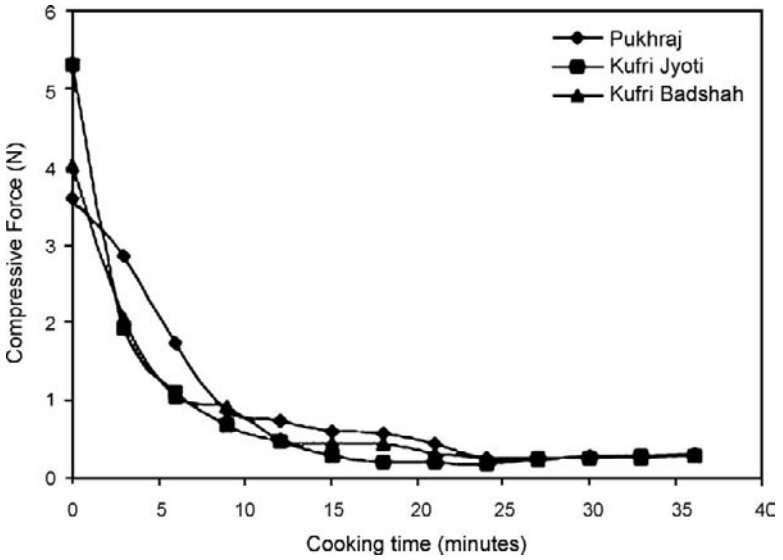

Figure 9.1: The effect of cooking time on compressive force of cooked potatoes. (Source: Kaur et al., 2002).

of potato). The potatoes with higher starch and dry matter content show higher water uptakes and loss during cooking than those with lower starch content. Mealy/waxy characteristics of the potatoes are determined by sensory analysis after cutting a cooked tuber in half and subjectively scoring the texture of the tuber as: waxy, slightly mealy, moderately mealy, and mealy. A positive correlation between dry matter content and mealiness of potatoes has been reported previously (O'Beirne et al., 1985). McComber et al. (1988) reported that the starch granules from the mealier potatoes gelatinized at a lower temperature.

9.3 Textural Characteristics

A detailed method to perform texture profile analysis (TPA) on raw and cooked potatoes has been described by Kaur et al. (2007). The raw or cooked cylindrical pieces are prepared as described

Table 9.1: Dry solids content (%), starch content (%), specific gravity and cooked texture of the raw tubers

Cultivar	Dry matter content (%)	Starch Content (%)	Specific gravity	Cooked texture
Nadine	14.63 ± 0.29	6.40 ± 0.26	1.057 ± 0.002	Waxy
Karuparera	19.03 ± 0.44	10.32 ± 0.39	1.059 ± 0.001	Waxy
Tutaekuri	21.57 ± 0.38	12.58 ± 0.33	1.074 ± 0.003	Mealy
Huakaroro	20.97 ± 0.26	12.05 ± 0.23	1.062 ± 0.003	Slightly mealy
Moemoe	21.97 ± 0.27	12.94 ± 0.24	1.069 ± 0.004	Moderately mealy

(Source: Singh et al., 2008)

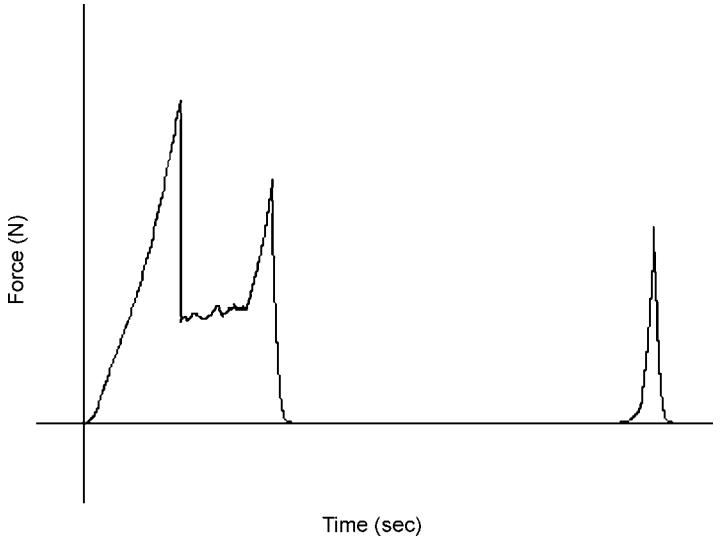

Figure 9.2: A typical texture profile analysis force-time curve for raw potato. (Source: Singh et al., 2008).

in the previous section. Each sample is compressed with a flat platen using a suitable load cell. The sample is preloaded with 0.5% of the total load. A slow crosshead speed is preferred and the maximum extent of deformation is generally 75% of the original length of the sample. Textural parameters (fracturability, hardness, adhesiveness, cohesiveness, gumminess, and springiness) are determined from the curve obtained through the computer software. A typical texture profile analysis force–time curve of a raw tuber is shown in Figure 9.2. The parameters derived from the textural profile analysis curves for raw potatoes vary considerably among different cultivars (Table 9.2). The raw tubers with high dry matter content generally show higher fracturability and hardness values. Differences in textural behavior among the potato cultivars may also be attributed to the differences in their microstructure (Kaur, 2004; Singh et al., 2005). Potatoes with closely packed small and irregular parenchymatous cells have been observed to be relatively hard and cohesive. In contrast, potatoes with large, loosely packed cells are generally less hard (Figure 9.3, Kaur, 2004).

Different textural properties of cooked potatoes, ranging from firmness to cohesiveness, in relation to mealiness, sogginess, stickiness, or gumminess, have been reported to be controlled by a number of factors, such as starch, pectic substances, and cell size. Cooked potato texture has also been associated with the dry solids content, specific gravity, amylose, sugars, proteins, and total nitrogen content of potato tuber. Many attempts have been made to show a relationship between the texture of the cooked potato and the physical or chemical properties of potato starch, which represents the predominant substance in the tuber. A highly significant correlation between the starch content of the raw tuber and various textural attributes of cooked potato,

Table 9.2: Texture profile analysis parameters of raw potatoes of different cultivars

Cultivar	Fracturability (Newton)	Hardness (Newton)	Cohesiveness	Springiness (Meter)	Gumminess (Newton)	Chewiness (Joules)
Kufri Chipsona-II	91 ± 2	132 ± 3	0.073 ± 0.002	0.0028 ± 0.0001	9.64 ± 0.50	0.027 ± 0.002
Kufri Chipsona-I	76 ± 2	105 ± 4	0.063 ± 0.001	0.0027 ± 0.0001	6.62 ± 0.35	0.018 ± 0.002
Kufri Lalima	91 ± 1	124 ± 2	0.072 ± 0.004	0.0033 ± 0.0003	8.93 ± 0.64	0.029 ± 0.004
Kufri Sindhuri	89 ± 2	115 ± 2	0.068 ± 0.002	0.0038 ± 0.0003	7.82 ± 0.37	0.030 ± 0.003
Kufri Anand	73 ± 1	99 ± 3	0.056 ± 0.001	0.0031 ± 0.0001	5.54 ± 0.27	0.017 ± 0.001

(Source: Kaur, 2004; Kaur et al., 2007)

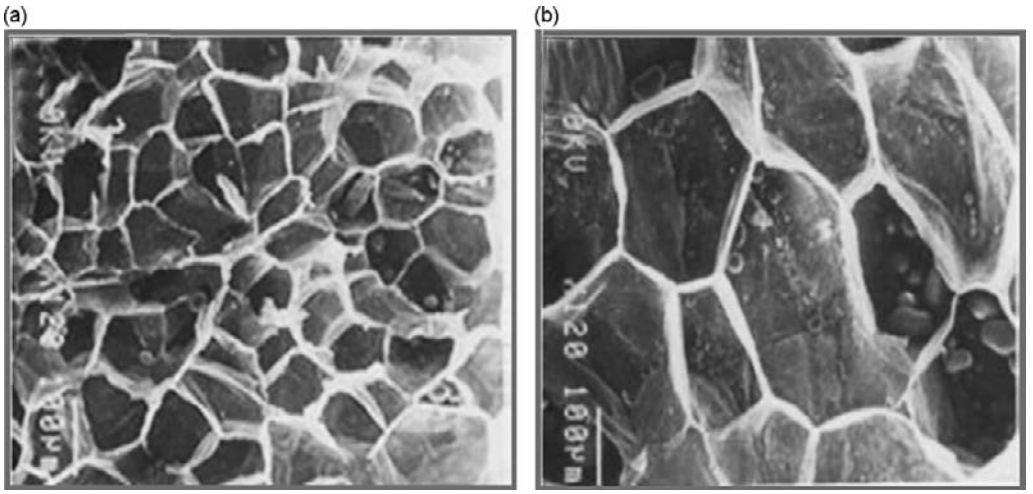

Figure 9.3: Scanning electron micrographs of fracture surface of raw potato parenchyma from different cultivars: (a) Small size cells, (b) Large size cells.
(Source: Kaur, 2004).

such as mealiness, consistency, sloughing, and sogginess, has been found by many researchers (Kirkpatrick et al., 1951; Unrau and Nylund, 1957). The properties of starch and the changes in these properties during cooking are generally considered to explain the variations in the texture. The internal pressures developed inside the cells resulting due to swelling of the gelled starch may result in a slight distension in the tuber cells causing them to round 'off' and thus separate from each other. Correlations between starch granule size and texture of cooked potatoes have been reported in the literature (Briant et al., 1945; Bu-Contreras and Rao, 2002). Mealy potatoes have higher specific gravities, starch, and amylose contents as well as a higher percentage of large starch granules (diameter >50 μm) than the waxy cultivars (Barrios et al., 1963; Kaur et al., 2002). It has been reported that tuber regions of low starch content cook softer than those containing more starch (Sharma et al., 1959). The cooked and cooled starch forms a stiff mass, which has a relatively low resistance to penetration. Textural changes in cooked potatoes are mainly associated with the gelatinization and retrogradation behavior of starch.

A typical TPA curve for cooked potato is illustrated in Figure 9.4, while the TPA parameters of cooked potatoes from different cultivars are summarized in Table 9.3. The texture of the cooked potatoes is expected to be dependent on the texture of raw potatoes. Fracturability, hardness, and cohesiveness are observed to be highest and chewiness and springiness to be lowest for potatoes with higher dry matter and starch content. McComber et al. (1987) reported higher cohesiveness values for mealy American potatoes. Potatoes with higher amylose content in their starches exhibit higher adhesiveness (Kaur et al., 2002). This may be due to the higher amylose contents in the starches of these potatoes. Linehan and Hughes (1969) have

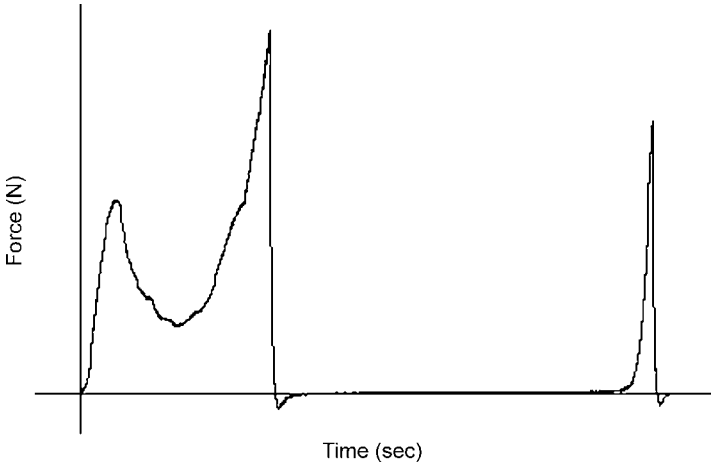

Figure 9.4: A typical texture profile analysis force-time curve for cooked potato. (Source: Singh et al., 2008).

observed a correlation between intercellular adhesion and amylose content during studies on the texture of cooked potatoes. During cooking, amylose leaches out through the weakened cell walls and acts as a cementing material between the cell walls of the tuber cells, thus leading to an increase in the intercellular adhesion (Linehan and Hughes, 1969). Other textural attributes like cohesiveness and springiness also differ considerably among the tubers of different cultivars (Table 9.3). The texture of cooked potatoes depends on various factors such as starch content, starch swelling power and gelatinization, pectin degradation, cell wall breakdown, and cell wall separation (Kaur et al., 2002). Cohesiveness of cooked potatoes was observed to be related to E_0, E_1, and E_3 elastic factors. The potato cultivars showing higher values of E_0, E_1, and E_3 also show higher cohesiveness values. The hardness and fracturability of raw as well as cooked tubers are found to be positively correlated with starch and dry matter content (Table 9.4). A highly significant correlation between the starch content of the raw tuber and various textural attributes has been found by many researchers (Kirkpatrick et al., 1951; Kaur et al., 2002). The fracturability of cooked tubers increases up to a few days of refrigerated storage and then decreases slightly thereafter. However, this was not true for hardness, which presented an irregular trend throughout the storage period in a study carried out on New Zealand potatoes (Singh et al., 2008). It has been suggested that fracturability and hardness are controlled mainly by starch content and starch behavior, but changes in potato cell wall and middle lamella pectic materials during heating may also influence these textural attributes (Kaur et al., 2002; Alvarez and Canet, 2002). Adhesiveness of the cooked tubers also increases progressively during refrigerated storage. The changes in the textural properties of the cooked tubers during storage may be attributed to the retrogradation of starch. The softening (%), calculated on the basis of changes in fracturability on cooking is another important textural parameter and differs

Table 9.3: Texture profile analysis parameters of cooked potatoes from different cultivars

Cultivar	Fracturability (Newton)	Hardness (Newton)	Cohesiveness	Springiness (Meter)	Adhesiveness $\times 10^{-4}$ (Joules)	Gumminess (Newton)	Chewiness (Joules)
Kufri Chipsona-II	3.2 ± 0.08	3.9 ± 0.22	0.088 ± 0.002	0.0011	2.14 ± 0.02	0.34 ± 0.02	0.0004
Kufri Chipsona-I	2.2 ± 0.12	3.3 ± 0.09	0.088 ± 0.003	0.0014	1.55 ± 0.06	0.29 ± 0.02	0.0004
Kufri Lalima	3.2 ± 0.15	4.7 ± 0.21	0.18 ± 0.003	0.002	1.98 ± 0.02	0.84 ± 0.05	0.0017
Kufri Sindhuri	3.4 ± 0.08	4.4 ± 0.25	0.12 ± 0.001	0.0019	2.22 ± 0.02	0.52 ± 0.04	0.001
Kufri Anand	2.7 ± 0.06	3.9 ± 0.20	0.065 ± 0.002	0.0014	1.76 ± 0.01	0.25 ± 0.02	0.0003

(Source: Kaur, 2004; Kaur et al., 2007)

Table 9.4: Pearson correlation coefficients for rheological and selected textural properties

	FrRaw	HdRaw	G_{heat}	G_{cool}	G'_{freq}
FrRaw					
HdRaw	0.968**				
G'_{heat}	0.955**	0.973**			
G'_{cool}	0.971**	0.958**	0.993**		
G'_{freq}	0.962**	0.970**	0.988**	0.986**	
Starch	0.903*	0.977**	0.915*	0.879*	0.912*
DM	0.901*	0.978**	0.922*	0.885*	0.916*

$*p \leq 0.05; **p \leq 0.01$. FrRaw = Fracturability of raw potatoes; HdRaw = Hardness of raw potatoes; G'_{heat} = Storage modulus during heating cycle at Tgel; G'_{cool} = Storage modulus during cooling cycle at 25°C; G'_{freq} = Storage modulus during frequency sweep at 20 Hz; Starch = Starch content; DM = Dry matter.
(Source: Singh et al., 2008)

significantly among the potato cultivars. The mealy cultivars show lower softening than the waxy or less mealy tubers. Starch characteristics such as amylose to amylopectin ratio, cell separation, and cell wall softening during cooking of potatoes have been reported to influence the degree of softening (Singh et al., 2008; Jarvis and Duncan, 1992). Raw potatoes are either stored under controlled conditions or processed for them to be consumed long after harvest. Post-harvest storage conditions can promote extensive changes in the chemical composition of potato tubers, thereby altering the quality characteristics of the final product (Spychalla and Desborough, 1990; McCay et al., 1987). Sugar and starch are the main components affected by post-harvest metabolism in potato tubers, which may ultimately affect their textural, sensory, and cooking properties. It has been shown that potatoes undergo considerable changes in texture depending on the storage temperature employed (Sowokinos et al., 1987; Kazami et al., 2000). The mechanisms of the changes occurring during storage at higher temperatures are different from those at lower temperatures (Bourne, 1982; Nourian et al., 2003). While cold storage may provide the necessary environment to prevent loss of weight, spoilage, and sprouting, the quality of potatoes continues to change as a result of physiological activity due to accumulation of reducing sugars and depletion of starch (Bourne, 1982; Nourian et al., 2003). The rate of sugar accumulation depends largely on the variety and temperature of storage and occurs most rapidly at cold temperatures. Hardness, cohesiveness, and gumminess of raw potatoes decrease progressively with increase in the post-harvest storage temperature. However, the decrease was observed to be significant only for potatoes stored above 12°C (Kaur et al., 2007). The textural parameters of the potato tubers stored at 16 and 20°C did not show significant differences among them (Kaur et al., 2007). Elevated storage temperatures have an adverse effect on the texture of raw tubers as they develop wrinkles and become slightly softer than those stored at lower temperatures. The softness and shrinkage of the potato tubers during storage may be attributed to the loss of both solids and water in the ratio in the original composition. Springiness and chewiness of the raw potatoes also decrease with the increase in the storage temperature. The

cooked potatoes from the produce stored at low post-harvest temperatures (4°C) show considerably different texture. The greater changes in the textural properties of these cooked tubers may be attributed to the changes in their starch content. Starch content of the potatoes has been reported to decrease with a decrease in storage temperature through the process of starch conversion to sugars at lower temperatures (Smith, 1987). Nourian et al. (2003) have also reported that the starch content decreases considerably during prolonged storage of the tubers at 4°C. The changes of starch to sugars at lower temperatures and the synthesis of starch from sugars at higher temperatures may be responsible for the change in the textural properties of the cooked potatoes stored at different temperatures.

9.3.1 Stress relaxation model

Davis et al. (1983) demonstrated that a generalized Maxwell model, consisting of three elastic and two viscous elements, could represent stress relaxation response for cooked potatoes. Leung et al. (1983) studied the textural and rheological properties of cooked potatoes, and correlated hardness of cooked potatoes, by sensory evaluation, with fracturability, hardness, and elastic elements of the stress relaxation model. The reported effects of heat treatment on the rheological properties of potatoes include tests on the effects of cultivar and maturity on potato texture (Madsen and Christensen, 1996; Taguchi et al., 1991) and on starch properties (Briant et al., 1945). For the stress relaxation test, the cylindrical potato pieces are cooked as described above in the case of cooking characteristics and TPA. The stress relaxation test can be performed on cooked potato pieces by using an Instron Universal Testing Machine or a texture analyzer. The unevenness of two ends of the sample is compensated for by preloading with 0.5% of the load. The stress relaxation curve of cooked potato samples obtained using texture analyzer (Figure 9.5) can be fitted with an equation consisting of three components, namely, an equilibrium stress and two exponential decay terms (Leung et al., 1983). The following equation represents the stress relaxation behavior of the cooked potato samples:

$$\sigma(t) = \varepsilon_0(E_0 + E_1 e^{-t/T_1} + E_2 e^{-t/T_2} + E_3 e^{-t/T_3}) \tag{9.1}$$

where σ = stress; t = time; E_0 = constant strain; $T_1 = \eta_1/E_1$; $T_2 = \eta_2/E_2$; $T_3 = \eta_3/E_3$; η_1, η_2, η_3 = viscosities; E_0, E_1, E_2, E_3 = elastic moduli.

Equation (9.1) can be represented by the Maxwell model (Mohsenin, 1970) consisting of four elastic elements (dashpots) as illustrated in Figure 9.6. The three Maxwell elements, each consisting of a spring and a dashpot in series, correspond to the three exponential terms in Equation (9.1). The lone elastic element corresponds to the equilibrium modulus (E_0) at infinite time. Kaur et al. (2002) reported stress relaxation behavior of three different potato cultivars (Table 9.5) which agrees with the earlier reported findings of Davis et al. (1983) and Leung et al. (1983). The tubers with higher elastic moduli obtained by stress relaxation data represent higher hardness.

Table 9.5: Stress relaxation test parameters of cooked potatoes from different cultivars

Cultivar	E_0 (Pa)	E_1 (Pa)	E_2 (Pa)	E_3 (Pa)	η_1 (PaS)	η_2 (Pas)	η_3 (Pas)
Pukhraj	1.612×10^4	1.715×10^5	1.476×10^5	1.185×10^5	1.011×10^6	0.0785×10^6	0.00978×10^6
Kufri Jyoti	6.481×10^5	1.858×10^5	2.247×10^5	2.138×10^5	8.835×10^5	2.967×10^5	0.2605×10^5
Kufri Badshah	5.172×10^4	2.013×10^5	0.94×10^5	2.013×10^5	1.263×10^6	0.078×10^6	0.05×10^6

(Source: Kaur et al., 2002)

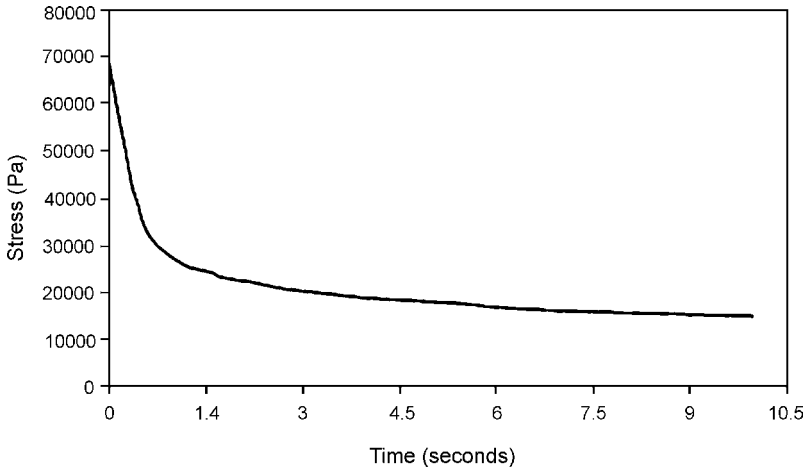

Figure 9.5: A typical stress relaxation curve of cooked potatoes. (Source: Kaur et al., 2002).

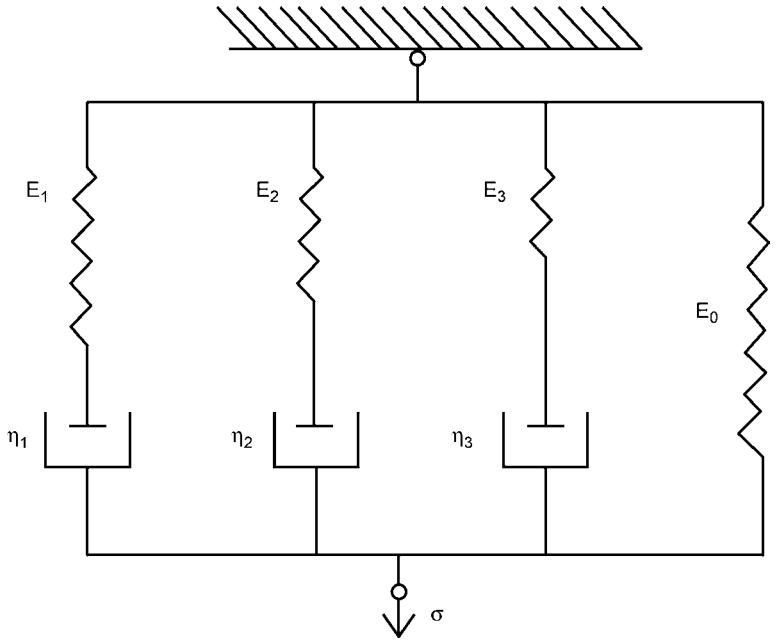

Figure 9.6: A seven element-Maxwell model representation for cooked potatoes. (Source: Kaur et al., 2002).

9.4 Rheological and Thermal Characteristics

9.4.1 Dynamic mechanical analysis

Rheological properties of foods can be studied using fundamental tests; empirical and imitative tests are used to determine textural characteristics. One advantage of fundamental tests is that they determine the properties of a material independent of the physical dimensions of the sample, the measurement procedure, or the equipment (Rao, 2007). Dynamic mechanical analysis is an effective analytical method because it applies very small strains on the samples (less than 0.1%) over a short time; as a result, the properties of solid foods can be studied with minimal physical and chemical changes. In addition, dynamic tests provide the ability to study the material's rheological properties at different frequencies. Another advantage that dynamic mechanical analysis provides is that one can detect changes due to cross-linking, phase separation, and molecular aggregation of polymer chains (Bu-Contreras, 2001; Rao, 2007).

Dynamic mechanical analysis is also called small-amplitude oscillatory testing because an oscillatory (sinusoidal) stimulus in the form of stress or strain is applied to the sample and a sinusoidal response (stress or strain) is measured. Materials have different responses to the deformation applied. The response could be elastic (Hookean solid) when the angle between the stimulus and the response is in phase. If the phase angle of the response is 90°, the material has a purely viscous response (also considered a Newtonian Liquid). Most materials have a response with a phase angle higher than zero but lower than 90°; such a response is considered to be viscoelastic because it contains both viscous and elastic characteristics. For compressional/elongational deformation, the symbol E is used for the moduli. The generated stress within the linear viscoelastic range is expressed in terms of the storage modulus E' (Pa) and a loss modulus E'' (Pa); E' is a measure of the magnitude of the energy that is stored in the material and E'' is a measure of the energy that is lost as viscous dissipation per cycle of deformation, respectively (Bu-Contreras and Rao, 2002). Bu-Contreras and Rao (2001) studied the starch content and the rheological properties of American RB and YG potatoes. Based on the solids content, the starch content was estimated to be 7.0% for the RB and 11.7% for the YG potatoes. Potato disks (13.3 mm diameter × 4.5 mm height) were cut from cylinders extracted from the center of a potato using a cork borer. In all experiments, values of E' were much higher than those of E''; therefore, only the trends of E' are discussed hereafter. The storage modulus of the raw sample, designated as E'_o, was measured at room temperature just before water was added to the submersion cup and it was used to normalize the E' data of the heated potatoes:

$$\text{Normalized Storage Modulus}(E'/E'_o) \tag{9.2}$$

The value of E'_o of the RB sample was 1.87 MPa and that of the YG sample was 2.01 MPa, respectively. Swelling of starch granules affected the storage modulus of the heated potato

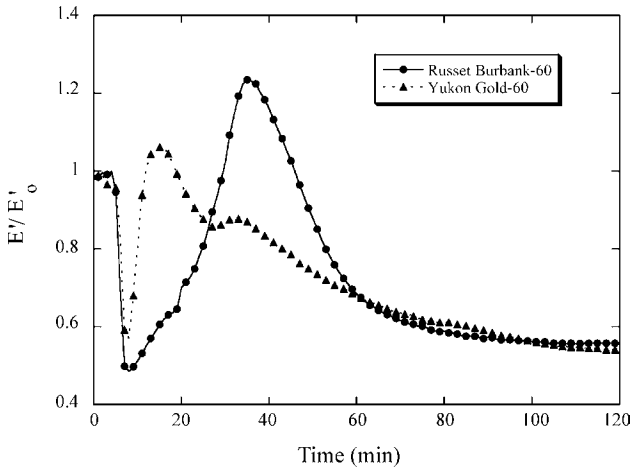

Figure 9.7: Profile of normalized storage modulus, E'/E'_o, of Russet Burbank potato disc as a function of heating time at 60°C; E'_o is the storage modulus of the raw sample = 1.87 MPa. (Source: Bu-Contreras, 2001).

samples, which in turn depended on the heating temperature and time. At all temperatures, immediately after the sample was immersed in water, the normalized storage modulus decreased for the first five minutes followed by an increase that depended on the heating temperature and time. The decrease in storage modulus after sample immersion was due to absorption of water by the potato sample causing the cells to separate, which resulted in the loss of rigidity (Bu-Contreras and Rao, 2001).

When samples were heated at the low temperatures: 40 and 50°C at which starch granules did not swell, the storage modulus of the RB and YG samples did not increase (not shown here). At 60°C, the normalized storage modulus of YG samples increased and reached a peak value of 1.1 in the first 10 minutes, while that of the RB samples reached a peak value of 1.25 after 35 minutes (Figure 9.7). After 60 minutes of heating, the normalized storage modulus values of both varieties were nearly equal and the modulus–temperature profiles were similar. At 70 and 80°C, the storage modulus of RB potato samples increased but that of the YG samples decreased significantly (not shown here). For example, at 80°C, the (E'/E'_o) values of RB were 30% higher than those of YG (Bu-Contreras and Rao, 2001).

9.4.2 Dynamic rheological analysis

Dynamic rheometers have been widely used to study the viscoelastic characteristics of liquid, solid, and semi-solid foods. However, to our knowledge, very few studies on the rheological characteristics of potato flesh using dynamic rheometry have been carried out. The dynamic rheological behavior of potato tubers may be helpful to have a quick idea about potato texture and

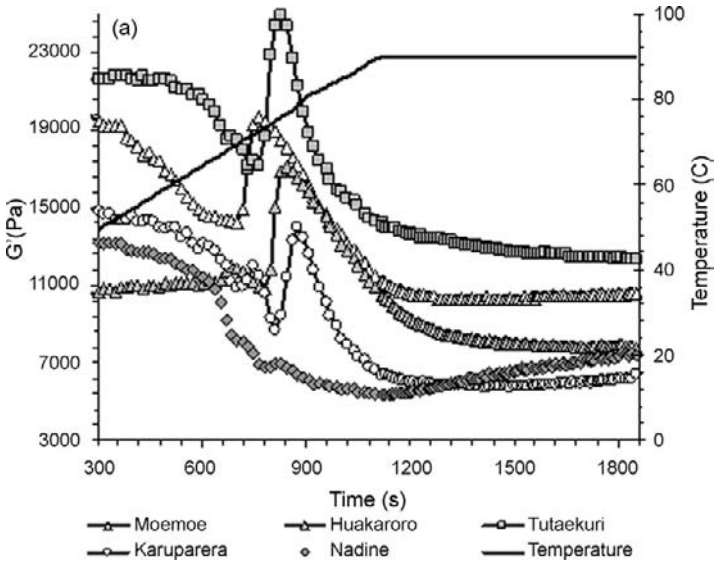

Figure 9.8a: Storage modulus (G') of raw tuber flesh during temperature sweeps (heating). (Source: Singh et al., 2008).

quality. Continuous measurement of the rheological changes in potato samples during heating can explain the role of starch in determining the texture.

Continuous monitoring of the dynamic rheological changes in potato samples during heating and cooling cycles on the rheometer may provide a crucial basis for the understanding of changes that occur during in-tuber gelatinization and retrogradation of starch in potatoes. The structural transitions associated with phase change in starch-containing food systems are reflected by changes in rheological profiles and are described by parameters such as G', G'', and *tan* δ ($=G''/G'$). Small-amplitude oscillatory three-step rheological measurements (temperature sweeps during heating and subsequent cooling, and a frequency sweep on the cooled sample) on the raw potato slices using a controlled stress rheometer have been reported by Singh et al. (2008). They determined dynamic rheological parameters such as storage modulus (G'), loss modulus (G''), and loss tangent (*tan* δ) for the potato tissue as functions of temperature and frequency. The softening of tissue and the swelling of starch granules in the potato tubers affects the rheological parameters, which in turn depended on the time and temperature of heating. Rheological profiles of the tubers from different potato cultivars of New Zealand are presented in Figures 9.8–9.11. During initial heating on rheometer, the G' of the potato tuber tissue decreases up to a certain time/temperature. With further heating, the G' increases to maxima and then drop. At early stages of heating, the tuber tissue lost its rigidity/strength owing to thermal softening, resulting in a decrease in the storage modulus (Bu-Contreras and Rao, 2001). With a further increase in temperature, G' increases steeply and reached maxima,

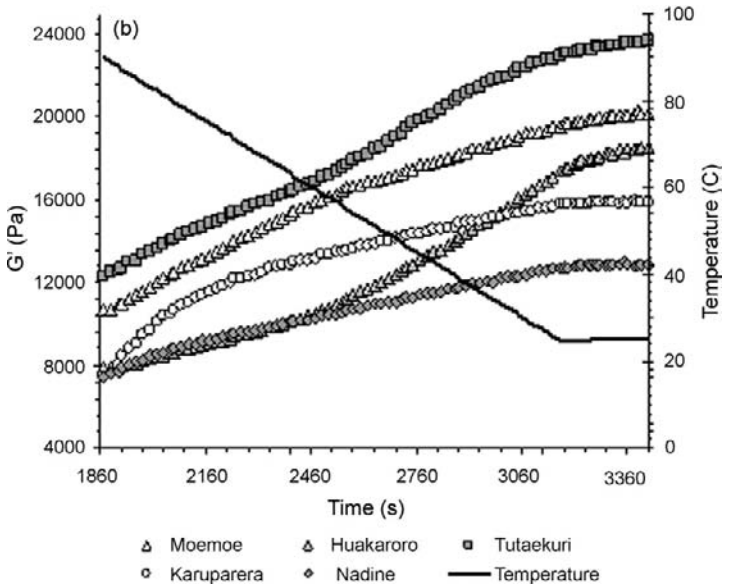

Figure 9.8b: Storage modulus (*G′*) of raw tuber flesh during temperature sweeps (cooling). (Source: Singh et al., 2008).

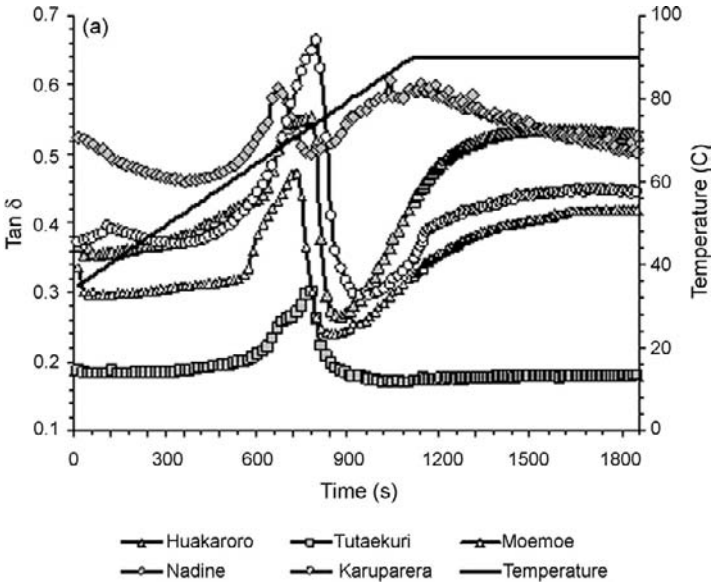

Figure 9.9a: Loss tangent (*tan δ*) of raw tuber flesh temperature sweeps (heating). (Source: Singh et al., 2008).

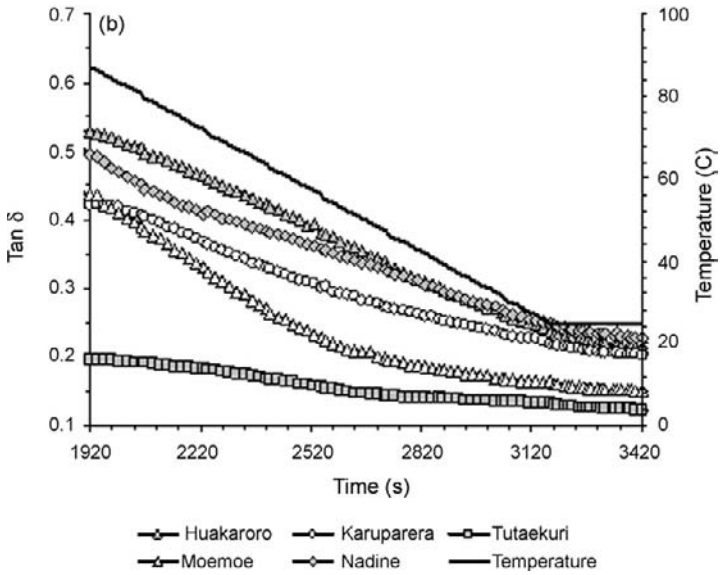

Figure 9.9b: Loss tangent (*tan δ*) of raw tuber flesh during temperature sweeps (cooling) (Source: Singh et al., 2008).

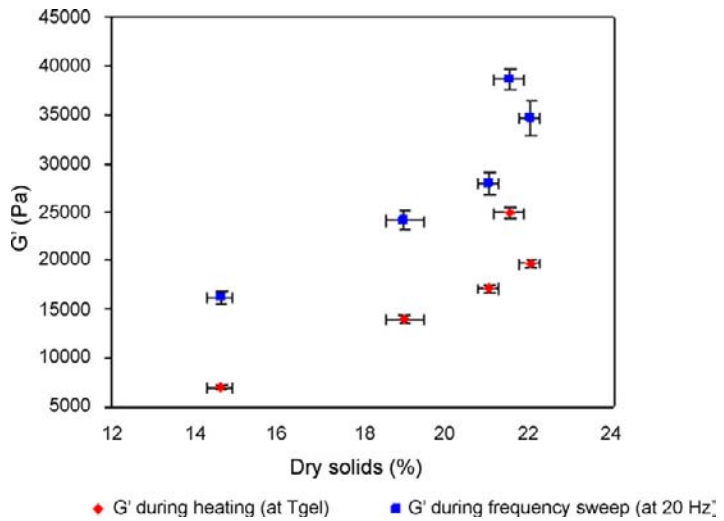

Figure 9.10: Effect of dry soilds (%) on the storage modulus (*G'*) of raw tuber flesh during temperature (heating) and frequency sweeps (Source: Singh et al., 2008).

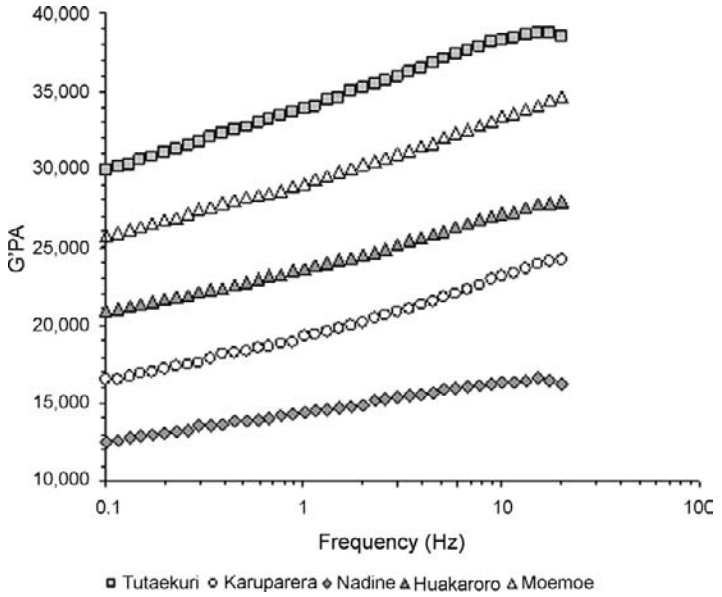

Figure 9.11: Storage modulus (G') of raw tuber flesh during frequency sweeps (Source: Singh et al., 2008).

owing to the swelling of starch granules and the formation of a network of swollen starch granules (gelatinization) in the tuber tissue (Figure 9.8a). Decrease in G' with further increase in temperature indicates the destruction of this gel structure with prolonged heating (Hsu et al., 2000). The rheological profile of the tissue from waxy potatoes does not show a distinct and clear gelatinization curve as observed for mealy cultivars, probably owing to their significantly lower dry matter and starch contents. The temperature at which G' was maximal (T_{gel}) shows a considerable variation depending on the starch characteristics of the cultivars. The mealy cultivars show higher peak G' than the waxy cultivars. Also the mealy potatoes require less time to complete gelatinization during heating than waxy potatoes (Bu-Contreras and Rao, 2001). A comparison between mealy cultivar Tutaekuri and a waxy cultivar Karuparera has been shown in Figure 9.8. In this figure, the gelatinization curve observed for a highly waxy cultivar Nadine is not sharp and distinct, in the temperature/time range studied. This may have been the result of this cultivar's very low starch content failing to change significantly the elastic character of the tissue on gelatinization. The other waxy cultivar, Karuparera, also exhibited a relatively small peak, which, as for Nadine, may also be attributed to its relatively low dry matter and starch contents. Generally, peak G' was directly related to dry matter and starch contents (Figure 9.10). During holding at 95°C, the G' of tubers showed a tendency to stabilize at a constant value. The G' of the heated tuber tissue increases during cooling from 90 to 25°C (Figures 9.8b, 9.9b). The mealy tubers exhibit higher G' values than waxy tubers. The continuous increase in G' during cooling reflects the gelation of the starch in the tuber tissue. Furthermore, retrogradation

of starch leached from granules, and interaction between starch molecules remaining inside granules, also reinforce the gel structure during cooling (Hsu et al., 2000).

The frequency dependence of G' can give valuable information about potato structure. A material that is frequency independent over a large frequency range is solid-like; a true gel system is such a material. In contrast, strong frequency dependence suggests a material structure with molecular entanglements that behaves more like a solid at higher frequencies and more like a liquid at lower frequencies (Ross-Murphy, 1984). The G' of the tuber tissues increases with increasing frequency, but the frequency dependence is not marked, indicating that the cooled gelatinized tuber tissues are predominantly solid-like in character (Figure 9.11). The storage modulus of potatoes is affected by starch swelling which plays a crucial role in determining the final firmness characteristics of heated potatoes (Bu-Contreras and Rao, 2002). During heating and in the presence of water, the starch granules swell and apply pressure to the cells of the potato samples that result in an increase in storage modulus. The rheological parameters such as storage moduli during heating, cooling, and frequency sweep show significant positive correlations with starch, dry matter content, fracturability, and hardness (Table 9.4). These correlations imply that the dynamic rheology of potatoes can be a useful tool to predict the texture of raw and cooked potatoes.

9.4.3 Differential scanning calorimetry (DSC) of potatoes

The DSC provides information on the thermal transitions taking place in a food. However, rheological changes are strongly influenced by the physical structure of a food; in a food containing starch, such as a potato, the rheological changes are strongly influenced by the state of the starch granules and the starch content. The DSC thermograms of unheated American RB potato samples had (not shown here) endothermic transition due to starch gelatinization over the range 68–74°C with a peak at 70.4°C, while those of the YG samples showed endothermic transition over 70.1 to 75.1°C with a peak at 72.3°C (Table 9.6) (Bu-Contreras, 2001).

9.4.4 Kinetics of firmness changes in potato

Because the firmness of potatoes seems to decrease rapidly at temperatures >70°C, in most studies, its values are modeled using first-order kinetics (Table 9.7). Defining P as a texture property, the first-order kinetics for texture degradation can be written as (Rao and Lund, 1986):

$$\frac{dP}{dt} = -k_f P \tag{9.3}$$

The temperature dependence of the rate constant can be expressed by the Arrhenius equation:

$$k_f = k_o \, exp(E_a/RT) \tag{9.4}$$

Table 9.6: Gelatinization temperatures of starch from RB and YG potato samples heated in water for 120 min at different temperatures

Sample	T_o (°C)	T_p (°C)	T_m (°C)
RB			
Raw	68.0	70.4	73.9
40°C	67.3	69.8	73.1
50°C	69.2	71.7	75.0
60°C	71.5	73.0	76.1
70°C	ND	ND	ND
80°C	ND	ND	ND
YG			
Raw	70.1	72.3	75.1
40°C	70.2	72.2	74.5
50°C	72.8	75.2	77.7
60°C	73.8	75.4	77.8
70°C	76.5	78.6	80.9
80°C	ND	ND	ND

T_o, T_p, T_m = Gelatinization initiation, peak and maximum temperatures, respectively.
(Source: Bu-Contreras and Rao, 2001)

Table 9.7: Fractional and first-order kinetic parameters for softening of potato

Variable	Value	Source
Fractional conversion kinetics		
k_o (per min)	1.18×10^8	Basis: Alvarez et al., 2001
E_a (kJ/mole)	64.18	
E_∞ (parameters c_1 to c_4 from Eq. 6)	0.53, 21.97, 63.78, 0.47	Basis: Nourian and Ramaswamy, 2003
k_o (per min)	1.29×10^{10}	
E_a (kJ/mole)	77.86	
E_∞ (parameters c_1 to c_4 from Eq. 6)	0.80, 8.35, 73.76, 0.20	
First-order kinetics		
k_f at 100°C (per min)	0.387	Basis: Harada et al., 1985 for maximum force
E_a (kJ/mole)	145	
k_f at 100°C (per min)	0.069	Basis: Solomon and Jindal (2003a, b) for tangent modulus
E_a (kJ/mole)	51.1	

At this stage, solution represents the kinetic effect and not diffusion. The diffusivity value used was $D_{eff} = 10^{-10}$ m^2/s.
(Source: Lee et al., 2007)

When a simple kinetic model is not enough, two separate first-order kinetic models for fast and slow degradation, respectively, have been used. But, it is difficult to define the time when the fast mechanism ends and the slow mechanism begins during cooking (Lee et al., 2007). However, the fractional conversion provides improved accuracy and reliability in determining

the texture degradation kinetics of vegetables (Rizvi and Tong, 1997). The fractional conversion model is derived from the first-order model. For Young's modulus as the property, the fractional conversion, f, is defined as:

$$f = \frac{E_o - E}{E_o - E_\infty} \tag{9.5}$$

where, E_o is initial modulus, E is modulus at time t, and E_∞ is the non-zero value of the modulus, obtained after long times. The non-zero retainable modulus, E_∞, is a function of temperature.

A first-order equation is written using this fraction as:

$$\frac{d(1-f)}{dt} = -k_f(1-f) \tag{9.6}$$

$$E_\infty(T) = c_4 + \frac{c_1}{1 + \left(\frac{T}{c_3}\right)^{c_2}} \tag{9.7}$$

where, c_1–c_4 are parameters to be determined by fitting the equation to experimental data. Table 9.7 contains values of the reaction rate constant and activation energy for the first-order kinetics and the fractional conversion models (Lee et al., 2007).

9.5 Conclusions

The textural and rheological characteristics of raw and cooked tubers are observed to be mainly dependent on their starch and dry matter contents. Other factors such as microstructure of potato tissue, amylose content may affect the textural and rheological attributes. Post-harvest storage temperature considerably affects the texture of raw and their cooked counterparts because of changes occur in starch to sugar ratio of potatoes. The dynamic rheological measurements of potato tubers may prove useful to monitor continuously the texture related changes that occur during heating.

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CHAPTER 10

Potato Starch and its Modification

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10.1 Introduction

Among all the carbohydrate polymers, starch is currently enjoying increased attention owing to its usefulness in different food products. The physico-chemical properties and functional characteristics of starch systems, and their uniqueness in various food products vary with the starch biological origin. Interest in new value-added products in the industry has resulted in many studies being carried out on starches. Starch exists naturally in the form of discrete granules within plant cells and is mainly composed of two polymers: amylose and amylopectin. Amylose is a linear polymer composed of glucopyranose units linked through α-D-(1 $\rightarrow$ 4) glycosidic linkages, while amylopectin is a highly branched and a high-molecular-weight polymer.

Potatoes in general are an excellent source of starch, which contributes to the textural properties of many foods. Potato starch can be used in food and other industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, and water-holding agent. But limitations, such as low shear and thermal resistance, and a high tendency towards retrogradation restrict its use in some industrial food applications. These limitations are generally overcome by starch modification, which can be achieved through derivatization, such as etherification, esterification, cross-linking, and grafting of starch; decomposition (acid or enzymatic hydrolysis and oxidization of starch); or physical treatment of starch using heat or moisture or pressure, etc. These treatments result in markedly altered gelatinization, pasting, and retrogradation behavior of potato starch. The Food and Drug Administration (FDA) regulates and reinforces the type and amount of each chemical used in starch modification.

In this chapter, we have presented a great deal of information on important physicochemical and functional characteristics of native potato starch in comparison with some cereal starches. In addition, we have also discussed various modification techniques being used to modify potato starch, with an emphasis on the post-modification changes (particularly after derivatization)

in its morphological, physico-chemical, rheological, and thermal behavior. The various factors that influence potato starch modification have also been discussed in detail.

10.2 Potato Starch vs. Cereal Starches

Potato starch exhibits different granular structure and composition, as opposed to cereal starches, which are responsible for the variation in functional behavior of these starches. Cereal starches exhibit the typical 'A' type X-ray crystalline pattern, whereas potato starch shows the B-form, and legumes the mixed state pattern 'C'. The 'A,' 'B,' and 'C' patterns are the different polymeric forms of starch that differ in the packing of amylopectin double helices. The structure of potato starch is discussed in more detail in Chapter 4.

10.2.1 Morphology

Starch granule morphology varies with plant genotype and cultural practices. It also depends on the biochemistry of the chloroplast/amyloplast and the physiology of the plant. When viewed under a microscope, the starch granules of potato differ in size and shape from those of cereal starches. Scanning electron micrographs (SEM) of starch granules from various plant sources are illustrated in Figure 10.1. Granule size differs considerably among different starches and ranges from 1 to 110 μm (Hoover, 2001). For potato starch, the average granule size ranges from 1 to 20 μm for small and 20 to 110 μm for large granules. The average size of individual maize starch granules ranges from 1 to 7 μm for small and 15 to 20 μm for large granules. Rice starch granules generally range from 3 to 5 μm in size. Wheat endosperm at maturity contains two types of starch granules: large A- (diameter 10–35 μm) and small B-type (diameter 1–10 μm).

The extent of variation in the granular structure of starches from cultivar to cultivar is also quite high in potatoes. Granule size distributions of starches of different potato cultivars studied through particle size analysis are given in Figure 10.2, and the distributions in terms of percentages of small, medium-size, and large granules in Table 10.1. Interestingly, the small potato starch granules are spherical or oval in shape, but the large ones are generally ellipsoidal to cuboidal or irregular in shape. This variation in shape with the size of potato starch granules could be related to granule packing during growth of the storage organs. Limited space availability in tuber cells may lead to alteration in shape of a growing granule. The starch granules are angular for maize, and pentagonal and angular for rice. A-type granules of wheat starch are disk-like or lenticular in shape and the B-type starch granules are roughly spherical or polygonal in shape.

Variations in amylose and amylopectin structures and their relative amounts in a granule play an important role in controlling starch granule size and shape. Activity of the enzyme granule bound starch synthase (GBSS) during growth may also affect starch granule morphology in

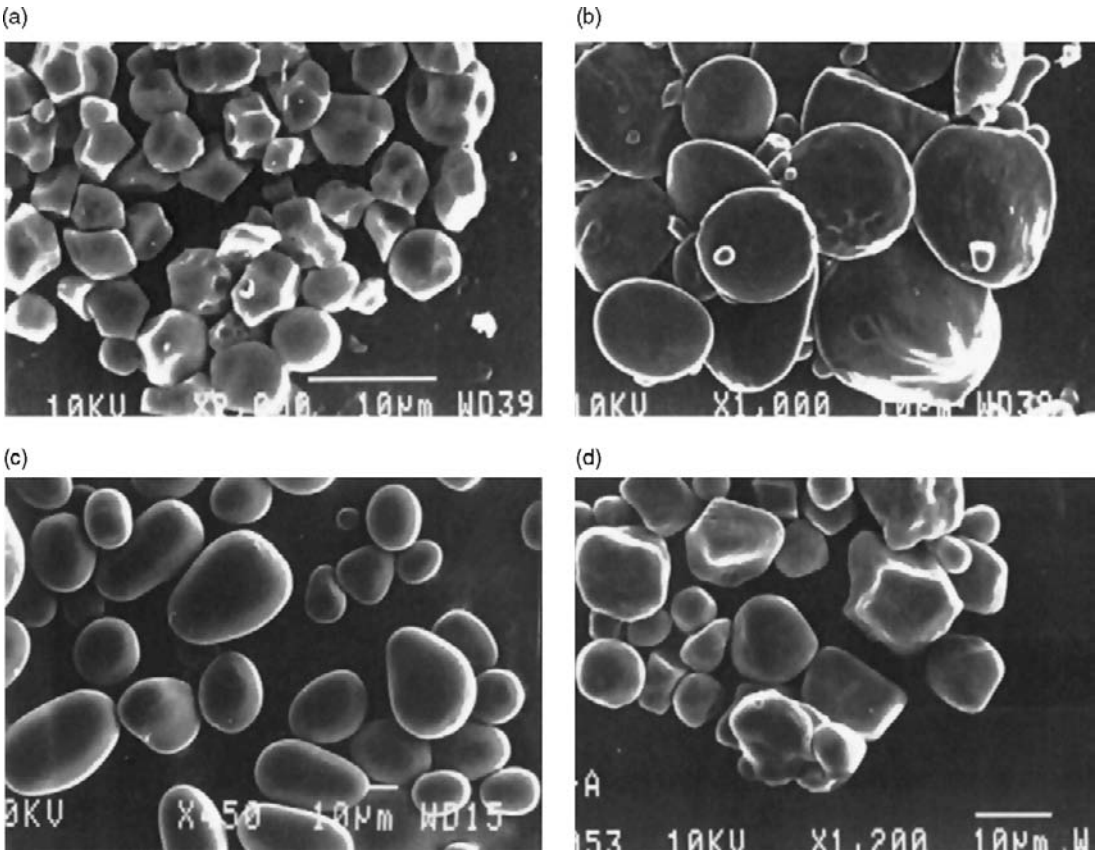


Figure 10.1: Scanning electron micrographs (SEM) of starches separated from different sources: (a) rice, (b) wheat, (c) potato, (d) maize (bar = 10 mm) (source: Singh et al., 2003).

potatoes (Blennow et al., 2002). The membranes and the physical characteristics of the plastids may also be responsible for providing a particular shape or morphology to starch granules during granule development (Jane et al., 1994; Lindebooma et al., 2004).

When observed under a scanning electron microscope, the surface of maize, rice, and wheat starch granules appears to be less smooth than that of potato starch granules. Li et al. (2001) observed the presence of 'pin holes' and equatorial grooves or furrows in large maize starch granules. Singh et al. (2006) have shown the presence of small protruberances and fragmentation on the surface of potato starch granules (Figure 10.3). Physico-chemical properties, such as transparency of the starch paste, enzymatic digestibility, amylose content, and swelling power have been significantly correlated with the average granule size of the starches separated from different potato cultivars (Kaur et al., 2007a, b).

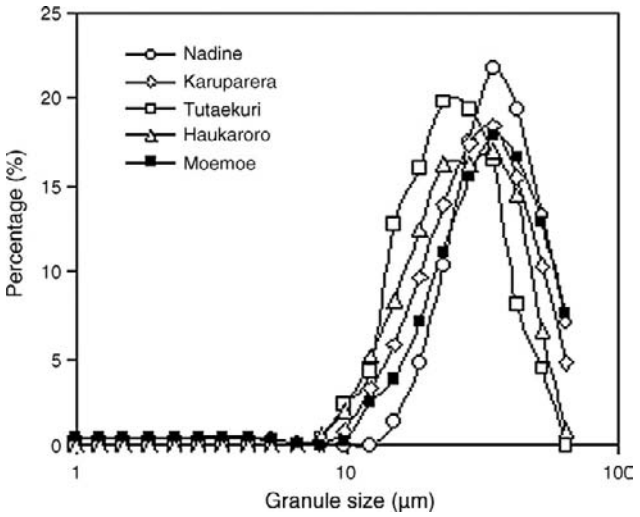


Figure 10.2: Particle size distribution of starches from some New Zealand potato cultivars (source: Singh et al., 2006).

Table 10.1: Morphological parameters of starches from different New Zealand potato cultivars: proportions of small, medium size and large granules; mean granule volume and granule specific surface area

Potato starch source	Small granules (%) (1–10 μm)	Medium granules (%) (11–25 μm)	Large granules (%) (>25 μm)	Mean volume (μm^3)	Specific surface area (m^2/g)
Nadine	4.4	16.4	79.2	10648	0.189
Karuparera	0.9	32.6	66.5	6859	0.198
Tutaekuri	2.7	52.8	48.5	3375	0.248
Huakaroro	2.7	42.1	55.2	4096	0.228
MoeMoe	5.1	24.5	70.5	5832	0.188

(Source: Singh et al., 2006)

10.2.2 Composition

Starch paste behavior in aqueous systems depends on the physical and chemical characteristics of the starch granules, such as mean granule size, granule size distribution, amylose/amylopectin ratio, and mineral content, etc. The amylose content of the starch granules varies with the botanical source of the starch (Table 10.2) and is affected by the climatic conditions and soil type during growth. The amylose content of potato starch varies from 23 to 31% for normal potato genotypes (Kim et al., 1995; Wiesenborn et al., 1994). However waxy potato genotypes, essentially without amylose, have also been reported (Hermansson and Svegmark, 1996). Also,

Table 10.2: Physico-chemical properties of starches from different botanical sources

Starch source	Amylose content (%)	Swelling power (g/g) (°C)	Solubility (%) (°C)	Organic phosphorus contents[a] (% dsb)			Light transmittance[e] (%, at 650 nm)
				Mono-P[b]	Lipid-P[c]	Inorganic-P	
Normal potato	20.1–31.0	1159 (95)	82 (95)	0.086 ± 0.007	ND[d]	0.0048 ± 0.0003	96
Normal maize	22.4–32.5	22 (95)	22 (95)	0.003 ± 0.001	0.0097 ± 0.0001	0.0013 ± 0.0007	31
Waxy maize	1.4–2.7	–	–	0.0012 ± 0.0006	ND	0.0005 ± 0.0001	46
High amylose maize	42.6–67.8	6.3 (95)	12.4 (95)	0.005 ± 0.001	0.015 ± 0.003	0.0076 ± 0.0006	–
Normal rice	5–28.4	23–30 (95)	11-18 (95)	0.013	0.048	–	24
Waxy rice	0–2.0	45–50 (95)	2.3-3.2 (95)	0.003	ND	–	–
High amylose rice	25–33	–	–	–	–	–	–
Normal wheat	18–30	18.3–26.6 (100)	1.55 (100)	0.001	0.058 ± 0.002	Trace	28
Waxy wheat	0.8–0.9	–	–	–	–	–	–

[a] *Calculated on the basis of integrated area of P-signals.*

[b] *Phosphate monoesters P-signals located at 4.0–4.5 ppm relative to external 80% orthophosphoric acid.*

[c] *Phospholipids P-signals located at -0.4–1.2 ppm.*

[d] *Not detectable.*

[e] *Calculated using 1% starch paste.*

(Source: Singh et al., 2003)

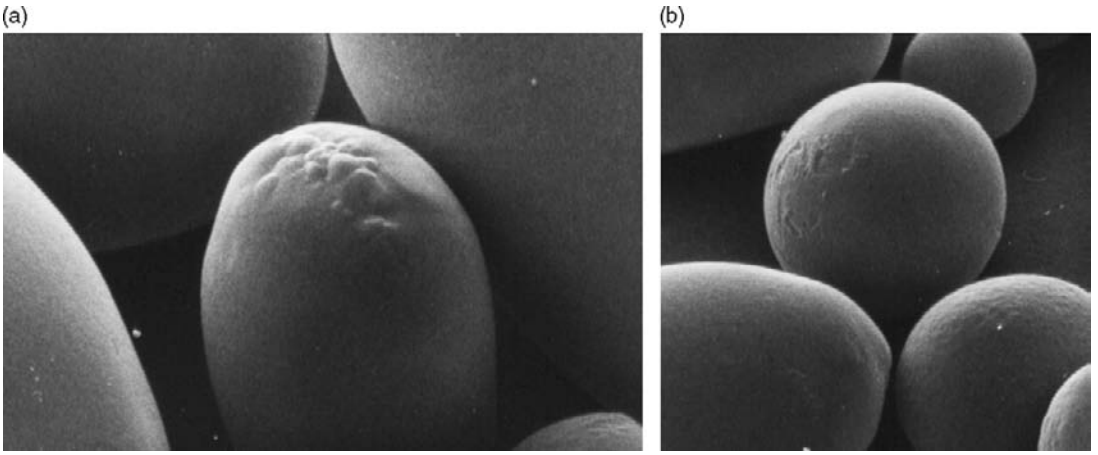


Figure 10.3: Scanning electron micrographs (SEM) featuring (a) the presence of some small size nodules or protuberances on some potato starch granules and (b) surface fragmentation on some potato starch granules (source: Singh et al., 2006).

large potato starch granules have a higher amylose content than small granules. The activities of GBSS, involved in the biosynthesis of linear components, and soluble starch synthase (SSS) and starch-branching enzymes (SBE), involved in the biosynthesis of branched components within the starch granule, may be responsible for the variation in amylose content among the various starches (Kossmann and Lloyd, 2000). Potato mutants lacking GBSS in potato have been reported to synthesize amylose-free starch (Hovenkamp-Hermelink et al., 1987). Also, the amylopectin fraction is synthesized at a faster rate than amylose during the early stages of starch granule growth owing to the high activities of SSS and SBE, which diminish during the later stages, while GBSS retains its activity throughout the growth period in association with the developing starch granule. Thus, the small granules, which are at initial stages of growth, may have higher SSS and SBE activities that result in lower amylose content. The variation in amylose contents among the starches from different and similar plant sources, in various studies (Table 10.2), could be due to the use of different starch isolation procedures and analytical methods used to determine amylose content.

Phosphorus is one of the non-carbohydrate constituents present in starches, which significantly affects the functional properties of starches. The phosphorus content varies from 0.003% in waxy maize starch to 0.09% in potato starch (Schoch, 1942; Table 10.2). Phosphorus is present as phosphate monoesters and phospholipids in various starches. Phosphate groups, esterified to the amylopectin fraction of potato starch, contribute to its high water-binding capacity, viscosity, transparency, and freeze–thaw stability (Craig et al., 1989). Phospholipids present in starch have a tendency to form complexes with amylose and long-branched chains of amylopectin, which limits the starch granule swelling, ultimately resulting in opaque and low-viscosity pastes. That is why wheat and rice starches with more phospholipids produce starch pastes with lower

transmittance than potato starches with less phospholipids. Phosphorus content and form in potato starch is influenced by growing conditions, temperature, and post-harvest storage of the potato tubers. It has been reported that 61% of the starch phosphate monoesters in potato starch are bound to C-6 of the glucose units, with 38% phosphate monoester on C-3 of the glucose, and possibly 1% of monoester on the C-2 position (Jane et al., 1996).

Potato starch contains fewer lipids than the cereal starches. Free fatty acids in rice and maize starches result in amylose–lipid complex formation, thereby contributing to their higher transition temperatures and lower retrogradation.

10.2.3 Swelling power and solubility

When the starch molecules are heated in excess water, their crystalline structure is disrupted and water molecules become linked to the exposed hydroxyl groups of amylose and amylopectin by hydrogen bonding, which causes an increase in granule swelling and solubility. The swelling power and solubility provide evidence of the magnitude of the interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction is influenced by the amylose to amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching, and conformation (Hoover, 2001). Potato starch exhibits much higher average swelling power than the cereal starches (Table 10.2). According to deWilligen (1976), maize and wheat granules may swell by up to 30 times their original volume and potato starch granules by up to 100 times their original volume, without disintegration. The higher swelling power and solubility of potato starch is probably due to the presence of a large number of phosphate groups on the amylopectin molecule. Repulsion between phosphate groups on adjacent chains increases hydration by weakening the extent of bonding within the crystalline domain. Small potato starch granules show greater hydration and swelling power than the large ones, which is mainly due to their higher specific surface area.

The differences in the swelling and solubility behavior of the starches between botanical sources and among the cultivars of any one botanical source are caused by differences in the amylose and the lipid contents, as well as the granule organization. Amylose plays an important role in restricting initial swelling because swelling proceeds more rapidly after amylose has first been exuded. The increase in starch solubility, with the concomitant increase in suspension clarity is seen mainly as the result of granule swelling, permitting the exudation of the amylose. The granules become increasingly susceptible to shear disintegration as they swell, and they release soluble material as they disintegrate. The hot starch paste is a mixture of swollen granules and granule fragments, together with colloidally and molecularly dispersed starch granules.

The mixture of the swollen and fragmented granules depends on the botanical source of the starch, water content, temperature, and shearing during heating. The extent of leaching of

solubles mainly depends on the lipid content of the starch and the ability of the starch to form amylose–lipid complexes, as the amylose involved in complex formation with lipids is prevented from leaching out. The cereal starches contain enough lipids to form lipid-saturated complexes with 7–8% of the amylose in the starch; hence the maximum amylose leached is about 20% of the total starch (Tester and Morrison, 1990). The higher solubility of potato starches may be attributed to the lack of starch–lipid inclusion complexing owing to absence of lipids.

10.2.4 Pasting/rheological properties

During gelatinization, the starch granule swells to several times its initial size, ruptures and simultaneously amylose leaches out and forms a three-dimensional network. Swelling of starch is the property of its amylopectin content, and amylose acts as both a diluent and an inhibitor of swelling (Tester and Morrison, 1990). Starch exhibits unique viscosity behavior with change of temperature, concentration, and shear rate (Nurul et al., 1999). This can be measured by the Brabender Visco-amylograph, and by the Rapid Visco-Analyser (RVA) pasting curves (Figure 10.4). The shape of the peak achieved is a reflection of the processes taking place during the pasting cycle. The height of the peak at the given concentration reflects the ability of the granules to swell freely before their physical breakdown. Potato starches are capable of swelling to a higher degree than cereal starches and are also less resistant to breakdown on cooking and hence exhibit viscosity decreases considerably after reaching the maximum value. The shape of the peak is, however, strongly influenced by the initial concentration of the starch suspension. The increase in viscosity during the cooling period indicates the tendency of various constituents present in the hot paste (swollen granules, fragments of swollen granules, colloidally and molecularly dispersed starch molecules) to associate or retrograde as the temperature of the paste decreases.

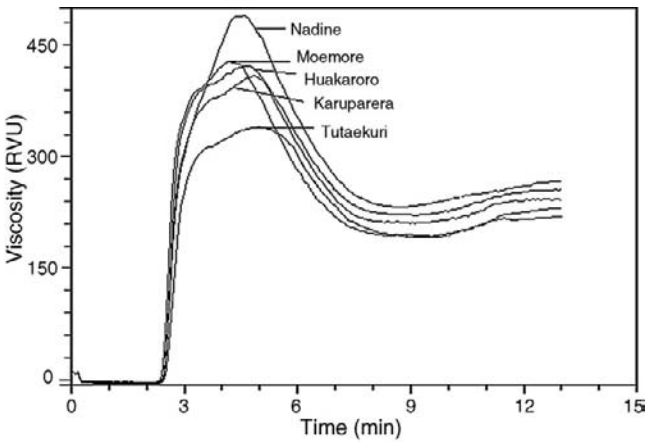


Figure 10.4: RVA pasting curves of starches isolated from different New Zealand potato cultivars (source: Singh et al., 2006).

Table 10.3: Rheological parameters of starches from different botanical sources during heating from 30 to 75°C, studied using dynamic rheometry

Source	TG' (°C)	*Peak* G' (Pa)	*Peak* G'' (Pa)	Breakdown in G' (Pa)	*Peak tan* δ
Potato[b]	62.7	8519	1580	3606	0.1565
Maize[a]	70.2	6345	1208	2329	0.1905
Rice[a]	72.4	4052	955	2831	0.1972
Wheat[a]	69.6	6935	1370	2730	0.1976

[a] *At 20% starch concentration.*

[b] *At 15% starch concentration.*

(Source: Singh et al., 2003)

Dynamic rheometry allows continuous assessment of dynamic moduli during temperature and frequency sweep testing of starch suspensions. The storage dynamic modulus (G') is a measure of the energy stored in the material and recovered from it per cycle while the loss modulus (G'') is a measure of the energy dissipated or lost per cycle of sinusoidal deformation (Ferry, 1980). The ratio of the energy lost to the energy stored for each cycle can be defined by the loss factor (*tan* δ), which is the ratio of G'' to G'. The G' of starch progressively increases greatly at a certain temperature (TG') to a maximum (peak G') and then drops with continued heating in a dynamic rheometer. The initial increase in G' is due to granular swelling to fill the entire available volume of the system to form a three-dimensional network of swollen granules. With further increase in temperature, G' decreases, indicating destruction of the gel structure during prolonged heating. This destruction is due to the melting of the crystalline regions remaining in the swollen starch granule (Eliasson, 1986).

The rheological properties of the different starches vary to a large extent with respect to the granular structure (Table 10.3). Maize starch has a lower peak G' and G'' than potato starch. A higher phosphate monoester content and the absence of lipids and phospholipids in the potato starch may also be responsible for the higher G' and G''. The phospholipids and the more rigid granules present in maize starch may be responsible for its lower G' of maize starch. The amylase–lipid complex formation during gelatinization of maize starch lowers the G' and G'' (Singh et al., 2002). The protein content of rice starch has been reported to be negatively correlated with peak viscosity and positively correlated with pasting temperature (Lim et al., 1999).

The extent of breakdown in starch pastes gives a measure of degree of disintegration of starch granules. The breakdown in G' is the difference between the peak G' at TG' and the minimum G' at 75°C. Potato starches generally show higher breakdown in G' than maize, rice, and wheat starches. The differences in the breakdown values of starches may be attributed to the granule rigidity, lipid content, and peak G' values. Amylose content is another factor, which affects the rheological and pasting properties of starch. Singh et al. (2007) reported higher G' values

for potato starches with higher amylose content during temperature sweep testing. Shewmaker et al. (1994) reported low paste viscosity for starch pastes made from potato genotypes containing low amylose content. Similarly, the starches isolated from waxy potatoes show lower G' and G'' and higher *tan* δ values (Kaur et al., 2002).

10.2.5 Gelatinization and retrogradation: thermal properties

The crystalline order in starch granules is often the basic underlying factor influencing its functional properties. Collapse of crystalline order within the starch granules manifests itself as irreversible changes in properties such as granule swelling, pasting and starch solubility loss of optical birefringence, loss of crystalline order, and uncoiling and dissociation of the double helices (Hoover, 2001). The gelatinization phenomenon starts at the hilum of the granule and the granule swells rapidly to the periphery. Gelatinization occurs initially in the amorphous regions as opposed to the crystalline regions of the granule, because hydrogen bonding is weakened in these areas. The order–disorder transitions that occur on heating an aqueous suspension of starch granules have been extensively investigated using dynamic scanning calorimetry (DSC). Starch transition temperatures (onset, T_o; peak, T_p; conclusion, T_c) of gelatinisation, and gelatinization enthalpy (ΔH_{gel}), measured by DSC have been related to degree of crystallinity. The onset temperature reflects the initiation of the gelatinization process, which is followed by a peak and conclusion temperature. After T_c, all amylopectin double helices have dissociated, although swollen granule structures will be retained until more extensive temperature and shear have been applied (Tester and Debon, 2000). A high degree of crystallinity provides structural stability and makes the granule more resistant to gelatinization, ultimately resulting in higher transition temperatures; and is affected by chemical composition of starch (Barichello et al., 1990).

Starches from different botanical sources, differing in composition, exhibit different transition temperatures and enthalpies of gelatinization. Singh et al. (2003) reported a great deal of research on the gelatinization parameters of starches from different botanical sources (Table 10.4). Granule shape, percentage of large and small granules, and presence of phosphate esters also affect the gelatinization enthalpy value of various starches (Singh et al., 2006; Yuan et al., 1993). The higher transition temperatures of maize and rice starch could be the result of their more rigid granular structure and the presence of lipids.

The gelatinization and swelling properties are controlled in part by the molecular structure of amylopectin (unit chain length, extent of branching, molecular weight, and polydispersity), starch composition (amylose to amylopectin ratio and phosphorous content), and granule architecture (crystalline to amorphous ratio) (Tester, 1997). T_p gives a measure of crystallite quality (double helix length), whereas enthalpy of gelatinization (ΔH_{gel}) gives an overall measure of crystallinity (quality and quantity) and is an indicator of the loss of molecular order within the granule (Tester and Morrison, 1990; Cooke and Gidley, 1992). Gernat et al. (1993) have

Table 10.4: Gelatinization parameters (studied using DSC) of the starches from different botanical sources

Source	Methodology	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)[a]
Potato	S:W[b] 1:2.3	59.72–66.2	62.9–69.6	67.28–75.4	12.55–17.9
Potato	S:W 1:3.3	57.0–68.3	60.6–72.4	66.5–78.0	13.0–15.8
Potato	S:W 1:1.5	57.2	61.4	80.3	17.4
Normal maize	S:W 1:1.5	62.3	67.7	84.3	14.0
Normal maize	S:W 1:3	64.1	69.4	74.9	12.3
Normal maize	S:W 1:9	65.7	71.0	–	12.0
Waxy maize	S:W 1:9	66.6	73.6	–	14.2
Waxy maize	S:W 1:3	64.2	69.2	74.6	15.4
High amylose maize	S:W 1:9	66.8	73.7	–	13.7
Rice	S:W 1:1.5	62.0	67.4	97.5	11.0
Rice	S:W 1:9	57.7	65.1	–	11.5
Rice	S:W 1:2.3	66.0–67.26	69.74–71.94	74.08–78.04	8.16–10.88
Rice	S:W 1:3	70.3	76.2	80.2	13.2
Waxy rice	DSC	66.1–74.9	70.4–78.8	–	7.7–12.1
Wheat	S:W 1:1.5	51.2	56.0	76.0	9.0
Wheat	S:W 1:2.3	46.0–52.4	52.2–57.6	57.8–66.1	14.8–17.9
Wheat	S:W 1:3	57.1	61.6	66.2	10.7

T_o = onset temperature; T_p = peak temperature; T_p = final temperature; ΔH_{gel} = enthalpy of gelatinization (dsb, based on dry starch weight).

[a]*enthalpy values are expressed in J/g of dry starch.*

[b]*starch (S): water (W).*

(Source: Singh et al., 2003)

stated that the amount of double-helical order in native starches is strongly correlated to the amylopectin content, and granule crystallinity increases with amylopectin content. This suggests that ΔH_{gel} should preferably be calculated on an amylopectin basis. However, ΔH_{gel} for different starches, given in Table 10.4, are not calculated in this manner. Because amylopectin plays a major role in starch granule crystallinity, the presence of amylose lowers the melting point of crystalline regions and the energy for starting gelatinization (Flipse et al., 1996). More energy is needed to initiate melting in the absence of amylose-rich amorphous regions (Krueger et al., 1987).

The potato amylopectin starches exhibit higher endothermic temperatures and enthalpies than the normal potato starches. The amorphous amylose in normal potato starches decreases the relative amount of crystalline material in the granule, thereby lowering the gelatinization parameters (Svegmark et al., 2002). However, the high amylose starches with longer average chain length exhibit higher transition temperatures (Jane et al., 1999).

The molecular interactions (hydrogen bonding between starch chains) after cooling of the gelatinized starch paste have been called retrogradation (Hoover, 2001). During retrogradation,

Table 10.5: Thermal properties during retrogradation of starches from different botanical sources

Source	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{ret} (J/g)	R (%)[h]
Potato[a]	59.72–60.70	63.26–64.58	67.28–70.34	6.42–8.61	51.50–62.16
Potato[b]	42.5	55.7	66.9	7.5	43.4
Normal maize[b]	39.0	50.1	59.4	5.8	47.6
Waxy maize[b]	40.2	51.3	60.2	7.3	47.0
High amylose maize[b]	44.1	ND[g]	115.4	9.9	61.0
Normal rice[b]	40.3	51.0	60.4	5.3	40.5
Normal rice[c]	37.05–38.43	49.80–52.59	62.42–65.92	–	–
Waxy rice[c]	36.72–37.25	50.65–51.26	62.56–62.93	–	–
Waxy rice[b]	43.2	50.6	55.2	0.8	5.0
Normal wheat[b]	38.6	47.6	55.7	3.6	33.7
Normal wheat[d]	29.8–31.7	41.8–42.7	–	7.0–8.5	–
Normal wheat[e]	30.9–32.6	41.2–42.6	–	8.1–9.7	–
Normal wheat[f]	20.4–20.6	33.2–33.7	50.0	10.1–10.6	–
Waxy wheat[f]	19.9–20.5	33.1–33.8	50.4–51.8	11.4–12.6	–

T_o = onset temperature; T_p = peak temperature; T_p = final temperature; ΔH_{ret} = enthalpy of retrogradation (dsb, based on dry starch weight).

[a]*storage at 4°C for two weeks.*

[b]*storage at 4°C for 7 days.*

[c]*storage at 4°C for 4 weeks.*

[d]*storage at 5°C for 2 weeks.*

[e]*storage at 5°C for 4 weeks.*

[f]*storage at 5°C for 4 weeks.*

[g]*Not detectable.*

[h]*Retrogradation (%) = $\Delta H_{gel}/\Delta H_{ret}$.*

(Source: Singh et al., 2003)

amylose forms double helical associations of 40–70 glucose units (Jane and Robyt, 1984) whereas amylopectin recrystallizes by the association of the outermost short branches (Ring et al., 1987). In retrograded starch, the value of enthalpy of gelatinization provides a quantitative measure of the energy transformation that occurs during the melting of recrystallized amylopectin as well as precise measurements of the transition temperatures of the endothermic event (Karim et al., 2000). The endothermic peak of starches after gelatinization and storage at 4°C appears at lower transition temperatures. Transition temperatures and retrogradation enthalpy (ΔH_{ret}) at the end of the storage period drop considerably, compared to transition temperatures and enthalpy (ΔH_{gel}) during gelatinization (Table 10.5). Starch retrogradation enthalpies and transition temperatures are usually 60–80% and 10–26°C lower, respectively, than those for gelatinization of starch granules (Baker and Rayas-Duarte, 1998). The crystalline forms for retrograded starch are different in nature from those present in the native starch granules and may be weaker than the latter, because recrystallization of amylopectin occurs in a less ordered manner during retrogradation than during granule formation in native raw starches.

The extent of decrease in transition temperatures and enthalpy is higher in stored potato starch gels than in cereal starch gels, which shows higher tendency of potato starch towards retrogradation (Kaur et al., 2007; Singh et al., 2008). The variation in thermal properties of starches after gelatinization and during refrigerated storage may be attributed to variation in the amylose to amylopectin ratio and the presence/absence of lipids. The amylose content has been reported to be one of the influential factors in starch retrogradation (Fan and Marks, 1998; Kaur et al., 2007). A greater amount of amylose has traditionally been linked to a greater retrogradation tendency in starches (Whistler and BeMiller, 1996) but amylopectin and intermediate materials also play an important role in starch retrogradation during refrigerated storage (Yamin et al., 1999). The intermediate materials with longer chains than amylopectin may also form longer double helices during re-association under refrigerated storage conditions. Retrogradation has also been accelerated by amylopectin with larger chain lengths (Yuan et al., 1993).

10.3 Potato Starch Modification

Modified starches are native starches that have been altered chemically or physically in order to improve their functional properties (viscosity, surface activity, enzyme resistance, etc.) for a specific use in industry (Ortega-Ojeda et al., 2005). Potato starch, like other starches, is modified to overcome limitations of the native starch, such as low shear, acid and thermal resistance, and its high tendency towards retrogradation. The larger size of the native potato starch granules and their high swelling capacity lead to exceptionally large (in volume) swollen granules, which not only result in a high viscosity but also give rise to a less smooth texture. Moreover, the higher fragility of the swollen potato starch granules makes them prone to disperse or solubilize on heating and shearing, resulting in weak bodied, stringy, and cohesive pastes. The processing of potato starch therefore results in overcooking.

Modification, which involves the alteration of the physical and chemical characteristics of the native potato starch to improve its functional characteristics, can be used to tailor it to specific food applications. The rate and efficacy of any starch modification process depend on the botanical origin of the starch; and on the size and structure of its granules. This also includes the surface structure of the granules, which encompasses the outer and inner surface depending on the pores and channels, which cause the development of the so-called specific surface (Juszczak, 2003). Potato starch modification can be achieved in three different ways: physical, conversion, and chemical (derivatization) (Table 10.6).

10.3.1 Physical modification

Physically modified potato starch is preferred in processed foods because of its improved functional properties over those of its native counterpart. Moreover, this modified strach can be safely used in different food products and other industrial applications. Different physical

Table 10.6: Some common potato starch modification types and preparation techniques

Modification	Types	Preparation
Physical	Heat/moisture/ pressure treatment	Heat-moisture treatment – Heating starch at a temperature above its gelatinization point with insufficient moisture to cause gelatinization Annealing – Heating a slurry of granular starch at a temperature below its gelatinization point for prolonged periods of time High-pressure treatment – Treating starch at ultra-high pressure (above 400 MPa), as an effect of which starch gelatinizes but shows very little swelling and maintains its granular character
	Pregelatinization	Pregels/instant/cold-water swelling starches prepared using drum drying/spray cooking/extrusion/solvent-based processing
Conversion	Partial acid hydrolysis	Treatment with hydrochloric acid or ortho-phosphoric acid or sulphuric acid
	Partial enzymatic hydrolysis	Treatment in an aqueous solution at a temperature below the gelatinization point with one or more food-grade amylolytic enzymes
	Alkali treatment	Treatment with sodium hydroxide or potassium hydroxide
	Oxidation/bleaching	Treatment with peracetic acid and/or hydrogen peroxide, or sodium hypochlorite or sodium chlorite, or sulfur dioxide, or potassium permanganate or ammonium persulfate
	Pyroconversion (dextrinization)	Pyrodextrins – Prepared by dry roasting acidified starch
Derivatization	Etherification	Hydroxypropyl starch – Esterification with propylene oxide
	Esterification	Starch acetate – Esterification with acetic anhydride or vinyl acetate Acetylated distarch adipate – Esterification with acetic anhydride and adipic anhydride Starch sodium octenylsuccinate – Esterification by octenylsuccinic anhydride
	Cross-linking	Monostarch phosphate – Esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate Distarch phosphate – Esterification with sodium trimetaphosphate or phosphorus oxychloride Phosphated distarch phosphate – Combination of treatments for monostarch phosphate and distarch phosphate
	Dual modification	Acetylated distarch phosphate – Esterification by sodium trimetaphosphate or phosphorus oxychloride combined with esterification by acetic anhydride or vinyl acetate Hydroxypropyl distarch phosphate – Esterification by sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide

(Source: Singh et al., 2007)

modification methods of potato starch include annealing (ANN); heat–moisture treatment (HMT); high-pressure treatment (HPT); a recently reported method, osmotic pressure treatment (OPT; Pukkahuta et al., 2007); and pre-gelatinization. Microwave heating and irradiation are some other physical modification methods currently employed.

10.3.1.1 Heat/moisture/pressure treatments

ANN represents 'physical modification of potato starch slurries in water at temperatures below gelatinization, whereas HMT 'refers to the exposure of starch to higher temperatures at very restricted moisture content (18–27%)' (Collado and Corke, 1999). Thus, both ANN and HMT are related processes, critically controlled by starch to moisture ratio, temperature, and heating time. Both these processes occur at above the glass transition temperature (T_g, a transition of the amorphous regions from a rigid glassy state to a mobile rubbery state, which leads to dissociation of double helices in crystallites) but below the onset of gelatinization (T_o) (Jacobs and Delcour, 1998). In both HMT and ANN, physical reorganization within starch granules is manifested. However, HMT requires higher temperatures to cause this reorganization because of low levels of water in the system, which lead to an elevation of T_g.

HMT leads to an increase in starch gelatinization temperatures and viscosity; and narrowing of the gelatinization range (T_c–T_o). These properties can be explained on the basis of more glassy amorphous regions within annealed potato starch granules, and a more ordered registration of amylopectin double helices restricts the ease of hydration of the starch granules during gelatinisation, and elevates gelatinization temperatures (Tester and Debon, 2000). Similar effects could be achieved by ANN, but, because of the lower temperature used, this treatment needs a much longer time, thus, may have some industrial implications, in terms of energy and time. In contrast, microwave treatment requires a shorter time to modify potato starch characteristics to the same extent as ANN and HMT (Lewandowicz et al., 2000). No major changes occur in granule morphology (size and shape) after these treatments. However, HMT converts the B-type X-ray diffraction pattern of potato starch to the A-type. Examples of HMT starches include pre-treatment of starches for infant foods and processing of potato starch to replace maize starch during shortages, to impart freeze–thaw stability and to improve its baking quality (Collado and Corke, 1999).

HPT is treating starch at ultra-high pressure (above 400 MPa), as an effect of which starch gelatinizes but shows very little swelling and maintains its granular character. This results in altered paste and gel properties (Stute et al., 1996). B-type starches such as potato are more pressure-resistant and need more pressure to gelatinize them completely than do the A- and C-type starches. (Kudla and Tomasik, 1992). Blaszczak et al. (2005) reported a decrease in transition temperatures after high-pressure treatment (using 600 MPa for 2 and 3 minutes) in potato starch. They also observed the that potato starch granule's surface was more resistant

to the treatment than its inner part. The inner part of the granule has been reported to be filled with a gel-like network, while the granular form of the starch is still retained. The high-pressure treatment causes reversible hydration of the amorphous phase followed by irreversible distortion of the crystalline region, which destroys the granular structure.

OPT is a new method of physical modification, in which potato starch is suspended in solution saturated with a salt such as sodium sulfate and heated (autoclaved) at temperatures above 100°C for different times. This treatment has been reported to have the same effects on the starch properties as HMT but the starch modified using OPT exhibits better homogeneity (Pukkahuta et al., 2007).

10.3.1.2 Pregelatinization

Physical modification can be employed to convert native potato starch to cold-water-soluble starch or small-crystallite starch. The common methods used in the preparation of this type of starch involve immediate cooking – drying of starch suspensions using drum drying, puffing, continuous cooking – puffing – extruding, and spray drying/injection and nozzle spray drying (Lewandowicz and Soral-Śmietana, 2004). Rehydration of drum-cooked starch at room temperature gives a paste of reduced consistency with a dull, grainy appearance and gels of reduced strength (Rajagopalan and Seib, 1992). Slight chemical treatments are also used in the preparation of this type of starch, such as heating of starch in aqueous alcohol solution or alcoholic alkali, or polyhydric alcoholic treatments. The granulated cold-water-soluble starches (GCWS) prepared by these methods exhibit different cold water solubilities, give greater viscosity and smoother texture, and have more processing tolerance than traditional pre-gelatinized starches. The alcoholic-alkaline treatment causes indentation and distortion of potato starch granular structure (Figure 10.5).

10.3.2 Conversion

10.3.2.1 Acid/enzymatic hydrolysis

Acid-thinned potato starch is normally prepared by controlled hydrolysis of native starch with mineral acid, either at room temperature (for several days) or at elevated temperature (but below gelatinization temperature) for several hours. The derived degradation products (glucose, high maltose syrups, and maltodextrins) have reduced hot-paste viscosity, increased solubility and gel strength and have wide applications in the food, paper, and textile industries. Acid modification can be achieved by both dry and wet processes. Dry processes involve dry roasting of starch in the presence of limited moisture and certain levels of acid. During dry roasting processes, hydrolysis of the glucosidic bond occurs along with molecular rearrangement that leads to the formation of dextrins. Dextrins are more soluble, have lower viscosity than the wet-hydrolysis products, and find applications in adhesives, gums, and pastes. In wet processes, acids are used at 1–3% concentration based on starch dry solids. The type of acid used for hydrolysis greatly

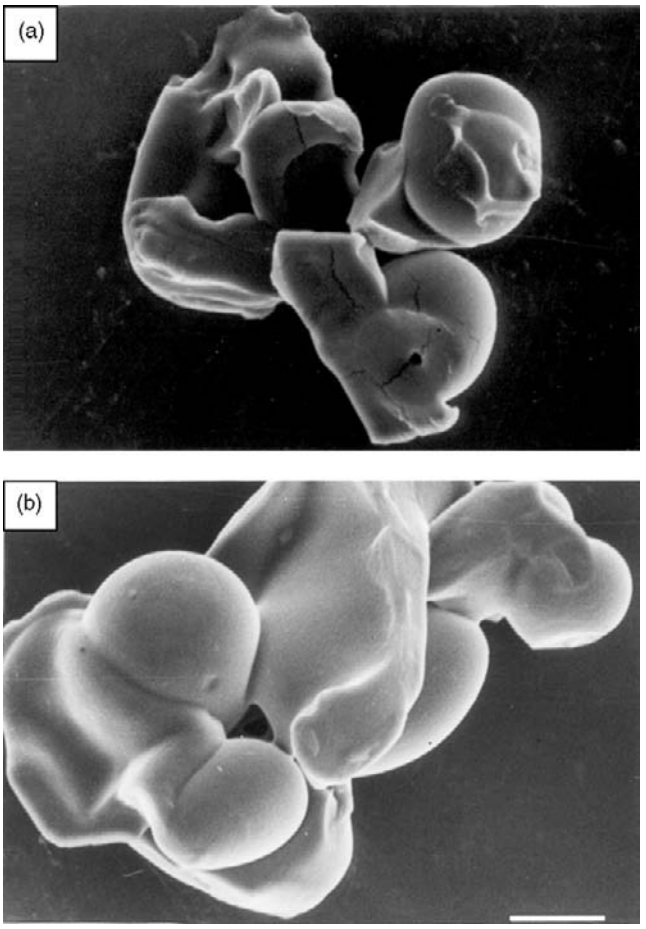


Figure 10.5: Scanning electron micrographs (a & b) showing granular indentation and fragmentation in granular cold water-soluble starches (source: Singh and Singh, 2003).

influences the molecular weight, alkali fluidity number, iodine binding capacity, and intrinsic viscosity of the converted starch. The molecular weight of starch decreases after modification, with ortho-phosphoric acid causing least, and hydrochloric and nitric acids the highest reduction (Singh and Ali, 2000).

Acid-modified starch can be used at a higher solids concentration for immediate gelling and provides gum or jelly with shorter texture and flexibility (Zallie, 1988). In acid modification, the hydroxonium ion attacks the glucosidic oxygen atom and hydrolyzes the glucosidic linkage. Acid depolymerization occurs first in the amorphous regions before hydrolysis of the crystalline regions of the starch granule. Also, acid attacks the granule surface first before entering the granule interior (Wang and Wang, 2001). Some of the problems encountered

during acid hydrolysis, such as random attack at the branch point (which could lead to an increase in linearity of the starch), high glucose yield, and acid removal later on, mean that enzymatic hydrolysis is preferred over acid hydrolysis. Acid modification in the presence of long-chain alcohols helps to reduce the degree of polymerization of amylopectin more effectively and also converts the crystalline regions into more amorphous regions that are prone to acid hydrolysis. Nageli dextrins, lintner starch, etc., are some of the acid-modified starch derivatives.

Enzymatic modification of potato starch on an industrial scale is generally carried out using starch hydrolyzing enzymes such as α-amylase, β-amylase, pullulanase, glucoamylase, and isoamylase (van der Maarel et al., 2002). These enzymes hydrolyze the α-1,4 or α-1,6 glycosidic bonds in amylose and amylopectin by first breaking the glycosidic linkage and subsequently using a water molecule as an acceptor substrate. Another group of enzymes, such as cyclodextrin glycosyltransferase and amylomaltase modifies starch by using the transferase reaction. These enzymes initially break the glycosidic linkage but use another oligosaccharide as an acceptor substrate instead of water to form a new glycosidic linkage. Cyclodextrins, cyclic oligosaccharides composed of six, seven, or eight glucose units linked by the α-1,4 linkage are produced by enzymatic hydrolysis followed by an enzymatic conversion by the action of cyclodextrin glycosyltransferase. These cyclodextrins are called α-, β-, and γ-cyclodextrins (French et al., 1954). These cyclodextrins have a polar hydrophilic exterior and an apolar hydrophobic interior, which makes them suitable candidates to form inclusion complexes with hydrophobic molecules of suitable dimension and configuration (Tharanathan, 2005). This property is made use of in food, pharmaceutical and agrochemical industrial applications in masking unpleasant odors and flavors, removal of undesirable components by their inclusion in the cyclodextrin cavity, etc. A novel thermoreversible gelling product has been prepared by hydrolysis of potato starch using amylomaltase, which may be a good plant-derived substitute for gelatin (van der Maarel et al., 2005).

10.3.2.2 Oxidation/bleaching

Bleached or oxidized starches are produced by reacting starch with a specified amount of reagent under controlled temperature and pH. The starches treated with low levels of reagents, such as hydrogen peroxide, peracetic acid, ammonium persulfate and sodium hypochlorite, in aqueous slurry are referred to as bleached starches.

The reagents used to oxidize starch include periodate, chromic acid, permanganate, nitrogen dioxide and sodium hypochlorite. pH, temperature, reagent concentration and starch molecular structure are the main factors controlling oxidation. During oxidation, hydroxyl groups on starch (at the C-2, C-3, and C-6 positions) are first oxidized to carbonyl and then to carboxyl groups. These bulky carboxyl groups result in low retrogradation of oxidized starch paste (Wurzburg, 1986a). The number of carboxyl groups on starch determines the extent of modification.

Oxidized starches show reduced viscosity, transition temperatures, and enthalpies of gelatinization and retrogradation (Adebowale and Lawal, 2003).

Oxidation also results in starch depolymerization, which is the cause of the low viscosity and improved clarity and stability exhibited by oxidized starches. Oxidized starches are used in foods as coating and sealing agents in confectionary, as an emulsifier and as a dough conditioner for bread, whereas bleached starches are used for improved adhesion of batter and breading mixes in fried foods.

10.3.3 Derivatization

Derivatization is the most widely employed method for industrial-scale starch modification; therefore we have discussed this modification in more detail.

10.3.3.1 Common types of derivatization

Derivatization involves the introduction of functional groups into starch resulting in markedly altered physico-chemical properties. The properties of some modified starches and their applications are presented in Table 10.7. The chemical and functional properties achieved when modifying starch by chemical substitution depend, inter alia, on starch source, reaction conditions (reactant concentration, reaction time, pH and the presence of catalysts), type of substituent, extent of substitution (degree of substitution, DS*; or molar substitution, MS**), and the distribution of the substitutent in the starch molecule (Kavitha and BeMiller, 1998; Richardson et al., 2000; Rutenberg and Solarek, 1984). (*DS represents the average number of hydroxyl groups on each anhydroglucose unit, which are derivitized by substituent groups. DS is expressed as average number of moles of substituent per anhydroglucose unit. As each anhydroglucose unit has three hydroxyl groups available for substitution the maximum possible DS is 3. **The substituent moiety on some starch esters and ethers can react further with the modifying reagent during the modification reaction, resulting in the formation of an oligomeric or possibly polymeric substituent. In these cases molar substitution is preferred, which represents the level of substitution as moles of monomeric substituent per mole of anhydroglucose unit. Thus, in contrast to degree of substitution, the value of MS can be greater than 3.)

Some chemical modification reactions of starch are presented in Table 10.8. The commonly employed methods of modifying potato starch for use in the food industry include etherification, esterification, and cross-linking. Acetylated potato starch with a low DS is commonly obtained by the esterification of native starch with acetic anhydride in the presence of an alkaline catalyst, whereas the food-grade hydroxypropylated starches are generally prepared by etherification of native starch with propylene oxide in the presence of an alkaline catalyst. Food-grade starches are hydroxypropylated to increase paste consistency and clarity, and to impart freeze–thaw and cold-storage stabilities (Xu and Seib, 1997). The hydrophilic hydroxypropyl groups introduced into the starch chains weaken the granular structure of starch by disrupting inter- and

Table 10.7: Some properties and applications of modified starches

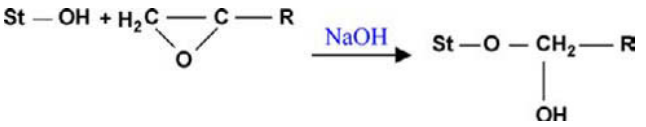
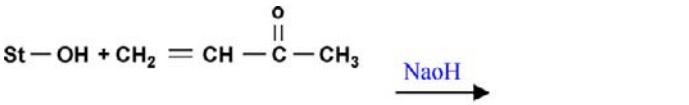
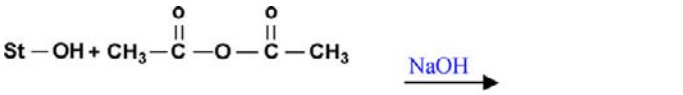
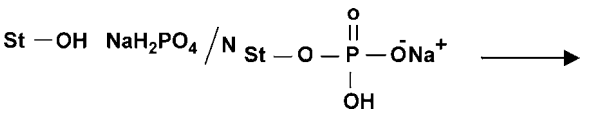
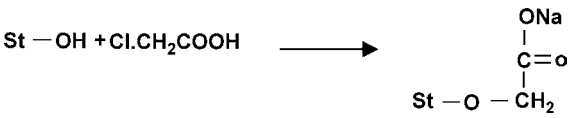
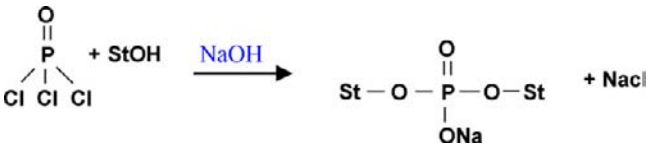
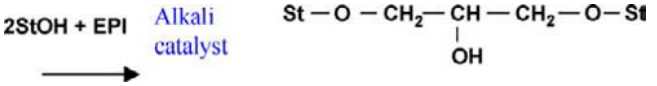
Types	Properties	Applications
Pregelatinization	Cold water dispersibility	Useful in instant convenience foods
Partial acid or enzymatic hydrolysis	Reduced molecular weight polymers, exhibit reduced viscosity, increased retrogradation and setback	Useful in confectionery, Batters and food coatings
Oxidation/bleaching	Low viscosity, high clarity, and low temperature stability	Used in batters and breading for coating various food stuffs, in confectionery as binders and film formers, in dairy as texturizers
Pyroconversion (dextrinization)	Low to high solubility depending on conversion, low viscosity, high reducing sugar content	Used as coating materials for various foods, good film forming ability and as fat replacers in bakery and dairy products
Etherification	Improved clarity of starch paste, greater viscosity, reduced syneresis and freeze-thaw stability	Used in wide range of food applications such as gravies, dips, sauces, fruit pie fillings and puddings
Esterification	Lower gelatinization temperature and retrogradation, lower tendency to form gels and higher paste clarity	Used in refrigerated and frozen foods, as emulsion stabilizers and for encapsulation
Cross-linking	Higher stability of granules towards swelling, high temperature, high shear and acidic conditions	Used as viscosifiers and texturizers in soups, sauces, gravies, bakery and dairy products
Dual modification	Stability against acid, thermal and mechanical degradation and delayed retrogradation during storage	Used in canned foods, refrigerated and frozen foods, salad dressings, puddings and gravies

(Source: Singh et al., 2007)

intra-molecular hydrogen bonding, leading to an increase in accessibility of the starch granules to water. Chemical modification of native granular starches by etherification also alters the gelatinization and retrogradation behavior of starches.

Cross-linking treatment is aimed to add chemical bonds at random locations in a granule, which stabilize and strengthen the granule. Starch pastes from cross-linked potato starches are more viscous, heavily bodied, and are less prone to breakdown with extended cooking times, increased acid content, or severe agitation. Cross-linking minimizes granule rupture, loss of viscosity, and the formation of stringy paste during cooking (Woo and Seib, 1997), yielding potato starch that is suitable for canned foods and other food applications (Rutenberg and Solarek, 1984). Nutritional benefits of cross-linked starch as a new source of dietary fiber have also been reported (Woo, 1999; Wurzburg, 1986c).

Table 10.8: Some common starch chemical modification reactions

Modification type	
Etherification Hydroxyalkyl starch (with alkylene oxide)	
Esterification Starch acetate (with vinyl acetate)	
  Starch acetate (with acetic anhydride)	
Starch phosphate (with orthophosphates)	
Carboxymethyl starch (with mono chloro acetic acid)	
Cross-linking With POCl3	
  With STMP	
  With EPI	

St = Starch; $POCl_3$ = Phosphorus oxychloride; STMP = Sodium tri-metaphosphate; EPI = Epichlorohydrin.
(Source: Singh et al., 2007)

Cross-linking is generally performed by treatment of granular potato starch with multifunctional reagents capable of forming either ether or ester inter-molecular linkages between hydroxyl groups on starch molecules (Rutenberg and Solarek, 1984; Wurzburg, 1986b). Sodium trimetaphosphate (STMP), monosodium phosphate (SOP), sodium tripolyphosphate (STPP), epichlorohydrin (EPI), phosphoryl chloride ($POCl_3$), a mixture of adipic acid and acetic anhydride, and vinyl chloride are the main agents used to cross-link food-grade starches (Woo and Seib, 1997; Yeh and Yeh, 1993). $POCl_3$ is an efficient cross-linking agent in aqueous slurry at pH > 11 in the presence of a neutral salt (Felton and Schopmeyer, 1943). STMP is reported to be an efficient cross-linking agent at high temperature with semi-dry starch and at moderate temperature with hydrated starch in aqueous slurry (Kerr and Cleveland, 1962). EPI is poorly soluble in water and partly decomposes to glycerol; thus water-soluble cross-linking agents such as $POCl_3$ and STMP are preferred. Moreover, it has also been reported that EPI cross-links are likely to be less uniformly distributed than STMP ones (Shiftan et al., 2000). So, the type of cross-linking agent greatly determines the change in functional properties of the treated starches. Cross-linking at low levels, although having a substantial effect on potato starch properties such as granule swelling (Rutenberg and Solarek, 1984) contributes little to water sorption properties (Chilton and Collison, 1974).

Starch phosphates, which are conventionally prepared, have been reported to give clear pastes of high consistency with good freeze–thaw stability and emulsifying properties, and may be grouped into two classes: monostarch phosphates and distarch phosphates (cross-linked starches). In general, monostarch phosphates (monoesters) can have a higher DS than distarch phosphates (diesters) because even a very few cross-links (in the case of diesters) can drastically alter the paste and gel properties of the starch. Starch phosphates are conventionally prepared through the reaction of potato starch with salts of ortho-, meta-, pyro-, and tripolyphosphoric acids and phosphorus oxychloride (Nierle, 1969; Paschall, 1964).

Dual modification, a combination of substitution and cross-linking, has been demonstrated to provide stability against acid, thermal, and mechanical degradation of starch and to delay retrogradation during storage. Dual modified starches are used commonly in salad dressings, canned foods, frozen foods, and puddings. The control of reaction conditions is important during preparation of dual-modified cross-linked/hydroxypropylated starches using different cross-linking reagents with different starch bases such as maize, tapioca, wheat, waxy maize, waxy barley, rice, and sago (Tessler, 1975; Wattanchant et al., 2003; Wu and Seib, 1990; Yeh and Yeh, 1993; Yook et al., 1993). The quantity of cross-linking reagent required to prepare a dual modified starch (with desirable properties) varies with the source of starch, the type of cross-linking reagent, the efficiency of the cross-linking reaction, the degree of substitution required and the specified range of final modified-starch properties. The effects of different reaction conditions such as starch base concentration, temperature, pH, and the concentration of the

catalyst salt play an important role during preparation of dual modified hydroxypropylated-crosslinked starch. The effects of chemical modifications on thermal, morphological, and pasting/rheological behavior of starches may be quantified using instrumentation such as DSC, SEM, Viscoamylograph/RVA and dynamic rheometer, respectively (Kaur et al., 2006; Singh et al., 2007).

10.3.3.2 Extent of derivatization

The rate and efficiency of this chemical modification process depends on the reagent type, botanical origin of the starch, and on the size and structure of the starch granules (Huber and BeMiller, 2001). This also includes the surface structure of the starch granules, which encompasses the outer and inner surface, depending on the pores and channels, leading to the development of the so-called specific surface (Juszczak, 2003). Channels that open to the granule exterior provide a much larger surface area accessible by chemical reagents, and provide easier access by the reagents to the granule interior. However, the reagent may diffuse through the external surface to the granule matrix in the absence of channels (Bemiller, 1997). Although starches from various sources exhibit fundamental structural similarities, they differ in the specific details of their microstructure and ultrastructure. These structural differences affect the chemical modification process to a great extent.

The DS and MS of some chemically modified starches prepared from different sources are presented in Table 10.9. Potato, maize, and rice starches show significant variation in their DS when acetylated under similar reaction conditions (Singh et al., 2004a, b). Factors such as amylose to amylopectin ratio, intragranule packing and the presence of lipids mainly govern the degree of substitution during acetylation of starches from different sources (Phillips et al., 1999).

The C=O bond of the acetyl group experiences a different molecular environment depending on whether it is a substituent on amylose or on amylopectin (Phillips et al., 1999). Acetylation occurs in all the amorphous regions and at the outer lamellae of crystalline regions, rather than throughout the crystalline regions of the whole starch granule, owing to poor penetrating ability of acetic anhydride in starch granules (Chen et al., 2004). Studies (Singh et al., 2004a) on acetylated potato starches suggested that the small-granule population with lower amylose content favors the introduction of acetyl groups and hence results in higher DS.

During hydroxypropylation, the hydroxypropyl groups are primarily introduced into the starch chains in the amorphous regions composed mainly of amylose (Steeneken and Smith, 1991). Amylose is modified to a greater extent than amylopectin in hydroxypropylated maize and potato starches, and the modification of amylopectin occurs close to the branch points because of the greater accessibility of the amorphous regions to the modifying reagent (Kavitha and

Table 10.9: DS[a] and MS[b] of some modified starches from different botanical sources

Starch source	DS (Acetylated)	MS (Hydroxypropylated)	DS (Cross-linked)
Normal Potato	0.115–0.238[d]	0.098–0.122[h]	0.07–0.26[l]
Normal Maize	0.104–0.184[d]	0.091–0.092[i]	0.09–0.25[l]
Normal maize	ND[c]	0.061–0.094[j]	ND
Waxy maize	0.081	0.067–0.127[j]	ND
Hi amylose maize	ND	0.078–0.119[j]	ND
Hybrid normal maize	0.030–0.040[e]	ND	ND
Normal wheat	0.035–0.131[f]	0.117–0.123[i]	0.004–0.020[m]
Normal rice	0.087–0.118[g]	>0.03[k]	0.025–0.035[n]
Normal rice	0.018	ND	ND
Waxy rice	0.016	ND	ND

[a] *DS = degree of substitution.*
[b] *MS = molar substitution.*
[c] *ND = Not detected.*
[d] *different levels of acetylation and starches from different potato cultivars.*
[e] *two levels of acetylation used.*
[f] *different levels of acetylation used.*
[g] *starches from different rice cultivars.*
[h] *starches from different potato cultivars.*
[i] *low MS values from two populations of starch granules.*
[j] *two levels of hydroxypropylation.*
[k] *two levels of hydroxypropylation.*
[l] *DS of starches phosphorylated using a mixture of monosodium and disodium phosphate.*
[m] *molar substitution values; three levels of cross-linking performed.*
[n] *different levels of cross-linking performed.*
(Source: Singh et al., 2007)

BeMiller, 1998). However, Richardson et al. (2001), after investigating the substituent distribution in hydroxypropylated potato amylopectin (PAP) starch suggested that the hydroxypropyl groups are homogeneously distributed on the amylopectin molecule. The modification reaction conditions and starch source may also affect the distribution of hydroxypropyl groups along the starch chain (Kaur et al., 2004; Steeneken and Woortman, 1994). Investigations carried out on hydroxypropylated PAP prepared in granular slurry or solution suggest that more substituents were located in close vicinity to branching points, which constitute the amorphous areas in the semicrystalline granule, than elsewhere. PAP starch hydroxypropylated in a granule slurry had a more heterogeneous substituent distribution compared with starch modified in a polymer 'solution' of dissolved starch (Richardson et al., 2001). The reactivity and concentration of reagents influence the degree of substitution of cross-linked starches. Also, the type of reagent used and the reaction conditions determine the ratio of mono- to di-type bonds (esters with phosphorus based agents, and glycerols with EPI) during cross-linking (Koch et al., 1982).

Characterization of substitution upon modification is important at both monomer and polymer levels (Richardson et al., 2003). The distribution of substituents at both monomeric and polymeric levels may be affected by the presence of granule pores and channels; the proportions of amylose and amylopectin and their arrangement; the nature of the granule surface and granule swelling (Bemiller, 1997; Kavitha and BeMiller, 1998). Techniques such as nuclear magnetic resonance (NMR) spectroscopy (Heins et al., 1998; Xu and Seib, 1997) or gas chromatography/mass spectrometry (Richardson et al., 2000; Wilke and Mischnick, 1997) may be helpful for the determination of the distribution of the substituent groups at the monomeric level. The methods used for the analysis of the substituent distribution along the polymer chains and the homogeneity/heterogeneity of substitution are based on partial degradation of the polymer by acid hydrolysis (Arisz et al., 1995; Mischnick and Kuhn, 1996) or by enzymatic degradation (Kavitha and BeMiller, 1998; Steeneken and Woortman, 1994; van der Burgt et al., 1998; Wilke and Mischnick, 1997).

10.3.3.3 Morphological properties of derivatized potato starch

Potato starch modification involves physical, chemical, and biochemical phenomena on the surface of contacting phases. Microscopy (light and SEM) has played an important role in increasing understanding of granular structure of modified starches. It has been used to detect structural changes caused by chemical modifications, and the most substituted regions in starch granules (Kaur et al., 2004; Kim et al., 1992). Most of the structural changes upon hydroxypropylation take place at the relatively less organized central core region of the starch granule, i.e. where the hydroxypropyl groups are most densely deposited (Kim et al., 1992).

The 'pushing apart effect' exerted by the bulky hydroxypropyl groups, especially in the central region of the granule, may lead to an alteration in granule morphology upon hydroxypropylation. Another possible explanation is that the starch granule itself is not structurally homogeneous from a physical and chemical point of view, since it has different physical regions (amorphous and crystalline) as well as different chemical compositions in each region (French, 1984). The treatment of potato starch granules with propylene oxide (10%, dwb) alters granule morphology (Figures 10.6a and b). Many of the less affected modified granules developed a depression that resulted later in slight fragmentation, indentation, and the formation of a deep groove in the central core region along the longitudinal axis in highly affected granules. These granules appeared as folded structures with their outer sides drawn inwards, giving the appearance of a doughnut (Figures 10.7a and b). Moreover, these altered regions were observed to be apparently larger in large granules compared with small granules in all the potato starches examined; this may be attributed to differences in the native granule architecture and fragility.

The peripheral regions and also the outer layer of the less affected starch granules remained unaltered, and the changes remained confined to the central core regions. By contrast, highly

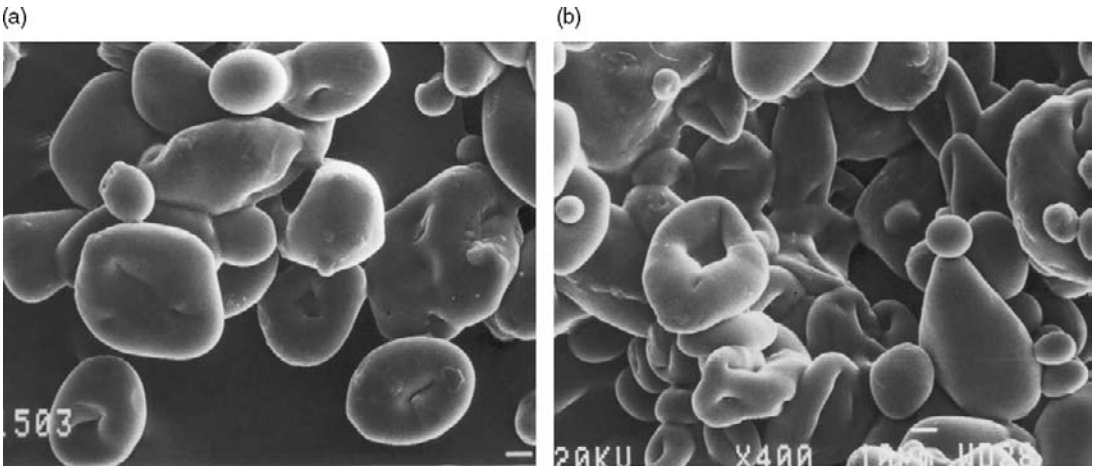


Figure 10.6: Effects of hydroxypropylation on the granule morphology of potato starches. (a) Potato starch granules after hydroxypropylation (at 10% propylene oxide concentration). (b) Effect of increased concentration of propylene oxide (15%) on the starch granule structure (source: Kaur et al., 2004).

affected starch granules developed blister-like appearances, cracks, and small protuberances on their surfaces, and a deep groove in the central core region; this suggests that the granule peripheral regions may be the last to be modified (Figure 10.7c) (Kaur et al., 2004). The granule structure is more substantially altered when the reaction is carried out with a higher concentration of propylene oxide (15% compared with 10%). Highly affected granules appear as if gelatinized, having lost their boundaries and fused together to form a gelatinized mass (Figure 10.6c). This effect was again more pronounced in larger granules (Kaur et al., 2004).

Huber and BeMiller (2001) reported that the material within the inner regions of potato starch granules was more susceptible to reaction (with propylene oxide) than that in the outer granule layers. Also, as potato starch granules do not possess channels, the reagent diffuses inwards through the exterior granule surface. Propylene oxide, being less reactive than other modification reagents, may diffuse into the granule matrix prior to reacting (Huber and BeMiller, 2001). The structural changes in hydroxypropylated starch granules become more evident with increasing MS (Kaur et al., 2004; Kim et al., 1992). Hydroxypropylation leads to alteration in the structure of the gelatinized potato starch gels also, which may be attributed to a weakened granule structure, leading to increased granule disruption during gelatinization. Hydroxypropylated potato starch gels showed extensive aggregation of granule remnants. Kaur et al. (2004) have also reported the appearance of numerous rod-shaped fuzzy clustered microfibrils in hydroxypropylated potato starch gels (after 30 days storage at 4°C) that could be easily distinguished from the other starchy material (Figure 10.8). The extensive phase separation occurring during long-term storage at 4°C may be responsible for the formation of these rod-shaped microfibrils.

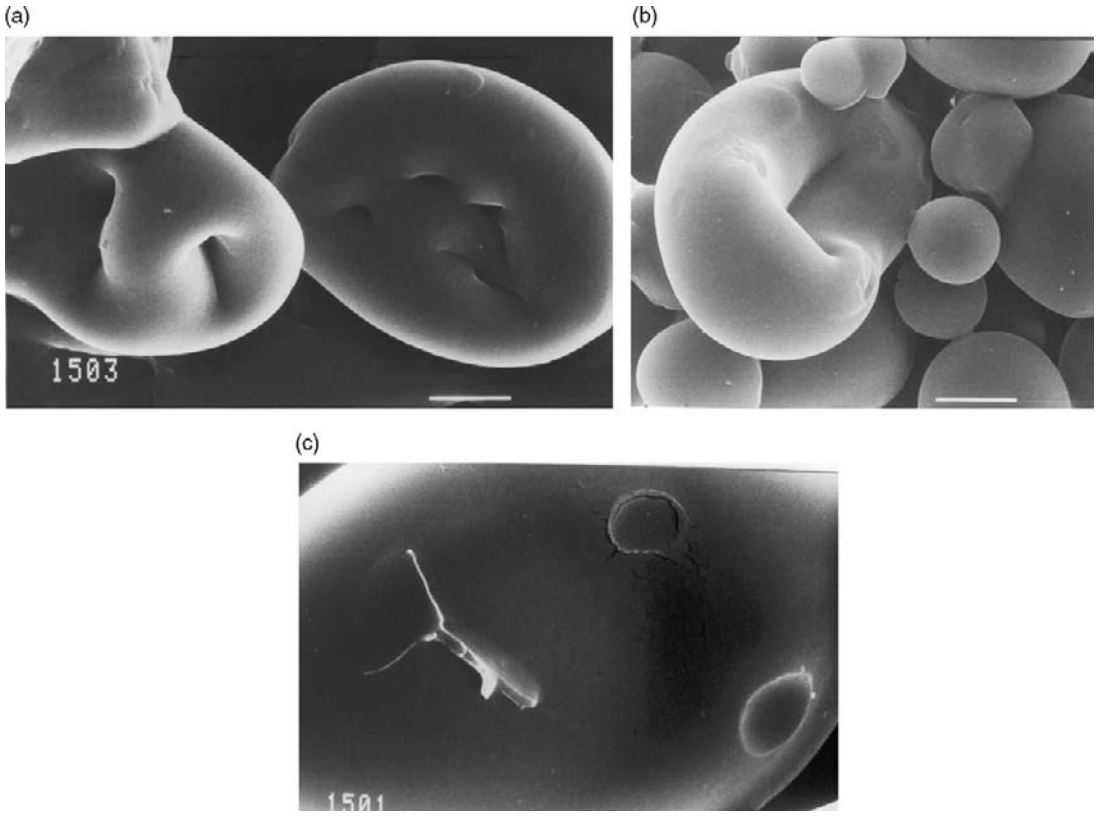


Figure 10.7: Effects of hydroxypropylation on the granule morphology of potato starches (at 10% propylene oxide concentration). (a), (b) Formation of deep groove in the central core region, folding of starch granules and formation of doughnut-like shape. (c) Formation of blister-like appearance and cracks on the starch granules (source: Kaur et al., 2004).

Slow cooling has also been reported to enhance the formation of non-spherulitic morphologies in starch gels (Nordmark and Ziegler, 2002).

Acetylation treatment has been found to alter the granule morphology, although to a lesser extent. Singh et al. (2004a) carried out acetylation of maize and potato starches using different concentrations of acetic anhydride, and reported that the acetylation treatment caused granule fusion in both maize and potato starches (Figure 10.9). The granule fusion was also observed to be more pronounced in potato starches with small granules (Singh et al., 2004b). Maize starch granules show a higher resistance towards acetylation treatment than potato starches. The addition of 4% acetic anhydride resulted in the fusion of granules in potato starches while maize starch granules tended to fuse at concentrations of acetic anhydride of 8% or higher (Singh et al., 2004a). The granule surface of maize and potato starch granules was observed

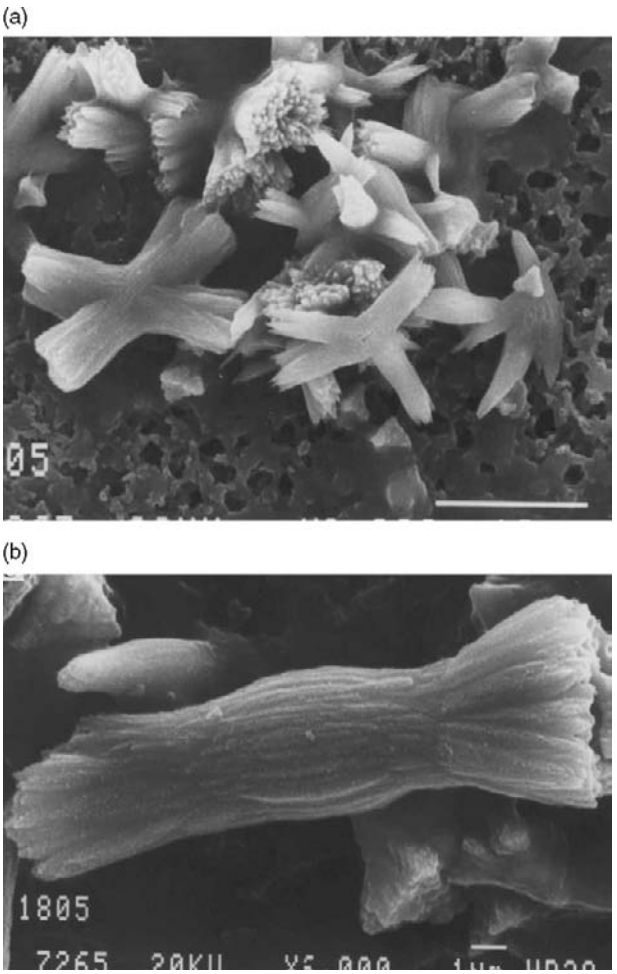


Figure 10.8: (a), (b) Rod-shaped microfibrils (showing longitudinal arrangement of crystalline structures) formed during long-term storage of hydroxypropylated gelatinized starch pastes (source: Kaur et al., 2004).

to become slightly rough upon acetylation (Singh et al., 2004b). The granule surface of rice starches becomes rough and the granules tend to form aggregates upon acetylation (Gonzalez and Perez, 2002). However, some granule fusion and deformation, and the rough appearance of the granule surface in the acetylated potato and maize starches might also be the result of surface gelatinization upon addition of NaOH to maintain alkaline conditions during acetic anhydride addition (Singh et al., 2004b).

Phosphorylated potato starches can be prepared using a mixture of mono- and disodium phosphate. The starch granule size is very responsive to the changes in the DS by phosphate groups: the higher the DS, the larger the granule size. The average increase in granule size has been

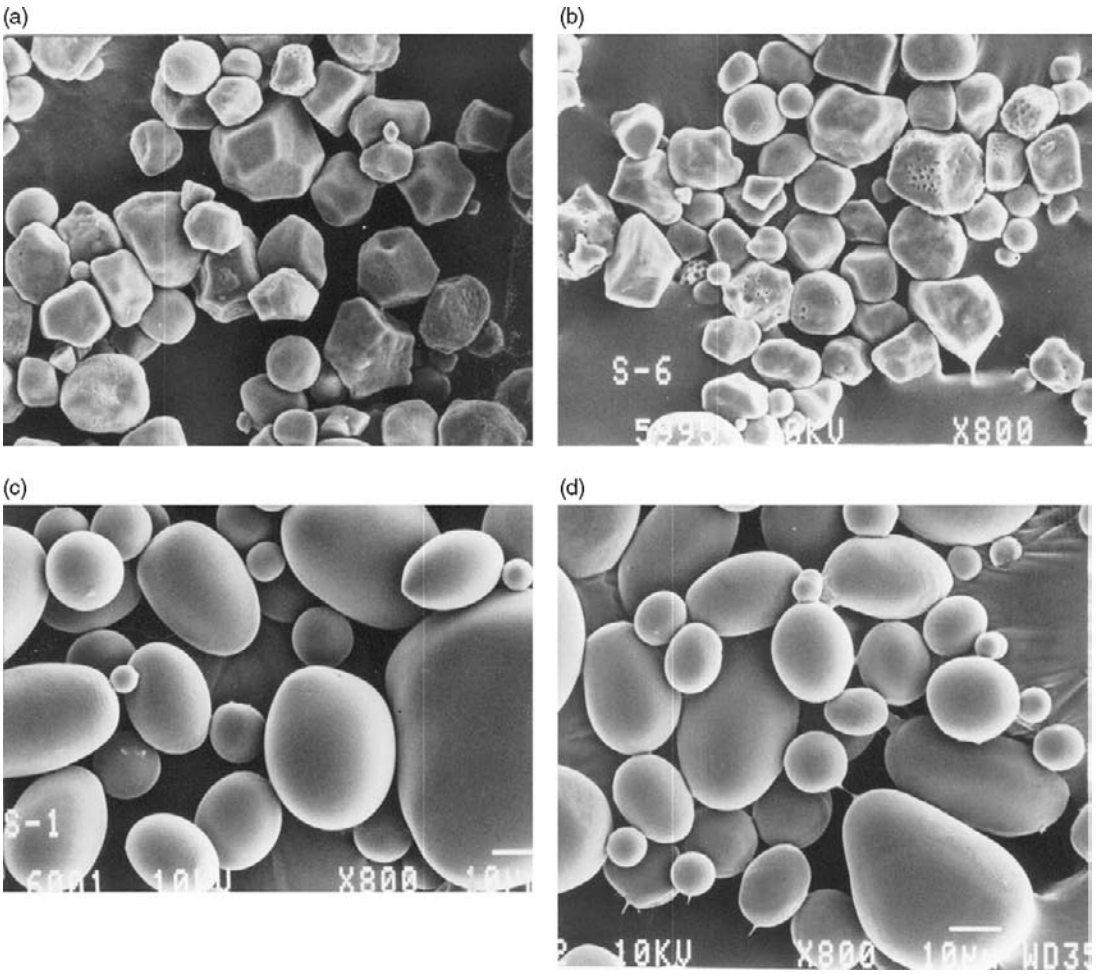


Figure 10.9: Effects of acetylation on the granule morphology of maize and potato starches. (a) Native maize starch granules. (b) Acetylated maize starch granules. (c) Native potato starch granules. (d) Acetylated potato starch granules (Singh et al., 2004a).

observed to be higher for rice and maize starches than for potato starch for a given DS. The increase may be due to the substitution by phosphate groups inside the modified granules, building certain repulsive forces that increase the sizes of inter- and intra-molecular spaces, allowing more water molecules to be absorbed. Phosphorylation does not cause any detectable change in the general granule shape (Sitohi and Ramadan, 2001). After being cross-linked using EPI and $POCl_3$, potato starch granules remain smooth and similar to native starch granules in morphology when viewed under SEM, suggesting that the modification does not cause any detectable morphological change (Kaur et al., 2006). Some chemical modifications of starch have been reported to increase granule surface area and granule porosity (Fortuna et al., 1999).

Table 10.10: Swelling power of some modified starches from different botanical sources

Starch source	SP[a] (g/g) (acetylated)	SP[a] (g/g) (hydroxypropylated)	SP[a] (g/g) (cross-linked)
Normal potato	60–71 (Native 58–70)[c,d]	33–39 (Native 28–31)[f]	20–25 (Native 28–31)[i]
Normal potato	ND[b]	ND	23–27 (Native 28–31)[j]
Normal maize	38 (Native 36)	≈ 20 (Native ≈ 6)[g]	ND
Waxy maize	ND	≈ 42 (Native ≈ 30)[g]	ND
Normal wheat	ND	9–16 (Native ≈ 6–8)[h]	ND
Normal rice	15–19 (Native 14–18)[e]	ND	≈ 9 (Native ≈ 18)[e]
Waxy rice	≈ 31 (Native ≈ 41)	ND	≈ 14 (Native ≈ 41)[e]

[a] *SP = Swelling power (g/g).*

[b] *not detected.*

[c] *The properties of corresponding native (unmodified) starches are given in brackets.*

[d] *starches from different potato cultivars.*

[e] *starches from different rice cultivars.*

[f] *starches from different potato cultivars.*

[g] *two levels of hydroxypropylation.*

[h] *two wheat starch granule populations.*

[i] *starches from different potato cultivars, cross-linking performed using $POCl_3$.*

[j] *starches from different potato cultivars; cross-linking performed using EPI.*

(Source: Singh et al., 2007)

Studies on waxy maize starches using different reagents ($POCl_3$ and propylene oxide) reported variations in the relative reaction patterns (Huber and BeMiller, 2001). Owing to the higher reactivity of $POCl_3$, the cross-links predominate on the granule surfaces, when using highly reactive $POCl_3$ (Gluck-Hirsch and Kokini, 1997; Huber and BeMiller, 2001) while the less reactive propylene oxide generally diffuses into the granule matrix prior to reaction (Huber and BeMiller, 2001; Kaur et al., 2004). Shiftan et al. (2000) reported that EPI cross-linking is not homogeneous and is concentrated in the non-crystalline domain of starch granules. Another factor that may influence the extent of cross-linking is the size distribution of the starch granule population (Hung and Morita, 2005). During cross-linking small granules have been reported to be derivatized to a greater extent than the large granules (Bertolini et al., 2003).

10.3.3.4 Physico-chemical properties of derivatized potato starch

The physico-chemical properties of starches, such as swelling, solubility, and light transmittance have been reported to be affected considerably by derivatization (Table 10.10). The change in these properties upon modification depends on the type of chemical modification. Chemical modifications, such as acetylation and hydroxypropylation increase, while cross-linking has been observed to decrease (depending on the type of cross-linking agent and degree of cross-linking) the swelling power and solubility of starches from various sources. The introduction of (bulky) acetyl groups into starch molecules by acetylation leads to structural reorganization

owing to steric hindrance; this results in repulsion between starch molecules, thus facilitating an increase in water percolation within the amorphous regions of granules and a consequent increase in swelling capacity (Lawal, 2004). Studies conducted on acetylated potato starches (Singh et al., 2004a, b) suggest a significant increase in swelling power and solubility upon acetylation in all these starch types. The extent of this increase was observed to be higher for potato starches. The degree of substitution introduced by acetylation mainly affects the intensity of change in the swelling power and solubility of starches. The structural disintegration caused by acetylation probably weakens the starch granules, and this enhances amylose leaching from the granule, thus increasing starch solubility (Lawal, 2004). Liu et al. (1999b) reported that the waxy starches show an increased swelling power upon acetylation because of the presence of mainly amylopectin with a more open structure than in non-waxy starch; this allows rapid water penetration, and increased swelling power and solubility. Swelling power and solubility of hydroxypropylated potato starches generally increase with an increase in MS. The decrease in associative forces within the starch granule due to hydroxypropylation may result in an increased penetration of water during heating that leads to the greater swelling (Kaur et al., 2004).

Cross-linking strengthens the bonding between potato starch chains, causing an increase in resistance of the granules to swelling with increasing degree of cross-linking. Higher concentrations of fast-acting cross-linking reagents such as $POCI_3$ result in greater reductions in the swelling potential as compared with slower-acting agents such as EPI (Gluck-Hirsch and Kokini, 2002). A large concentration of $POCl_3$ cross-links at the surface of the granule causes the formation of a hard outer crust that restricts granule swelling (Huber and BeMiller, 2001). Inagaki and Seib (1992) also reported that the swelling power of cross-linked waxy barley starch declined as the level of cross-linking increased. Cross-linked starches exhibit lower solubility than their native equivalents, and solubility decreases further with an increase in the concentration of cross-linking reagent, which may be attributed to an increase in cross-link density (Kaur et al., 2006).

Derivatization alters the light transmittance of potato starch pastes to a considerable extent. Acetylation and hydroxypropylation have been reported to increase the light transmittance of potato starches (Kaur et al., 2004; Singh et al., 2004a, b). The chemical substitution of the –OH groups on the starch molecules by acetyl moieties hampers the formation of an ordered structure following gelatinization, and thus retards retrogradation, resulting in a more fluid paste with improved long-term clarity (Lawal, 2004). The high retention of water entering the starch granule results in a greater swelling power and favors the clarity of pastes and gels. Potato starch source, native starch granule size distribution, amylose content, and degree of substitution are the important factors affecting light transmittance of acetylated potato starches. Singh et al. (2004b) reported a higher light transmittance of acetylated potato starches compared to acetylated maize starches acetylated under similar reaction conditions. Highly cross-linked potato starch pastes

Table 10.11: Solubility (%) in DMSO of some modified starches from different botanical sources

Starch source	SB[a] (after 4 hrs)	SB (after 8 hrs)	SB (after 16 hrs)	SB (after 24 hrs)
Normal potato (Hydroxypropylated)[d,b]	≈ 76 (Native ≈ 57)	≈ 83 (Native ≈ 60)	≈95 (Native ≈ 65)	≈ 99 (Native 75)
Normal rice (Hydroxypropylated)[e]	≈ 35 (Native ≈ 10)	≈ 80 (Native ≈ 20)	≈ 95 (Native ≈ 35)	≈ 98 (Native ≈ 55)
Normal rice (Cross-linked)[f]	≈ 08 (Native ≈ 10)	≈ 10 (Native ≈ 20)	≈ 20 (Native ≈ 35)	≈ 26 (Native ≈ 55)

[a] *Solubility in DMSO (%).*
[b] *values reported as % transmittance in DMSO.*
[c] *The properties of corresponding native (unmodified) starches are given in brackets.*
[d] *starches from different potato cultivars used.*
[e] *two levels of hydroxypropylation used.*
[f] *different levels of cross-linking used.*
(Source: Singh et al., 2007)[c]

generally show lower light transmittance than their counterpart native starches. Incomplete gelatinization and reduced swelling of cross-linked starches is mainly responsible for their reduced paste clarity (Kaur et al., 2006; Morikawa and Nishinari, 2000a; Reddy and Seib, 2000; Zheng et al., 1999).

The solubility of native, cross-linked and hydroxypropylated potato starches in dimethyl sulfoxide (DMSO) varies to a significant extent (Table 10.11). Hydroxypropylation results in a significant increase in the solubility of potato starches in DMSO (Kaur et al., 2004; Yeh and Yeh, 1993). Yeh and Yeh (1993) compared the solubilites (in DMSO) of hydroxypropylated and cross-linked rice starches with those of native rice starch, and observed higher and lower solubilities for hydroxypropylated and cross-linked rice starches, respectively. Differences in granule morphology, amylose content, and degree of substitution of native and modified potato starches, respectively, have been reported to affect the solubility in DMSO (Kaur et al., 2004). Sahai and Jackson (1996) reported that the solubility of starch in methyl sulfoxide varies significantly with granule size, presumably reflecting inherent structural heterogeneity within granules.

10.3.3.5 Pasting/rheological properties of derivatized potato starch

Derivatization leads to a considerable change in the rheological and pasting properties of potato starch (Table 10.12). Potato starch paste viscosity can be increased or reduced by applying a suitable chemical modification. Again, modification method, reaction conditions, and starch source are the critical factors that govern the rheological/pasting behavior of potato starch pastes.

Control of reaction conditions such as pH during acetylation may allow reduction of secondary hydrolysis reactions and facilitate the incorporation of acetyl groups. Such modification

Table 10.12: Rheological properties of some modified potato starches during heating from 30 to 75°C studied using a dynamic rheometer[a]

Modification type	*TG′* (°C)	*Peak G′* (Pa)	*Peak G″* (Pa)	*Peak tan δ*
Hydroxypropylated[c,d] (using 10 % propylene oxide)	–	5951 (3288)	1083 (693)	0.182 (0.211)
Acetylated[b,e] (using 10 % acetic anhydride)	54.9 (55.2)	90220 (85790)	53726 (49529)	0.596 (0.591)
Cross-linked[c,f] (using 0.1 % $POCl_3$)	62.2 (63.6)	4450 (3288)	850 (693)	0.191 (0.211)
Cross-linked[c,f] (using 0.2 % $POCl_3$)	64.8 (63.6)	2750 (3288)	616 (693)	0.224 (0.211)
Cross-linked[c,f] (using 0.25 % EPI)	63.9 (63.6)	3120 (3288)	660 (693)	0.211 (0.211)
Cross-linked[c,f] (using 1 % EPI)	64.9 (63.6)	2800 (3288)	630 (693)	0.225 (0.211)

[a] *The properties of corresponding native (unmodified) starches are given in brackets.*
[b] *At 20% starch concentration.*
[c] *At 15% starch concentration.*
[d] *Kaur et al. (2004).*
[e] *Singh et al. (2004b).*
[f] *Kaur et al. (2006).*

enhances the water-holding capacity of the starch matrix and the development of more organized structures, leading to a higher resistance to deformation; thus a higher peak viscosity can be achieved (Betancur-Ancona et al., 1997). Acetylation influences interactions between potato starch chains by steric hindrance, altering starch hydrophilicity and hydrogen bonding and resulting in a lower gelatinisation temperature and a greater swelling of granules, the latter resulting in an increased peak viscosity (Liu et al., 1997; Singh et al., 2004a, b). The amylose/amylopectin ratio is considered to be the prime determinant of the change in the paste viscosity upon acetylation. The substituent groups restrict the tendency of the starch molecules to realign after cooling, thus facilitating lower setback values (Betancur-Ancona et al., 1997). The hot paste viscosity (HPV) and cold paste viscosity (CPV) of the acetylated starches have also been observed to be higher than in the case of the unmodified starch. This may be attributed to physical interaction between more swollen but weaker granules (Gonzalez and Perez, 2002).

Hydroxypropylation can influence interaction between the potato starch chains through different possible mechanisms: (1) by steric hindrance, which prevents the close association of chains and restricts the formation of inter-chain hydrogen bonds; and (2) by changing the hydrophilicity of the starch molecules and thus altering bonding with water molecules. The observed effects of hydroxypropylation are consistent with an overall reduction in bonding between starch chains and a consequent increase in the ease of hydration of the starch granule. Gelatinization can thus commence at a lower temperature, and greater swelling of the granule will lead to an

increased peak viscosity (Liu et al., 1999a). Kim et al. (1992) reported that with increase in MS, the pasting temperature of potato hydroxypropylated starches decreased and their peak Brabender viscosity increased. The extent of change in potato starch functional properties upon hydroxypropylation has also been observed to be influenced by the amylose content and starch granule size distribution.

The greater strength of the cross-linked granule limits the breakdown of viscosity under shear, giving a higher HPV, and persistence, or resistance to breakdown, of the swollen potato starch granules, and on cooling, results in a higher CPV. Gluck-Hirsch and Kokini (2002) studied the relative effects of different cross-linking agents on the physical properties of starches and reported that $POCl_3$ has the ability to impart a greater viscosity than other agents. $POCl_3$-treated granules have a more rigid external surface than STMP- and EPI-treated granules owing to the concentration of cross-links at the surface of the granule.

The rheological properties of modified potato starches exhibit significant differences from those of native starches when subjected to temperature sweep testing using heating and cooling cycles on a dynamic rheometer (Kaur et al., 2004; Singh et al., 2004b). Parameters such as G' and G'' of acetylated, hydroxypropylated, and cross-linked starches from different sources increase to a maximum and then drop during heating, confirming that these starches follow the same general rheological pattern as native starches. The temperature of maximum G'' drops significantly on acetylation or hydroxypropylation, while it increases after cross-linking (Kaur et al., 2004, 2006; Singh et al., 2004b). Changes may be explained on the same basis as for the changes in thermal and pasting properties caused by modification. Acetylation of maize and potato starches results in increased G' and G'' maxima and a decreased $tan\ \delta$ maximum. Acetylated starches with higher DS exhibit a correspondingly higher increase in G' and G'' maxima during heating. These changes occur for the same reasons that acetylation causes an increase in peak pasting viscosity (see above) (Betancur-Ancona et al., 1997; Singh et al., 2004a).

Acetylated maize and potato starches showed slightly lower G' and G'' as compared with their native starch gels, during the cooling cycle of heated starch pastes on the rheometer. This confirms the lowered tendency of these modified starches to retrograde (Betancur-Ancona et al., 1997; Singh et al., 2004a). Similarly, increases in the peak G' and G'' of hydroxypropylated potato starches during heating occurs owing to the decrease in associative forces within the starch granules caused by the introduction of hydroxypropyl groups; this introduction results in greater water penetration and swelling and a consequent increase in G'. Hydroxypropylated potato starches with a higher MS and large granules exhibit higher peak G' and G'' during heating than do the native starches (Kaur et al., 2004). Cross-linking of starches leads to a significant alteration in their dynamic rheological behavior. A fairly high degree of cross-linking results in a lower peak G', owing to the lower degree of swelling and consequent lower degree of intergranular interaction. Potato starches treated with a relatively low concentration of $POCl_3$

showed a higher maximum G', and lower *tan* δ maximum, than their counterpart native starches, whereas those treated with higher concentrations showed an opposite effect (Kaur et al., 2006). Strengthening bonding between starch chains by cross-linking will increase the resistance of the granule towards swelling, leading to lower paste viscosity, which suggests that the concentration of the cross-linking reagent affects the structure within the granule, perhaps by affecting the distribution of the introduced cross-links. Cross-link location has also been reported to have varied effects on different properties of cross-linked potato starches (Muhrbeck and Eliasson, 1991; Muhrbeck and Tellier, 1991; Yoneya et al., 2003). The extent of change in the rheological properties upon cross-linking also varies significantly for starches from different sources depending on the granule size distribution. Potato cultivars with a higher percentage of large irregular starch granules showed a higher susceptibility towards cross-linking and exhibited greater changes in their functional properties upon cross-linking (Kaur et al., 2006). These characteristics could also be explained on the basis of differences in the amylose to amylopectin ratio, amylopectin branch chain length, and degree of crystallinity between larger and smaller granules (Noda et al., 2005; Singh and Kaur, 2004).

Dual-modification results in starch pastes with higher peak viscosity and greater stability than in the case of native starch pastes (Wu and Seib, 1990). However, the effect of dual-modification depends upon the preparation procedure. Cross-linking followed by hydroxypropylation has been reported to yield starches that are more shear and heat stable than the native starch. This may be due to the structural change in the granules after the first modification (cross-linking). Cross-linking reduces the degree of subsequent hydroxypropylation, but prior hydroxypropylation increases the degree of subsequent cross-linking. Moreover, the cross-linked and then hydroxypropylated (XL-HP) starch exhibited lower pasting temperature and viscosity than the hydroxypropylated and then cross-linked (HP-XL) starch, suggesting the locations of the cross-links in the two types of starch are different. The cross-links in XL-HP starch were also found to be more resistant to attack by enzymes and chemicals (Reddy and Seib, 2000). The reactivity of the modifying agent during dual modification may vary towards different starch sources. The cross-linked hydroxypropylated waxy wheat starch gave pasting curves showing higher viscosities than those of cross-linked hydroxypropylated waxy maize starch (Reddy and Seib, 2000). Therefore, by appropriate choice of the native starch source (potato, maize, wheat, etc.) and of the type of chemical modification, concentration of the modifying reagent, modified starches with very useful rheological properties can be obtained (Dubois et al., 2001; Kaur et al., 2004; Singh et al., 2004a, b).

10.3.3.6 Gelatinization and retrogradation (thermal properties) of derivatized potato starch

DSC studies have shown that modification alters thermal transition temperatures and the overall enthalpy (ΔH_{gel}) associated with gelatinization (Tables 10.13 and 10.14). Upon hydroxypropylation, the reactive groups introduced into the starch chains are capable of disrupting the inter- and intra-molecular hydrogen bonds, leading to an increase in accessibility by water that lowers

Table 10.13: Transition temperatures of some modified starches from different botanical sources

Starch source	T* (°C) (Acetylated)	T (°C) (Hydroxypropylated)	T (°C) (Cross-linked)
Normal potato	48–67 (Native 57–69)[a]	51–62 (Native 57–68)[f]	60–68 (Native 57–68)[l]
Normal potato	55–64 (Native 59–70)[b]	ND	59–68 (Native 57–68)[m]
Normal maize	65–77 (Native 69–77)[c]	59–75 (Native 65–81)[g]	ND
Normal maize	66–75 (Native 70–78)[d]	ND	ND
Waxy maize	64–69 (Native 68–72)	61–79 (Native 63–84)[g]	65–75 (Native 62–76)[n]
Waxy maize	ND*	ND	67–72 (Native 68–73)[o]
Hybrid normal maize	71–84 (Native 60–78)	ND	ND
Hi-amylose maize	ND	66–95 (Native 67–105)[g]	ND
Normal wheat	ND	55–71 (Native 63–84)[h]	63–76 (Native 63–84)[p]
Normal wheat	ND	46–55 (Native 55–67)[i]	61–72 (Native 57–58)[q]
Waxy wheat	ND	ND	62–66 (Native 61–66)[o]
Normal rice	60–72 (Native 66–78)[e]	64–83 (Native 63–84)[j]	70–87 (Native 71–87)[r]
Normal rice	ND	57–92 (Native 63–92)[k]	ND
Waxy rice	60–78 (Native 60–78)	ND	61–78 (Native 60–78)
Waxy rice	ND	ND	55–66 (Native 53–67)[n]

T = Transition temperatures of different starches (°C); ND* not detected, The properties of corresponding native (unmodified) starches are given in brackets; [a]different levels of acetylation, [b]starches from different potato cultivars; [c]different levels of acetylation, [d]starches from different potato cultivars; [e]starches from different rice cultivars; [f]starches from different potato cultivars; [g]two levels of hydroxypropylation, [h]three levels of hydroxypropylation; [i]two wheat starch granule populations, [j]two levels of hydroxypropylation; [k]three levels of hydroxypropylation; [l]starches from different potato cultivars used and cross-linking performed using $POCl_3$; [m]starches from different potato cultivars used, cross-linking performed using EPI); [n]cross-linking performed using EPI; [o]cross-linking performed using $POCl_3$; [p]cross-linking performed using $POCl_3$; [q]three levels of cross-linking performed using $POCl_3$ and onset transition temperatures only. [r]different levels of cross-linking performed using $POCl_3$.*

(Source: Singh et al., 2007)

the temperature of gelatinization. In potato starch, increasing the level of MS results in decreases in ΔH_{gel}, T_o, T_p and T_c and a widening of the gelatinization temperature range (T_c–T_o) (Perera et al., 1997). An increase in gelatinization temperature range upon hydroxypropylation of different potato starches has been reported by Kaur et al. (2004), which could be attributed to an increase in homogeneity within both the amorphous and crystalline regions of the starch granules. A progressive shift of the biphasic gelatinization endotherms to lower temperatures as well as a broadening and shortening of the gelatinization endotherm with increasing MS have also been observed for hydroxypropylated rice starch, indicating increased internal plasticization and destabilization of the amorphous regions of the granules (Seow and Thevamalar, 1993).

The decrease in ΔH_{gel} on hydroxypropylation suggests that hydroxypropyl groups disrupt double helices (owing to the rotation of these flexible groups) within the amorphous regions of the potato starch granules. Consequently, the number of double helices that unravel and melt during gelatinization would be lower in hydroxypropylated than in unmodified starches (Perera

Table 10.14: Enthalpy of gelatinization of some modified starches from different botanical sources

Starch source	$^{*}\Delta H_{gel}$ J/g (Acetylated)	ΔH_{gel} J/g (Hydroxypropylated)	ΔH_{gel} J/g (Cross-linked)
Normal Potato	10.1–11.4 (Native 12.1)[a]	10–11 (Native 11.7–12.9)[d]	12.7–14.7 (Native 11.7–12.9)[i]
Normal potato	10.1–11.8 (Native 12.8–3.2)[b]	ND	12.7–14.4 (Native 11.7–12.9)[j]
Normal maize	08.52 (Native 10.9)[a]	ND	ND
Normal maize	08.9–09.7 (Native 10.6)[b]	07.1–8.4 (Native 11)[e]	ND
Hybrid normal maize	09.8–10.6 (Native 14.3)	ND	ND
Hi-amylose maize	ND[**]	6.4–8.4 (Native 13.7)[e]	ND
Waxy maize	14.8 (Native 15.6)	08–8.7 (Native 13.6)[e]	15.2 (Native 15.3)[k]
Normal wheat	ND	04.7–05 (Native 6.8)[f]	6.6–7.4 (Native 06.8)[l]
Normal wheat	ND	02.2 (Native 06.2)[g]	–
Waxy wheat	ND	ND	12.8 (Native 13.2)[k]
Normal rice	08.1–11.4 (Native 08.1–11.9)[c]	08–09 (Native 10.4)[h]	11–13 (Native 10.4)[m]
Waxy rice	08.5 (Native 09.8)	ND	10.3 (Native 09.8)

$^{*}\Delta H_{gel}$ enthalpy of gelatinization of different starches (J/g); ND[**] = Not detected; The properties of corresponding native (unmodified) starches are given in brackets; [a]different levels of acetylation; [b]starches from different potato cultivars; [c]starches from different rice cultivars; [d]starches from different potato cultivars; [e]two levels of hydroxypropylation; [f]three levels of hydroxypropylation; [g]two wheat starch granule populations; [h]two levels of hydroxypropylation; [i]starches from different potato cultivars; cross-linking performed using $POCl_3$; [j]starches from different potato cultivars and cross-linking performed using EPI; [k]cross-linking performed using $POCl_3$; [l]three levels of cross-linking performed using $POCl_3$; [m]different levels of cross-linking performed using $POCl_3$.

(Source: Singh et al., 2007)

et al., 1997). Decreases have been recorded in gelatinization temperatures and gelatinization enthalpies of amylose extender mutant (*ae*), waxy mutant (*wx*), and normal maize starch upon hydroxypropylation (Liu et al., 1999a). The decrease was observed to be greater for high amylose maize starches (66% amylose) as compared with waxy maize starch (3.3% amylose). Substitution on granular starch occurs mainly in the amorphous regions, which promotes swelling in these regions and thus disrupts the crystalline phase, which melts at a lower temperature than in the case of the unmodified starch.

Potato starches with higher degrees of acetylation show greater decreases in the transition temperature and enthalpy of gelatinization. Weakening of the starch granules by acetylation leads to early rupture of the amylopectin double helices, which accounts for the lower values of T_o, T_p, and T_c (Adebowale and Lawal, 2003). Decreases in the thermal parameters are consistent with fewer crystals being present after modification (owing to damage to the crystals during acetylation) and with a co-operative melting process enhanced by added swelling (Singh et al., 2004b). The extent of the lowering of transition temperatures and ΔH_{gel} on acetylation has been observed to be different for starches from different sources. Potato starches show a greater

decrease in thermal parameters upon acetylation compared with maize and rice starches (Singh et al., 2004a, b; Sodhi and Singh, 2005). The differences in granule rigidity, presence/absence of lipids, degree of substitution, and amylose to amylopectin ratio between these starches affect the extent of changes in thermal parameters.

Cross-linking also alters the thermal transition characteristics of starch, the effect depending on the concentration and type of cross-linking reagent, reaction conditions, and the botanical source of the starch. An increase in gelatinization temperature has been observed for cross-linked potato starches; these phenomena are related to the reduced mobility of amorphous chains in the starch granule as a result of the formation of intermolecular bridges. The level of phosphate cross-links has a strong influence on the DSC properties of starches. Choi and Kerr (2004) reported that cross-linked starches prepared using a relatively low concentration of the $POCl_3$ had gelatinization parameters similar to those of native starches, while cross-linked starches prepared using higher reagent concentrations showed considerably higher T_c and ΔH_{gel} values. Cross-linking at lower levels reduces the proportion of the starch that can be gelatinized, resulting in a lower value of ΔH_{gel} (Yook et al., 1993). Yoneya et al. (2003) also reported that potato starches treated with 100 ppm of $POCl_3$ displayed significantly lower gelatinization temperatures and lower ΔH_{gel} than other treated samples (with 40–5000 ppm). Decreasing or increasing the degree of phosphate cross-links caused a gradual increase in T_o, T_p, and ΔH_{gel} of cross-linked potato starch samples compared with the values of these properties for the 100 ppm-treated sample.

Chatakanonda et al. (2000) conducted studies on cross-linked rice starches (using as reagent a mixture of STMP and STPP) that showed deep and sharp endotherms, $\approx 30^{\circ}$C wide. Also, the gelatinization temperature increased significantly (by up to 5°C) with an increase in the degree of cross-linking, while enthalpy showed no significant change ($\approx$ 15% decrease), suggesting complete melting of crystalline regions in spite of cross-linking. The introduction of phosphate cross-links into the starch by STMP alone tightened the molecular structure, leading to an increase in the gelatinization temperature (Chatakanonda et al., 2000). The enthalpy of cross-linked normal rice starch was observed to decrease while waxy rice starch showed an increase in enthalpy after cross-linking (Liu et al., 1999b). These findings suggest that the type and concentration of the reagent and the amylose/amylopectin ratio of starch during cross-linking significantly affects the extent of change in thermal properties. Dual-modified (substituted and cross-linked) normal potato starches are available commercially with varying degrees of hydroxypropylation and cross-linking. The temperatures and enthalpies of gelatinization of the dual-modified (hydroxypropylated/cross-linked or acetylated/cross-linked) starches are generally lower than those of the unmodified starches (Reddy and Seib, 2000).

The crystallinity of starch granules is disrupted during derivatization (Saroja et al., 2000), and this leads to a greater degree of separation between the outer branches of adjacent amylopectin

chain clusters in modified starches compared with those in native starches. Consequently, double-helix formation (during storage) between adjacent amylopectin chains of the modified starches is much slower and less extensive owing to the introduction of functional groups upon chemical modification. Retrogradation of starch is suppressed by cross-linking using an STMP and STPP mixture, as indicated by the lower enthalpy of retrogradation after storage which may be because of the restricted mobility of cross-linked amylopectin branches owing to the presence of phosphate groups (Chung et al., 2004). Thermal behavior during DSC heating of stored native starch gels has been reported to be more conspicuous than that of hydroxypropylated phosphate cross-linked potato starch (HPS) dispersions as the retrogradation phenomenon of HPS dispersions was barely observed by DSC measurements even when a high concentration of starch (33%) was used (Morikawa and Nishinari, 2000b). Moreover, it has been reported that the effect of phosphate cross-linking on starch is more pronounced than that of hydroxypropylation with respect to retrogradation. Yook et al. (1993) concluded that the tendency towards retrogradation was greatly reduced by both hydroxypropylation and cross-linking, and they showed a synergistic effect of these treatments in retarding the retrogradation of gelatinized rice starch gels. Hydroxypropylated-crosslinked starch exhibited significantly higher onset and peak gelatinization temperatures, enthalpy of gelatinization, and lower retrogradation than crosslinked-hydroxypropylated starch, confirming the previous assumption that the locations of cross-links are different in the two kinds of modified starch (Yook et al., 1993). Hydroxypropylation followed by cross-linking provides starch with better storage stability in food applications.

10.4 Nutritional and Toxicological Aspects

Many modified starches made for food use contain only small amounts of substituent groups and have been used as safe food ingredients. During acetylation and hydroxypropylation of food-grade starches, the level of monosubstitution groups introduced is relatively low. The maximum permitted levels of substitution for starch acetates, starch phosphates, and hydroxypropylated starches are 2.5, 0.4, and 10% (FAC, 1980). Similarly, cross-linked food starches containing one substituent cross-linking group per 1000 or more anhydroglucose units are considered safe (Wurzburg, 1986c). The legislative approval for the use of novel starch derivatives in processed food formulations is still under debate, but several tailor-made starch derivatives with multiple modifications are being prepared and characterized (Tharanathan, 2005). Some of the starch derivatives are being increasingly used as fat replacers or fat substitutes. These derivatives are either partially or totally undigested, therefore contributing zero calories from the food on consumption (Tharanathan, 1995). Many studies have suggested that the physiological effects of chemically modified starches are affected by the type of modification (Ebihara et al., 1998). The chemical modification of starch by acetylation improves the satiating, glycemic, and insulinemic properties of the meal (Raben et al., 1997). Phosphorylated/cross-linked starches are slowly digestible and are thought to provide nutritional benefits for humans (Sang and Seib, 2006).

Also the slowly digesting modified starches could be used for the treatment of certain medical conditions (e.g. glycogen storage disease and diabetes mellitus) (Wolf et al., 1999).

10.5 Conclusions

Progress in understanding the high value of chemically modified starches has encouraged the starch industry to produce modified starches using different modification reagents and starch sources. Some factors such as starch composition, concentration and type of reagent, and reaction conditions may affect the reactivity of starch during chemical modifications such as acetylation, hydroxypropylation, and cross-linking. The heterogeneity of the granule population within a single starch source may also affect the extent of modification. The changes observed in the morphological, physico-chemical, pasting/rheological, and gelatinization and retrogradation (thermal) properties of the starches after modification may provide a crucial basis for understanding the efficiency of the starch modification process at industrial scale.

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CHAPTER 11

Fried and Dehydrated Potato Products

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11.1 Introduction

Potatoes are the fourth most important vegetable crop in the world and the main utilization of processed potatoes includes table stock (31%), frozen French fries (30%), chips and shoestrings (12%), and dehydrated items (12%) (Miranda and Aguilera, 2006). Potatoes are grown in approximately 80% of all countries and worldwide production stands in excess of 300 million tons/year, a figure exceeded only by wheat, maize, and rice. As the eating habits of consumers have become more sophisticated, much attention has been given to the quality of fried and dehydrated food, which is greatly affected by process- and/or storage-induced changes. Processed potato products such as potato chips have been popular salty snacks for 150 years and their retail sales in the US are about $6 billion/year representing 33% of the total sales of this market (Clark, 2003; Garayo and Moreira, 2002).

Chemical composition of potato varies with cultivar, location of growth, agricultural practices, maturity at harvest, and subsequent storage history, among others. Potatoes are mainly made up of water (~75% on average). Starch, comprising 65–80% of the dry matter content of the potato tuber, is calorically the most important nutritional component. The composition of potato starch is about 21% amylose, 75% amylopectin, 0.1% protein and 0.08% phosphorus. The content of reducing sugars is closely related to the final color and acrylamide formation of the fried potatoes. For example, a maximum of 1 g/kg reducing sugars has been suggested as a way to diminish significantly the formation of acrylamide after frying. On the other hand, selecting of cultivars to fry that contain low levels of asparagine (another acrylamide precursor) may result in low-acrylamide fried potatoes (Friedman, 2003). Finally, the two main factors that influence the sugar content of potatoes during post-harvest storage are variety and storage temperature. For frying, potatoes with high solids content (20–22%) are preferred, because they result in better texture, higher yields, and lower oil absorption in the finished product (Lesinska and Leszczynski, 1989).

Deep-fat frying is one of the oldest and most common unit operations used for cooking foods by immersing them in an edible oil or fat heated above the boiling point of water (Farkas et al.,

1996). This complex unit operation involves significant microstructural changes; in fact, most of the desirable characteristics of fried foods are derived from the formation of a composite structure: a dry, porous, crispy and oily outer layer or crust, and a moist cooked interior or core, whose microstructures form during the process (Bouchon et al., 2001). The high temperatures (around 160 and 180°C) cause water evaporation, which is transferred from the food towards the surrounding oil, whereas oil is absorbed by the food replacing part of the released water. This process results in products with a unique flavor–texture combination (Mellema, 2003).

On the other hand, drying is a widely used method of fruit and vegetable preservation. Water is removed to a final concentration, which assures microbial stability of the product and minimizes chemical and physical changes (Lewicki and Jakubczyk, 2004). Nowadays, drying is regarded not only as a preservation process, but also as a method for increasing added value of foods (Ramos et al., 2003). Processing of plant raw materials causes irreversible changes in the tissues of fruit and vegetables. These changes are particularly visible after heat treatment (Lisińska and Golubowska, 2005). In this chapter, the most important issues related to unit operations used to process potatoes such as frying and dehydrating will be shown.

11.2 Fried Potato Products

Deep-fat frying is a complex unit operation involving high temperatures, significant microstructural changes both to the surface and the body of the chip, and simultaneous heat and mass transfer resulting in flows in opposite directions of water vapor (bubbles) and oil at the surface of the piece (Bouchon et al., 2003). In fact, most of the desirable characteristics of fried foods are derived from the formation of a composite structure: a dry, porous, crisp and oily outer layer or crust, and a moist cooked interior or core. The crust is the result of several alterations that mainly occur at the cellular and sub-cellular level, and are located in the outermost layers of the product. Deep-fat frying can be defined as well as the process of drying and cooking through contact with hot oil (Sahin et al., 1999). High heat transfer rates are largely responsible for the development of the desired sensorial properties in fried products (Hubbard and Farkas, 1999). Although data are still inconclusive, it appears that longer times and lower frying temperatures lead to higher final oil contents in fried potato products.

Potato chips are very thin pieces (1.27–1.78 mm thick) of potatoes fried to a final oil and moisture contents of ∼35% and 1.7%, respectively (Moreira et al., 1999). For potato chip processing, raw potatoes are washed, peeled, and cut in slices whose shape and width vary according to consumer preferences. Then, the cut slices are washed to remove the starch excess and dried to eliminate surface moisture. Some processing plants use blanching to improve the final product color. Then the slices are fried until reaching a final moisture content of almost 1.8% and the oil excess over the fried chips is removed and salt in the proper quantity is added. Finally, potato chips are cooled and classified according to their size before being packaged (Bouchon, 2002). Potato chips are very thin pieces (1.27–1.78 mm thick) of sliced raw potatoes that are fried to a

final oil content of 33–38% in total basis (Moreira et al., 1999). Potato chips contain a significant amount of fat, reaching in many cases ~1/3 of the total food product by weight (Mellema, 2003). This ensures a high level of satiety, but can also pose a risk. Especially over the last decade the desirability of the reducing fat content of deep fried products has been recognized.

On the other hand, yearly production of French fries in the US is higher than 17.4 billions of pounds and constitutes almost 44% of the total processed potato. French fries represent a composite structure formed by two regions: (i) an external dehydrated and crispy region where oil is located; and (ii) a humid and cooked core region free of oil. The external crust is very similar to the structure of a fried potato slice or potato chip (Bouchon, 2002; Pedreschi et al., 2001; Pedreschi and Aguilera, 2002). For French fry processing, firstly the raw potato strips are blanched in hot water and dried with hot air until reaching a moisture content of around 60% (total basis). Then, the dried potato strips are fried in hot oil (160–190°C), cooled, frozen, and finally packaged (Bunger et al., 2003). The final preparation of the par-fried frozen potatoes could be accomplished by a final frying or baking. The final oil and moisture content of French fries are of almost 15% and 38%, respectively (Aguilera and Gloria, 2000; Saguy and Dana, 2003).

Factors which affect heat and mass transfer during frying are the thermal and physical–chemical properties of the food and oil, the food geometry, and the oil temperature. Many variables are involved in the traditional frying process of potatoes:

1. Variables dependent of the process: (a) temperature and time of frying; (b) frying method: batch or continuous; (c) potato variety.

2. Variables dependent of the oil type: (a) oil composition; (b) additives.

3. Variables dependent of the raw material: (a) surface/volume relations; (b) fat content, (c) moisture content. The interaction of these variables determines the characteristics of the fried product (Navas, 2005).

In deep-fat-fried potato products, both health and sensory aspects should be addressed to meet consumer demand. Consumer trends are moving toward healthier foods and low-fat products, creating the necessity of developing technologies to reduce the amount of oil in end-fried products (Bouchon et al., 2003). In order to obtain low-fat fried potatoes, it is useful to understand the mechanisms involved during the frying process, so that oil migration into the structure can be minimized. Numerous studies have shown that most of the oil is confined to the surface region of the fried potatoes (Bouchon and Aguilera, 2001; Bouchon et al., 2001; Pedreschi et al., 1999), and there is evidence that it is mostly absorbed after frying during the cooling period (Aguilera and Gloria-Hernandez, 2000; Bouchon et al., 2003; Ufheil and Escher, 1996).

Bouchon et al. (2003) explained that three different oil fractions can be identified as a consequence of the different absorption mechanisms in fried potato cylinders, that is: (i) structural

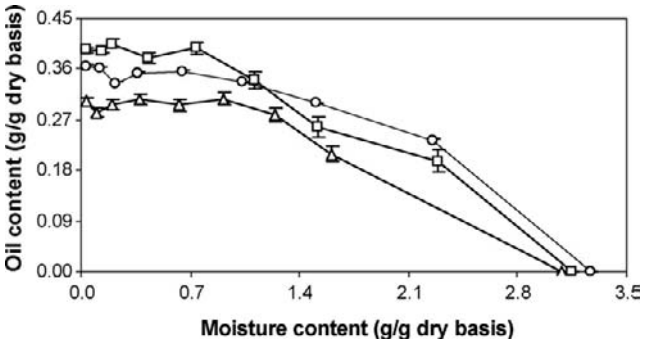

Figure 11.1: Oil uptake versus moisture content during frying of potato slices. Lines connect experimental data corresponding to the same frying temperature. □ 120°C, O 150°C, Δ 180°C (reprinted with permission from Lebensmittel-Wissenschaft und-Technologie, 2006, 39, 285–291).

oil (STO), which represents the oil absorbed during frying; (ii) penetrated surface oil (PSO), which represents the oil suctioned into the food during cooling after removal from the fryer; and (iii) surface oil (SO), which is the oil that remains on the surface (Figure 11.1). These authors showed that a small amount of oil penetrates during frying because most of the oil was picked up at the end of the process, suggesting that oil uptake and water removal are not synchronous phenomena. These findings have been confirmed by Pedreschi et al. (2008) and Durán et al. (2007).

Oil content versus moisture content in dry basis for control slices fried at 120, 150, and 180°C is shown in Figure 11.2. There is a clear effect of the frying temperature on oil uptake at moisture contents ≤ 1 g water/g dry solid; the higher the frying temperature the lower the oil content – average values of 0.39, 0.35, and 0.30 g/g dry basis for 120, 150 and 180°C, respectively. Figure 11.3 shows kinetics of total oil (TO) uptake and their different fractions (PSO, STO,

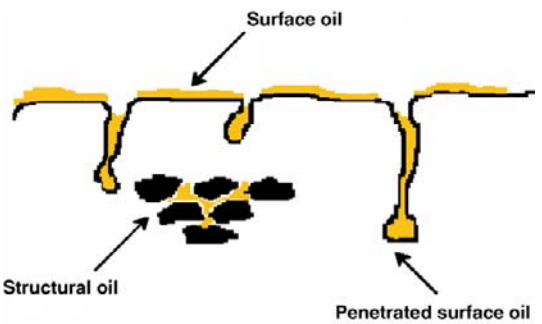

Figure 11.2: Diagram showing the three locations of oil in the crust of a fried potato piece according to Bouchon et al. (2003) (used by permission of the Journal of Food Science, Institute of Food Technologists).

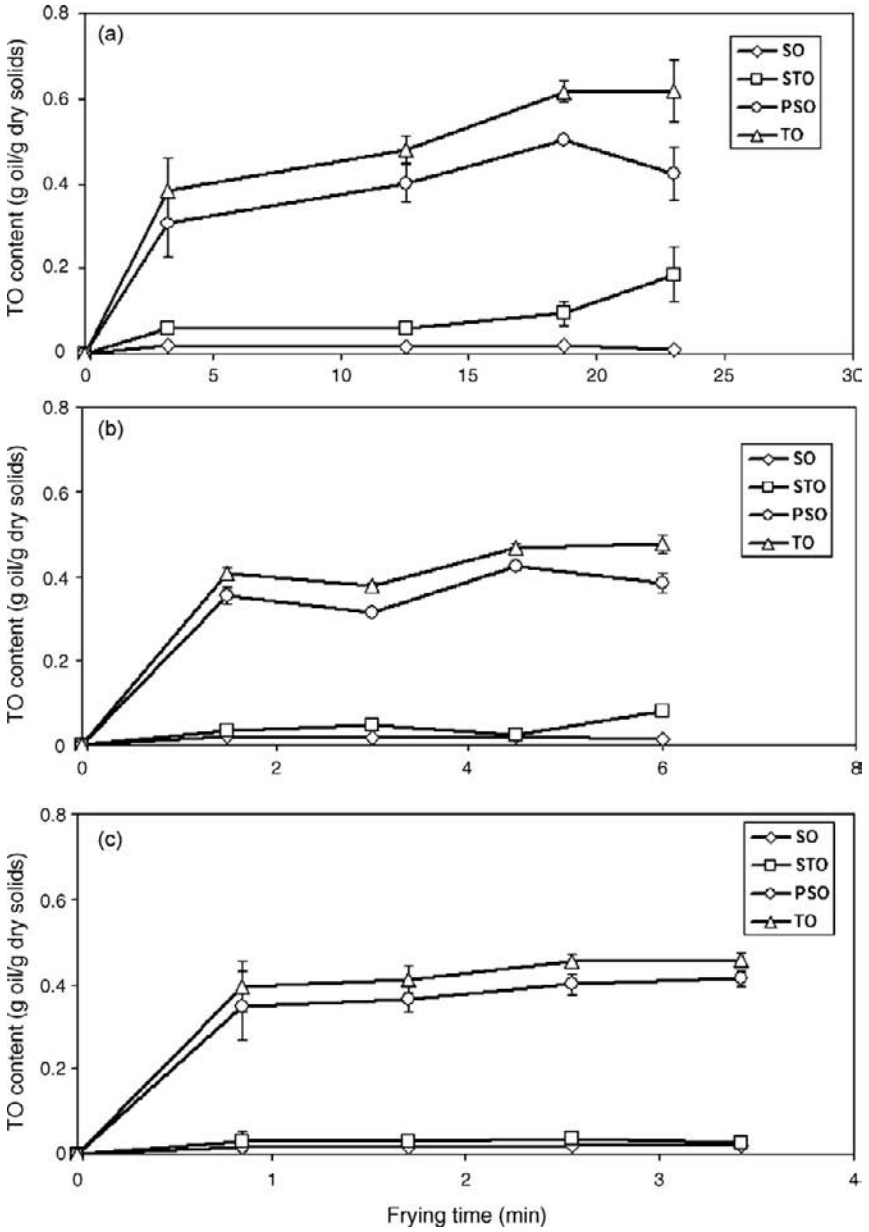

Figure 11.3: Kinetics of oil uptake fractions and total oil in potato slices during frying at: (a) 120°C; (b) 150°C; (c) 180°C. TO: total oil; PSO: penetrated surface oil; SO: surface oil; STO: structural oil (reprinted with permission from J. Food Eng., 2008, 87, 200–212).

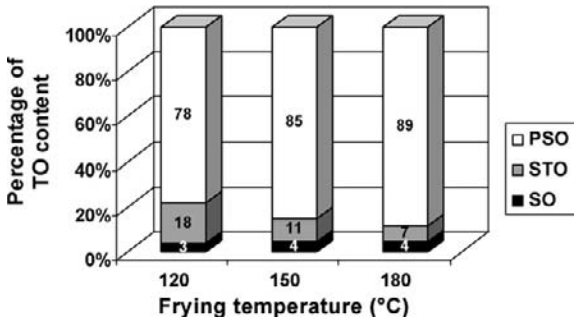

Figure 11.4: Average oil fractions absorbed by potato slices during frying at 120, 150, and 180°C. TO: total oil; PSO: penetrated surface oil; SO: surface oil; STO: structural oil (reprinted with permission from J. Food Eng., 2008, 87, 200–212).

and SO) in the structure of potato chips fried at 120, 150, and 180°C. At very short frying times (between 1 and 4 minutes) almost 75% of the total oil content of final control potato chips (chips with ∼ 1.8% of moisture content in total basis) is absorbed. After that time interval, the TO content remained almost constant until the moisture content (total basis) reached 1.8%. As the frying temperature decreased, the frying time required to reach that final moisture content and the TO increased. Similar results have been found in pre-treated potato chips, fried potato cylinders, and tortilla chips (Bouchon et al., 2003; Durán et al., 2007; Moreira et al., 1997). This result suggests that the total oil in potato chips is absorbed almost in the initial stage of frying once the potato slices are placed inside the hot oil. Almost 75% of the total oil content of the fried product (chips with ∼ 1.8% moisture content) was attained soon after immersion in the frying oil (between 1 and 6 minutes).

The distribution of oil fractions during frying of potato at three oil temperatures slices is shown in Figure 11.4. PSO constituted the highest fraction of TO during frying of the control; the higher the frying temperature, the higher the percentage of PSO based on the TO content (e.g. 78, 85, and 89% for 120, 150, and 180°C respectively). When the slices were removed from the fryer, a higher temperature difference develops between the surface and the interior, which, in turn, generates a higher negative pressure in the pore space leading to more oil penetration into their microstructure during cooling. This fact confirms that the oil absorption is principally a surface phenomenon (Aguilera and Gloria-Hernández, 2000; Bouchon et al., 2003; Durán et al., 2007; Ufheil and Escher, 1996). STO is the second important fraction in the TO content during frying of both control and blanched slices (Figure 11.4). As the frying temperature increases, STO penetration drops, since the higher internal pressures developed at higher temperatures make it more difficult for the frying oil to penetrate the potato structure whilst inside the fryer. The percentage of STO ranged between 7–18% for potato slices. This confirms that a small amount of oil penetrates during frying, because most of the oil is picked up at the end of the process, suggesting that oil uptake and water removal are not synchronous

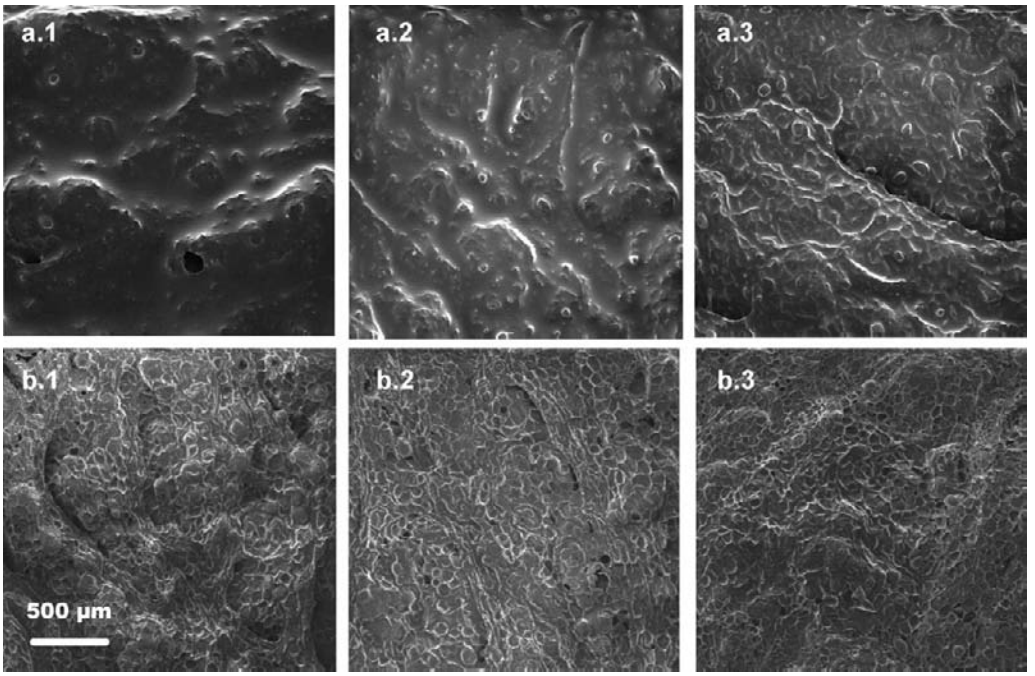

Figure 11.5: SEM images of the surface of control (a) and petroleum ether washed control for one second after frying (b) potato chips (moisture content ~ 1.8%, total basis) fried at: (1) 120°C; (2) 150°C; (3) 180°C (reprinted with permission from J. Food Eng., 2008, 87, 200–212).

phenomena. The SO fraction was the lowest constituent of TO content (approximately 4% of the total oil content) and remained almost independent of the frying temperature (Pedreschi et al., 2008).

Scanning electron microscopy (SEM) is a powerful tool used to study the surface topography of potato chips. Figure 11.5 shows that potato chips lose a considerable quantity of surface oil after they are washed in petroleum ether, which allows clear observation of the cellular microstructure of the surface (Figures 11.5b1, 11.5b2 and 11.5b3) (Pedreschi et al., 2008).

The other important quality parameters in fried potatoes are: moisture content, texture (crispness and shrinkage), color and, of course, flavor. Researchers have reported different means to improve quality in fried products. The blanching step previous to frying improves the color and texture (Fan et al., 2005; Shyu and Hwang, 2001). Shyu and Hwang (2001) immersed apple slices in fructose solution prior to frying, which resulted in a reduction of absorbed oil after frying. Pre-drying of potatoes is a common way to reduce fat uptake in fried potatoes (Krokida, et al., 2001; Moyano et al., 2002). Besides, properties of the surface of the potatoes are highly relevant for fat uptake, so the application of a coating is a promising route to reduce oil content (Mellema, 2003).

Finally, in order to improve the quality of potato chips, vacuum frying may be an option for production of fruits and vegetables with low oil content and the desired texture and flavor characteristics. It is defined as the frying process that is carried out under pressures well below atmospheric levels (Garayo & Moreira, 2002). Due to the pressure lowering, the boiling points both of the oil and the moisture in the foods are lowered. Vacuum frying has some advantages that include: (1) reduction of the oil content in the fried product; (2) preservation of the natural color and flavors of the product due to the low temperature and oxygen content during the process; and (3) reduction of adverse effects on oil quality. Granda et al. (2004) concluded that vacuum frying can also reduce the acrylamide content in potato chips.

In April 2002, Swedish researchers shocked the food safety world when they presented preliminary findings of acrylamide in some fried and baked foods, most notably potato chips and French fries, at levels of 30–2300 μm/kg. Reports of the presence of acrylamide in a range of fried and oven-cooked foods have caused worldwide concern because this compound has been classified as probably carcinogenic in humans with significant toxicological effects, namely neurotoxic and mutagenic (Rosen and Hellenäs, 2002; Tareke et al., 2002). French fries and potato crisps exhibit relatively high values of acrylamide 424 μg/kg and 1739 μg/kg, respectively.

It has been stated that acrylamide is generated during a side reaction of the Maillard reaction. Crucial participants in this Maillard reaction in fried potatoes are an amino acid (asparagine) and reducing sugars (fructose and glucose) (Mottram and Wedzicha, 2002). Asaparagine provides the backbone of the acrylamide molecule, while reducing sugars are essential co-reactants in the formation of the N-glycoside intermediates, which lead to the formation of acrylamide. Fried products, especially French fries and crisps, belong to the food category with probably the highest concentration of acrylamide recorded so far. The reason for this strong susceptibility to acrylamide formation is the abundance of free asparagine present in potato (Zyzak et al., 2003). Besides, fried potato color is the result of the Maillard reaction as well, that depends on the superficial reducing sugar content, and the temperature and frying period (Marquez and Añon, 1986). Consequently, for frying temperatures between 120 and 180°C, a linear correlation was found between acrylamide content and color not only in potato chips but also in French fries (Pedreschi et al., 2005, 2007). Figure 11.6 shows that the color difference parameter (ΔE) presented good correlation with the acrylamide content of the fried potato strips previously treated in the different ways. As the frying temperature increases from 150 to 190°C, the resultant chips get more red and darker as a result of non-enzymatic browning reactions that are highly dependent on oil temperature and frying time. Blanching reduces the ΔE values of French fries, probably due to the leaching out of reducing sugars previous to frying inhibiting in this way non-enzymatic browning reactions and leading to lighter and less red French fries (Pedreschi et al., 2004, 2006, 2007).

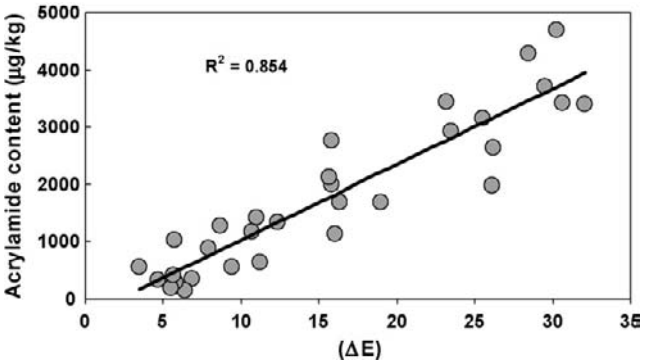

Figure 11.6: Acrylamide content vs. color difference parameter Δ*E* for potato strips fried at 150, 170, and 190°C for different pre-treatments (reprinted with permission from J. Food Eng., 2007, 19, 1287–1294).

Acrylamide appears to form as a result of a reaction between specific amino acids, including asparagine, and sugars found in foods reaching high temperatures during cooking processes. The process is known as the Maillard reaction and occurs at temperatures above 100°C. Variation in suitability of potato tubers for processing is not influenced only by cultivar and storage conditions, but also by differences in normal cultural practice and growing conditions. A low amount of reducing sugars in the tuber is necessary to prevent the non-enzymatic Maillard reaction between sugars and free amino acids during frying. The Maillard reaction is responsible for the development of undesirable dark-colored compounds, melanoidins, with a bitter taste. On the other hand, it is essential for its contribution to the color and flavor of fried potatoes. Potato variety, composition, and field site had a noticeable influence upon acrylamide formation (Haase et al., 2003).

Recently, research has focused on possible mechanisms of acrylamide formation in foods in order to develop strategies to minimize its formation. Some international research groups have separately confirmed a major Maillard reaction pathway for acrylamide formation (Mottram and Wedzicha, 2002; Stadler et al., 2002; Weiβhaar and Gutsche, 2002). Significant amounts of acrylamide are formed by the high-temperature reaction of glucose and the common amino acid asparagine (Coughlin, 2003). Since potato tubers contain a high amount of asparagine, it is now thought that this Maillard reaction is most likely responsible for the majority of the acrylamide found in potato chips and French fries. The potential for acrylamide formation is strongly related to the sugar content such as glucose and fructose (Biedermann et al., 2002; Pedreschi et al., 2006). For instance, some authors reported that the reduction of the sugar content by blanching or soaking could decrease acrylamide concentration by about 60% in potato chips (Haase et al., 2003; Pedreschi et al., 2004). Potato processing conditions (pre-treatments, temperatures, and

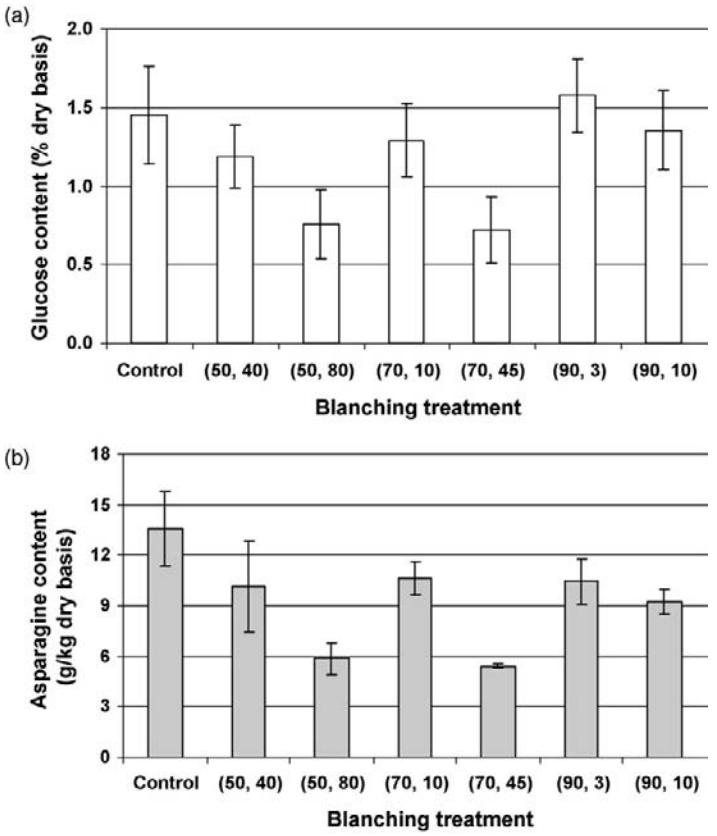

Figure 11.7: (a) Glucose content of potato strips blanched in hot water at different temperature–time combinations before frying. (b) Asparagine content of potato strips blanched in hot water at different temperature–time combinations before frying. First numbers inside parenthesis indicate the blanching temperature (°C); second numbers indicate the blanching time (min). Control corresponds to unblanched potato strips (reprinted with permission from J. Food Eng., 2007, 19, 1287–1294).

times) had a noticeable influence upon acrylamide formation. Asparagine is the free amino acid present in the highest amount in potatoes (93.9 mg/100g) (Martin and Ames, 2001). Asparagine content in potatoes depends on factors like variety, location, fertilization, storage, and processing (Davis, 1997; Hippe, 1998).

Blanching as pre-treatment has a great influence in diminishing acrylamide formation in potato pieces after frying (Pedreschi et al., 2004, 2007). Not only glucose but also asparagine content (Figures 11.7a and 11.7b) decreased drastically as the temperature and time of blanching increased leading to French fries with less acrylamide after frying. Acrylamide formation increased significantly in blanched samples when the frying temperature was increased

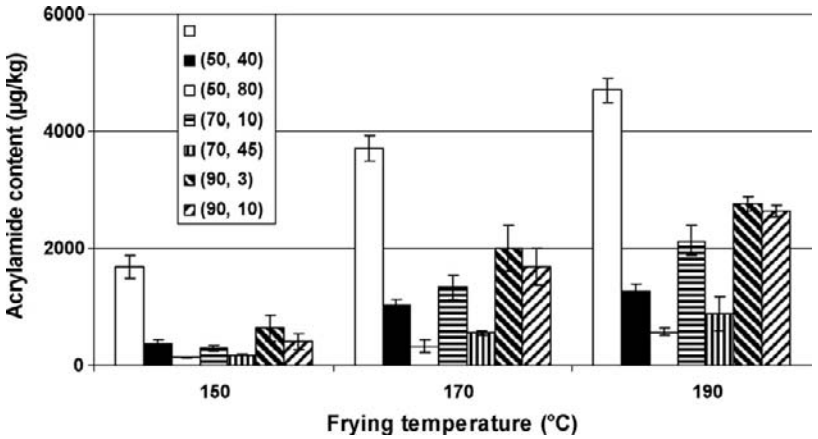

Figure 11.8: Acrylamide content of potato strips blanched at different temperature–time combinations after being fried at 150, 170, and 190°C. First numbers inside parenthesis indicate the blanching temperature (°C); second numbers indicate the blanching time (min). Control corresponds to unblanched potato strips (reprinted with permission from J. Food Eng., 2007, 19, 1287–1294).

(Figure 11.8). For instance, acrylamide contents were 287, 1338, and 2128 μg/kg after frying at 150, 170, and 190°C, respectively, in the case of potato slices blanched at 70°C for 10 min.

11.2.1 Blanching

Long-time blanching treatments such as that of 50°C for 80 min and 70°C for 45 min resulted in the lowest levels of acrylamide formation (342 and 538 μg/kg as average values for the three frying temperatures tested). These two blanching treatments after frying at 190°C, lead to the lowest acrylamide contents (564 and 883 μg/kg, respectively).

Currently, a substantial body of research has been carried out worldwide to build a greater understanding of acrylamide, how it is formed in foods, what the risks are for consumers, and how to reduce occurrence levels. Although many possible ways to reduced acrylamide content have been confirmed, the corresponding effects in sensory attributes in most of the reduction studies in fried potatoes have not been clearly reported yet (Zhang and Zhang, 2007).Dehydrated Potato Products

Drying is a widely used method of fruit and vegetable preservation. Water is removed to a final concentration, which assures microbial stability of the product and minimizes chemical and physical changes (Lewicki and Jakubczyk, 2004). Nowadays, drying is regarded not only as a preservation process, but also as a method for increasing added value of foods (Ramos

et al., 2003). Processing of plant raw materials causes irreversible changes in the tissues of fruit and vegetables. These changes are particularly visible after heat treatment (Lisińska and Golubowska, 2005).

Conventional air-drying is the most frequently used dehydration operation in the food industry (Krokida et al., 2003). Potato drying kinetics is greatly affected by air temperature, relative humidity, air velocity, and material size (Krokida et al., 2003; Mulet et al., 1989). Drying causes notorious physical and structural modifications of potato tissue. The most pronounced macroscopic modification is the shrinkage and deformation of food pieces undergoing drying. Transient thermal and moisture gradients develop tensional and compressional stresses. Stresses cause tissue breakage and fracturing during drying (Lewicki and Pawlak, 2005). Wang and Brennan (1995) showed that surface layers of potato slabs dried by air convection are severely damaged at short times, while the inner structure appears apparently intact. Further drying induces formation of cracks, the inner tissue is pulled apart and numerous holes are produced (Lewicki and Pawlak, 2005; Ramos et al., 2003). Loss of water and segregation of components occurring during drying, could cause rigidity, damage and disruption of cell walls, and even collapse of cellular tissue. These changes are associated with volume reduction of the product. Fast drying leads to cracking, resulting in final rigid products with more volume and a surface crust; slow drying rates result in uniform and denser products (Brennan, 1994).

Texture has been recognized as one of the most important quality attributes in dried potatoes which contribute to the consumer acceptance. Texture of potatoes is affected by drying processes and it is strongly associated with composition and structure of cells walls (Ramos et al., 2003). As previously stated, water loss is accompanied by loss of internal pressure; cellular tissue shrinks and becomes soft. This pressure is known as turgor pressure and plays an important role in the rheological and textural properties of the potato tissue.

Puncture and compression tests have been extensively used to measure the textural and viscoelastic properties of dried foods (Nisha et al., 2006; Ramos et al., 2003). The puncture test records the force required to push a probe into a food and it can be measured by the initial slope and the maximum breaking force extracted from the force–distance curve. Troncoso and Troncoso and Pedreschi (2007) studied the kinetics of textural changes during convection drying at different air temperatures (50, 60, 70, and 80°C) of potato slices by using four mathematical models which linked the dimensionless textural parameter obtained by a puncture test, maximum force F^*_{MAX} with drying time. F^*_{MAX} allows the study of global textural changes in potato tissue not only during the softening but also during the hardening stage (Krokida et al., 2000; Ramos et al., 2003). This parameter reflects physical and structural modifications of potato during drying which are closely linked to the turgor pressure of the tissue and the rigidity of cell walls (Ramos et al., 2003). Regardless of the different drying temperatures, the trend of F^*_{MAX} with drying time was almost the same, showing a progressive and significant decrease

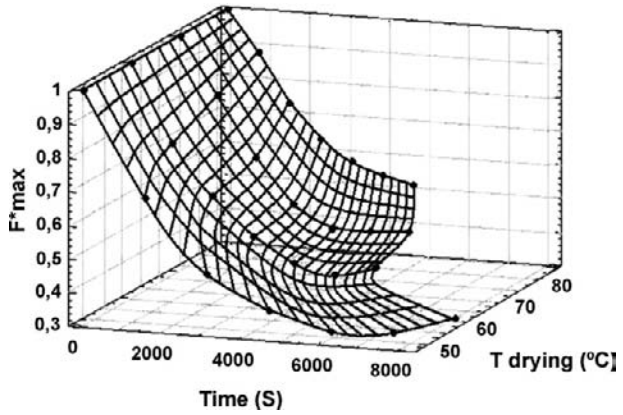

Figure 11.9: Changes in the F^*_{MAX} values of potato slices that air-drying at the different air temperatures tested (reprinted with permission from J. Food Eng., 2007, 82, 577–584).

in its value as the exposure time increased especially at higher temperatures. The drying of potato slices originates the softening of the tissue. For high drying temperatures, the initial velocity of reaction of the softening process is fast showing a clearly pronounced negative slope (Figure 11.9). Then, the reaction velocity diminishes. Change in F^*_{MAX} with moisture content during drying at different air temperatures is shown in Figure 11.10. F_{MAX} in potato disks decreases with moisture content and it is affected significantly by the drying temperature ($P > 0.05$). On the other hand, Figure 11.10 shows that for moisture contents lower than ~1.1 g/g dry solid, F_{MAX} increases principally due to the low drying temperatures leading to irreversible changes in the product microstructure which results in the disruption of lamella media and increasing of cristalinity (Krokida et al., 2000).

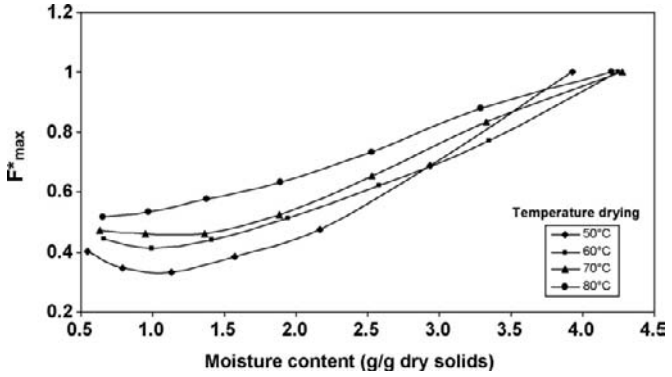

Figure 11.10: Changes in the F^*_{MAX} values with moisture content at different drying temperatures (reprinted with permission from J. Food Eng., 2007, 82, 577–584).

Wang and Brennan (1995) modeled transport phenomena during potato drying. They use a mathematical model which considers simultaneous heat and moisture transfer permitting moisture and temperature distributions to be predicted during air drying of potato slices. This model took into account the effect of the moisture–solid interaction at the drying surface by means of sorption isotherms of food. Non-constant physical and thermal properties were also incorporated in the model. Experimental data and model calculations showed good agreement. On the other hand, Mayor and Sereno (2004) modeled the shrinkage of potatoes and other vegetables during convective drying using either empirical or fundamental models in order to give a physical description of the shrinkage mechanism and behavior during drying. Empirical models were obtained from regression analysis of potato shrinkage date and fundamental models were based on a physical interpretation of the structure of food materials. Average relative deviations between experimental and predicted values of shrinkage in potato and other vegetables were found in more cases less than 10%.

Krokida et al. (2001a) studied the effect of the method of drying on the color of dehydrated products. Five different methods of drying were used: conventional, vacuum, microwave, freeze, and osmotic drying. Changes in color parameters a* (redness) and yellowness (b*) followed a first-order kinetic model. Freeze drying was the method that best preserved the color of potatoes, avoiding extensive darkening caused by the other methods of drying. On the other hand, Severini et al. (2005) studied drying in potato cubes pre-treated with a blanching. Blanching was alternatively performed in hot water, hot sugar–saline solution, by microwaves in distilled water, or by microwaves in saline solution. Drying was alternatively carried out in an air cabinet, a microwave oven, or a belt drier. In terms of process speed, color retention, and water absorption capacity, the best results were obtained combining microwaving blanching with dehydration on the belt dryer.

During air drying, potatoes undergo several simultaneous physical and structural modifications which are undesirable (McMinn and Magee, 1997). Thus, potato cylinders when dried in a convective dryer showed that moisture removal was accompanied by almost negligible internal porosity (< 10%), with air temperature representing the principal controlling factor. The dried potato cylinders suffered significant volumetric shrinkage which displayed a linear correlation with moisture content and air temperature. Also, Wang and Brennan (1995) studied structural changes of potatoes during drying with light microscopy. They observed that the degree of shrinkage of potato during low-temperature drying is greater than at high-temperature drying. Shrinkage also affects the physical properties of materials, such as the density and porosity. In the early stage of drying the density increased as the moisture content decreased, reaching a peak and then decreased with further decrease in moisture content. Total porosity increased steadily as moisture content decreased in the early stages of drying and then sharply toward the end of drying. The percentage changes in thickness, length, and width of the potato samples during drying increased linearly with decreasing moisture content.

Air drying could be used as a pre-treatment in frying operations and some authors have reported that they could reduce oil absorption in potato chips and increase texture in potato chips significantly (Pedreschi and Moyano, 2005b). Also, drying at high temperatures could cause the case-hardening phenomenon which consists of a surface hardening leading to a diffusion diminishing since there is an increasing in the resistance to water loss in potato pieces (Moyano and Berna, 2002). Leeratanarak et al. (2006) found that blanched potato slices dried faster than that corresponding to unblanched or raw potato slices. These authors concluded that the softening of the potato tissue during blanching reduces the case-hardening phenomenon after drying. Pedreschi and Moyano (2004) studied the effect in fried potato slices of blanching in hot water at 85°C for 3.5 min plus a drying treatment at 60°C until the potato pieces reached a moisture content of approximately 60%. Final potato chips blanched and pre-dried presented 24% less oil uptake than final blanched chips without pre-drying.

Potato could be dried to obtain potato flakes which are formed by potato starch granules. Potato starch granules are ellipsoidal, with a typical dimension of between 10 and 100 μm. They contain approximately 23% in wet basis of amylose and have amylopectin with β-type crystallinity. The granules are naturally low in lipids, but high in phosphates (Cheyne et al., 2005). Cooking processes are essential to allow starch to be metabolized by humans. They involve the action of heat, moisture and often mechanical action, and are a subset of actions termed starch conversion (Mitchell et al., 1997). There are some patented processes for making dehydrated potato products from potato flakes so that they can be sulfite-free by mixing the potato flakes with a sufficient amount of water to increase their moisture content to a predetermined level, lowering the temperature of the moistened potato flakes to a temperature and for a period of time sufficient for retrogradation of free soluble starch contained in the potato flakes, reducing the size of the potato flakes in a manner to minimize breakage of potato cell walls, and drying the resulting potato product. The dehydrated potato product can be reconstituted without using a boiling liquid to make a dough for French fries, chips, or to make a mashed potato product.

The process of converting potatoes into potato flakes involves many stages. Producing good-quality potato flakes is the result of controlling the operating parameters of all the equipment involved in the production process. Perfect process control and high-quality material and ingredients will assure excellent quality of the finished product. Yield ratio of ∼ 6.7:1 and rehydration ratio of potato flakes to potato mash is 1:5. Potato flakes can be easily reconstituted with cold water, which led to its widespread use as an ingredient. This product has very good demand and wide application in industries, restaurants, fast-food joints, catering services, feeding programs, etc. Bouchon and Pyle (2004) use low-leach potato flake as the major ingredient in formulating restructured potato chips, since it is frequently used in the manufacturing of pellets or die-cut sheet snacks due to its high stickiness. In addition, it is a desirable ingredient because of the expanded texture and rapid palate clearance that it confers on finished products, mainly because the starch in potato flake is fully gelatinized (Bouchon, 2002).

11.4 Conclusions

Potato is one of the most important processed vegetables. Frying and dehydration are important unit operations used commonly by industry to process potatoes. Oil uptake and acrylamide content are major issues in fried potatoes related to the health and safety of consumers. Oil uptake in fried potatoes diminishes as the frying temperature increases. Most of the oil is absorbed when the fried potato pieces are removed from the fryer and penetrate into the microstructure during the cooling process. Acrylamide is a toxic compound (carcinogenic in animals), which is formed during frying mostly by the Maillard reaction mechanism in which asparagine reacts with reducing sugars such as glucose and fructose. Several methods to mitigate acrylamide formation have been studied such as blanching, soaking, acid addition, amino acid addition, etc. Since the flavor and taste of fried potatoes depend on the Maillard reaction, the major challenge is to produce fried potatoes with low acrylamide content without affecting their traditional flavor, taste, and other sensorial attributes. Drying of potato produces strong structural changes in the microstructure making crucial physical properties such as porosity, volume, thickness change abruptly with moisture loss affecting negatively the aspect of the final product.

Acknowledgments

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CHAPTER 12

Post-harvest Storage of Potatoes

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12.1 Introduction

Potatoes are widely grown and consumed in temperate as well as tropical countries. The potato crop season varies in different countries. In most countries, potatoes are raised as one single crop. Therefore, depending upon the end-use, e.g., consumption or seed purposes, and to allow for later use, a comprehensive understanding of factors affecting the storage of potatoes becomes imperative to successfully store potatoes for three to ten months. As a perishable commodity, potato tubers undergo active metabolism during the post-harvest period. Their high moisture content and metabolic rate lead to both losses of mass and quality. The primary causes of these losses are biotic (e.g., growth responses like sprouting (weight loss) and quality changes due to pest and diseases (blight, soft rot), and biochemical changes (weight loss due to respiration)) and abiotic, thermal, (low-temperature sweetening), water (pressure flattening), atmospheric composition (black heart), light (greening) and mechanical (skinned tubers) stresses. The quality loss of potatoes during storage depends on storage management and the treatments received as early as seed storage as well as during growth and at harvest. These losses can be minimized and tubers can be stored successfully by proper crop management, and proper storage design and conditions (Booth and Shaw, 1981; Gottschalk and Ezhekiel, 2006; Rastovski et al., 1987).

This chapter discusses the various factors that affect the quality of potatoes during post-harvest storage such as (1) maturity stage of crop (early/late), intended use (table stock/processing/seed), (2) pre-harvest conditions of crop, (3) harvest and handling conditions, (4) health of the crop such as incidence of pests and diseases, (5) biochemical changes, (6) storage preparations and conditions, and (7) management of storage environment.

12.2 Maturity Stage of Tubers

Potatoes are destined to either the fresh market (boiled, baked) or for further processing (chips, French fries). The potato crop can be separated into late- or early-crop based on their maturity at

harvest. Maturity is a complex physiological and morphological condition which is influenced by several factors including: respiration, carbohydrate changes, dry matter content, moisture loss (defined by skin thickness and skin set), dormancy, and sprouting. The mature or late-crop potatoes are harvested after the tubers have reached physiological maturity and immature, early crop or new potatoes are harvested when the tubers are still increasing in size. Besides attainment of full size, tuber physiological maturity is indicated by the soluble sugar content reaching a minimum and the starch content being at a maximum, and full development of a thickened periderm layer (skin) below the epidermis (ability of the tuber skin to resist abrasion i.e., skinning during harvest; Brecht, 2003).

Early-crop potatoes are harvested to primarily meet the demand for fresh market and to obtain high prices, while some are used for processing, e.g., chipping. Although early-crop tubers are quite perishable and more susceptible to damage than mature potatoes, due to their immature skins, they are more succulent. The respiration rate of immature tubers at harvest is about four to five times greater than for mature tubers. Early harvested potatoes are usually stored only briefly, if at all. However, early potatoes which are free from serious bruising and decay may be held 4–5 months at 4°C for table use provided that they are cured 4 or 5 days or longer at 13–18°C to heal wounds prior to storage. However, it is recommended that early-crop potatoes be sold immediately due to poor storability and typically high early season prices. Early-crop potatoes, if used for chipping, should be chipped directly from the field since storage even at moderate temperatures of 10–13°C for a few days can cause excessive reducing sugar and undesirable dark chips (Brecht, 2003).

Most of the late-crop potatoes are used for processing or stored for the fresh market use. A high-quality fresh market potato tuber will be turgid, well-shaped, uniform brightly colored as well as free from adhering soil, mechanical damage, greening, sprouts, diseases, and other physiological defects such as black spot, black heart, freezing injury, sugar end browning, hollow heart, and internal necrosis. Potatoes for processing are graded based on size in combination with individual quality and size requirements of each company (Brecht, 2003; Kleinkopf, 1995; Walsh, 1995).

12.3 Pre-harvest Factors Affecting Post-harvest Storage

The quality of the potato crop that is stored ultimately determines the quality of the stored product. A good storage management cannot enhance quality out of storage if the health of the tubers is compromised during pre-harvest conditions. Storage behavior depends on growing conditions and production management and practices. The various pre-harvest growth conditions that influence the quality of stored tubers are moisture stress, nutrient status of the soil, and the incidence of pests and diseases (Booth and Shaw, 1981; Burton, 1989; de Haan, 1987; Gottschalk and Ezhekiel, 2006).

12.3.1 Moisture stress

Moisture requirement of the potato crop varies with cultivar maturity characteristics, plant population, water-holding capacity of the soil, climatic condition, and whether the tubers are grown for 'seed' or 'consumption' ('table' and 'processing') markets. Moisture stress during tuber growth, particularly during the early stages of development is associated with the occurrence of sugar-end tubers in some varieties such as Russet Burbank (Eldredge et al., 1996; Iritani, 1981; Iritani and Weller, 1973). When above normal temperatures occur during the growing season, excess reducing sugars (glucose and fructose) are formed in the stem end of tubers. These reducing sugars react with free amino acids during frying to form brown or black fry ends. For processors this results in reduced processing efficiency and economics, and in some cases an unusable product. The sugar-end disorder is also known as dark ends, translucent ends or, in more severe incidences when stem-end tissue breakdown occurs, jelly ends (usually during storage). Currently, there is no reliable method to identify the onset of this disorder. Evaluation of tubers following an environmental stress has shown that the formation of excess reducing sugars may not occur until weeks or even months after the stress. Sugar-end tubers are typically irregularly shaped with pointed stem ends. However, ideally shaped tubers may also have sugar-ends. Several factors including moisture stress, high temperatures, drying winds, higher than desirable fertilization levels and soil types have been shown to cause sugar-ends. Of these factors, heat and moisture stress appear to be the most important. The tuber should remain hydrated, especially those in the top six inches of soil after vine kill. If the tubers are dehydrated it can cause pressure flattening or internal sprouting during storage especially those stored for the medium to long term (Lewis, 2007). The severity of flattening varies depending on the variety. Internal bruising is referred to as black spot which results from a physical impact to the tuber.

12.3.2 Nutrient status

Cultural practices such as fertilizer management can affect quality of tubers during storage. For example, high nitrogen fertility increases yield but usually decreases storage quality (Booth and Shaw, 1981). High or excessive nitrogen may delay maturity of potato crop, lower dry matter of the tubers, and result in poorer skin set. During a 2-year experiment to study the effect of nitrogen application, it was found that tubers from untreated isopropyl N-(3-chlorophenyl) carbamate (CIPC), and treated with low nitrogen rates, sprouted earlier and produced more sprout weight than those from the high nitrogen treatments. In CIPC-treated tubers, sprouting was closely related to nitrogen rate with tubers from the low nitrogen rates sprouting earlier and producing higher sprout weight (Thornton et al., 1994). In a sand culture study, potatoes were grown to maturity in the greenhouse to study the effects of factorial application of four levels, each of potassium (K) (2, 4, 8, and 12 meq L^{-1}) and sulfur (S) (1, 2, 4, and 6 meq L^{-1}), on yield, quality, and storage behavior of tubers. Potassium applied with sulfur, besides increasing the yield and other quality parameters such as dry matter and protein contents, improved the shelf life of tubers, as determined by the percent weight loss of tubers after storage for four weeks at

room temperature. The best interaction with lowest tuber moisture loss being 12 meq K L^{-1} x 6 meq S L^{-1} (Moinuddin and Umar, 2004). It should be noted that there is a direct correlation between dry matter content and internal bruising such that potato tubers with high dry matter of more than 22% are more prone to shattering and bruising (personal communication, Dr. Coffin).

12.4 Harvesting and Handling Factors Affecting Post-harvest Storage

Conditions of harvesting affect the quality of tubers. However, the susceptibility of tubers to external damage during harvesting varies based on cultivar, stage of maturity of crop, soil and weather conditions, harvester skills, and design of harvesting and handling equipment (Booth and Shaw, 1981; de Haan, 1987; Gottschalk and Ezhekiel, 2006). Harvesting immature tubers, when the soil conditions are too wet or dry, and during too warm weather conditions can affect the quality of tubers. Soil moisture at harvest is an important factor with regard to bruising. The soil should be just moist enough (typically between 60 and 75%) to carry the harvested potato and soil to the secondary conveyor on the harvester where the soil should separate completely from the tubers. Tuber hydration level influences the type and amount of bruising. When tubers are dehydrated, blackspot bruise is more prevalent, whereas hydrated tubers have a tendency to have more shatter bruise. An intermediate level of hydration results in the least amount of tuber bruising. A light application of irrigation water 1–4 days before harvest to condition the soil may not impact tuber hydration. It is also important that potatoes should be harvested when pulp temperatures are between 10 and 16°C. Tuber pulp temperature is directly influenced by soil temperature. Cold tuber pulp temperatures increase both blackspot and shatter bruise, but the type of bruise damage also depends on tuber hydration level. Cold, hydrated tubers tend to shatter bruise more readily, whereas warm, dehydrated tubers develop blackspot bruise more easily. Tuber pulp temperature in each field should be checked before starting harvest with the help of an accurate tuber pulp thermometer and using it regularly to monitor pulp temperatures is important. Warm tubers, >16°C, may actually have less bruising than cold tubers, but cooling tubers in storage is difficult and tubers with warm pulp temperatures are more susceptible to rot (Bohl, 2003). When tubers are harvested under warm conditions the respiration rate of the tubers increases. Leak or shell rot, caused by *Pythium* spp. (primarily *P. ultimum*), is an important soil-borne pathogen that directly affects tuber integrity in storage. *Pythium* primarily invades the tubers through harvesting wounds, and post-harvest rot often develops in transit or in storage. Infection is most likely when tuber pulp temperatures are above 19°C. Avoid harvesting when the weather is hot and humid, and avoid exposing tubers to heat after harvest (Lewis, 2007). When tubers are harvested at very wet conditions, the chances of getting pink rot also increase. Pink rot is caused by *Phytophthora ethroseptica* fungus and is widespread and often infects tubers under wet soil conditions. Pink rot usually starts from the stem end and progresses uniformly through the tuber. Eyes can also be infected directly. External lesions are purplish black. The margin of lesions may be limited by a dark line. With time, the cut

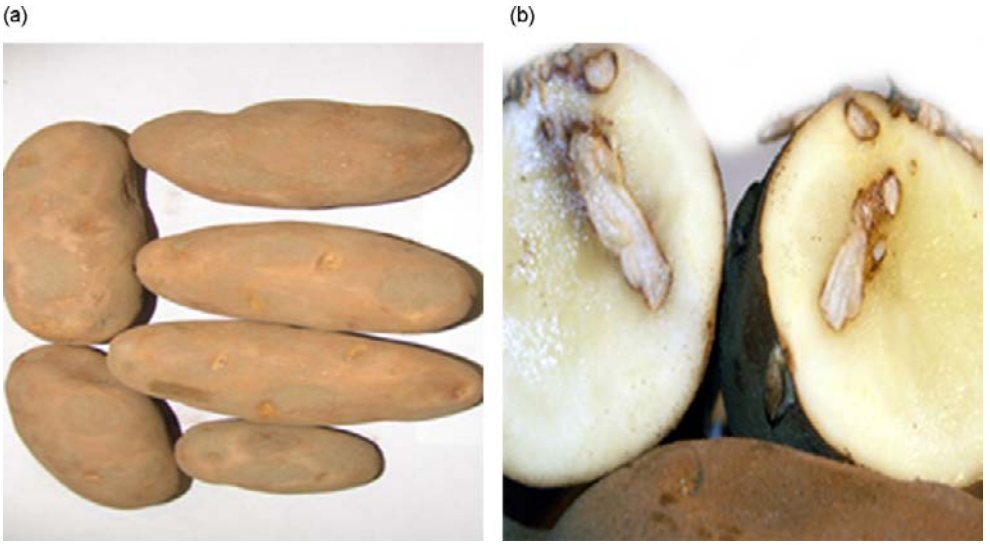

Figure 12.1: Development of pressure flattening (a) and internal sprouting (b) of stressed/ dehydrated tubers stored for medium to long term storage (reproduced with permission from Lewis 2007).

surface will become dark brown or black and will smell of ammonia. To reduce the incidence of pink rot, overwatering should be avoided late in the season particularly if temperatures stay above 22°C. Too dry soil conditions will result in dehydrated tubers which in turn will result in pressure flattening or internal sprouting during storage (Figures 12.1a and 12.1b). Internal sprouting can also occur due to too a low concentration of sprout inhibitor applied to tubers (personal communication, Dr. Coffin). Within a variety, the degree of damage to outside skin is influenced by dry matter content, maturity, and turgidity of the tubers. Harvesting tubers at an immature stage leads to skinned tubers (Figure 12.2). A direct correlation exists between

Figure 12.2: Development of skinned tubers during harvesting due to mechanical injury and or immature tubers (reproduced with permission from Lewis 2007).

incidence of internal bruising and content of dry matter whereby high dry matter leads to high bruising. Dry matter is influenced by growing conditions and variety. Variety, soil type, and temperature also influence tuber shape and skin strength which in turn greatly influence the damage to outside skin. The morphology of the periderm and its susceptibility to damage may also affect the extent of wound and other pathogens. Blight (*Phytophothora infestans)*, dry rot (*Fusarium solani* var.*caeruleum),* and skin spot (*Oospora pustulans)* infections are influenced by skin thickness. Spores are unable to penetrate the intact periderm (Scott and Wilcockson, 1978). Flaccid or flabby, limp tubers are more prone to bruising. Thus their susceptibility to damage increases with storage time (Booth and Shaw, 1981). Immature tubers will result in skinned tubers and increase the potential to develop dry rot. Tubers sustain mechanical damage during harvesting with poorly adjusted equipment, transport, and often during grading. Poorly adjusted equipment, such as excessive dropping heights, high belt speeds, and failure to coat the grading riddles with rubber, etc., results in increased bruises and soil on the tubers. Soil on the tuber prevents air circulation and exchange through the pile and at the surface of tuber, increases rot, black heart, and dehydration (Meijers, 1987a). Blue discoloration (internal bruising) or black spot can normally be identified only after peeling, hence it is not possible to remove the affected tubers from a lot of unpeeled potatoes unless external damage has occurred. For potatoes destined for processing, extra costs can be incurred to eliminate discolored chips and fries as well as damaged tubers. Dry cleaning (brushing) is required immediately after lifting of potatoes while trying not to cause mechanical damage. Direct exposure of tubers to sunlight after harvest should be avoided since such exposure stimulates undesirable greening in potatoes and possible increases in glycoalkaloids, and overheating of tubers which in severe cases results in cell death and blackening. Mechanical damage can also occur during handling operations such as grading, packing, and transporting. Up to three-fourths of total tuber damage occurs during harvest time with possible substantial injuries occurring each time tubers are handled. Potatoes are more susceptible to mechanical injury at low temperatures of about 5°C. Under certain conditions, injury may be reduced by raising the temperature of susceptible tubers before grading. Injury to the surface of the periderm accelerates moisture losses and exposes the interior of the tuber to pathogens which can result in dry and soft rots during storage. Curing, a pre-storage treatment, is critical to limit weight losses and prevent the penetration of microorganisms (see Section 12.8.4).

12.5 Health of the Tuber (Incidence of Pests and Diseases)

Tubers are initially or externally infected with spores in the field. Even a small quantity of diseased tubers in a lot can potentially result in the disease spreading and the whole lot being damaged during storage. Harvesting, handling, and transportation may also lead to mechanical damage to tubers which can result in the entry of pathogenic organisms. For example, the inoculum of *Fusarium* may enter the tubers through wounds caused during harvesting. It is recommended that tubers be examined for pests and diseases prior to storage. Grading of tubers

is important before storage in order to eliminate the diseased or infected tubers (Booth and Shaw, 1981; Burton, 1989; Gottschalk and Ezhekiel, 2006; Meijers, 1987a).

12.6 Biochemical Changes Influencing the Quality of Tubers During Storage

Biochemical processes/factors that affect the weight loss and quality of tubers during storage include respiration, water loss, sprouting, incidence of pests, and diseases and temperature.

12.6.1 Respiration

As a living organism, potato tuber requires energy through respiration. During respiration, the sugars in the tubers are converted to carbon dioxide and water with the help of oxygen thereby releasing energy as given by the following reaction.

$$C_6H_{12}O_6 + 6O_2 6CO_2 + 6H_2O + \text{energy(heat)}$$

Sugars used as substrates for respiration are produced from the starch hydrolysis which results in the loss of dry matter. Thus respiration is an indirect source of weight losses during storage. The rate of respiration is controlled by factors such as storage temperature and is lowest at about 5°C. The respiration rate increases slowly up to about 15°C above which respiration begins to increase sharply. Reducing the temperature to 3°C also results in a sharp increase in respiration due to the high concentration of reducing sugars formed by the breakdown of starch. The activity of the enzyme invertase, which hydrolyzes sucrose into glucose and fructose, is also high at lower storage temperature. At 0°C, the rate of respiration is same as that at 20°C. At 10°C, the dry matter loss by respiration represents approximately 1–2% of fresh weight during the first month and about 0.8% per month thereafter, but rising to about 1.5% per month when sprouting is well advanced. It is, therefore, important to keep the respiration rate low, preferably storing potatoes at a temperature of around 5°C (Booth and Shaw, 1981; Burton, 1966; Rastovski, 1987a; van Es and Hartmans, 1987a). Unfortunately most varieties used for processing into chips and French fries cannot be successfully stored below 9°C due to build up of sugars (low temperature sweetening; Burton, 1966, 1978).

Another important effect of tuber respiration is the production of respiratory heat and its subsequent influence on storage temperatures and ventilation systems. A significant amount of heat is produced during respiration and if this heat is not removed then the temperature of the potatoes could increase. To maintain the temperature of the potatoes at a specific level, the heat evolved by the potatoes during respiration must be removed by cooling. It is also important to have a steady supply of fresh air during storage to provide the oxygen needed in respiration and to remove CO_2 released during respiration. Excessive accumulation of CO_2 may cause blackheart eventually resulting in rot and also affect the processing quality of stored potatoes by affecting

the chip color. The CO_2 concentration outdoors is often 360–380 ppm. In a well-maintained potato storage, it often ranges from 1200–1500 ppm. If concentrations remain above 5000 ppm, it may be an indicator of storage rot and/or insufficient air exchanges/refreshing. Accumulation of CO_2 up to 3% in well-sealed stores is reported to result in unacceptable fry color. Therefore, regular ventilation of stores for 2–4 hours per day is recommended (Gottschalk and Ezhekiel, 2006; Veerman and van Loon, 1997; personal communication, Dr. Coffin).

The respiratory quotient (RQ), which is the ratio of volume of O_2 absorbed per hour to volume of CO_2 released per hour, should ideally be 1 when the O_2 supply is not limiting. It has been reported that the CO_2 to O_2 ratio value is approximately 0.8 during the early period of storage and 1.3 when sprout growth started (Burton, 1989; Gottschalk and Ezhekiel, 2006; Isherwood and Burton, 1975).

12.6.2 Water loss

Another physiological loss during storage is water loss. Tubers which have lost large amounts of water succumb to mechanical damage, such as bruising and blue/black discoloration, greater peeling losses, reduction of culinary quality leading to economic loss. The marketability of tubers will be affected if there is a water loss in excess of 10% because of the poor unattractive shriveled appearance. Water loss during potato storage depends on heat production in potatoes, the temperature of the potatoes, relative humidity, quality of potato skin, variety, sprout growth, and duration of ventilation (Booth and Shaw, 1981; Rastovski, 1987a). If a grower is storing a healthy crop of tubers, an objective is to maintain the relative humidity at approximately 90–92% in order to reduce desiccation of potatoes during air circulation. The rate of water loss from any particular sample of potatoes is proportional to the water vapor pressure deficit (WVPD) or drying power of the surrounding air. The rate of water loss under any given WVPD is influenced by the periderm or outer skin layer of mature potatoes. The periderm limits the rate of evaporation from a tuber to below that from a free water surface, exposed to the same WVPD. The dead cork cells do not allow for water to easily migrate to the surface where it would evaporate in response to the WVPD. About 98% of the water loss appears to occur in this way and only about 2% by direct diffusion in the gas phase through the lenticels (Burton, 1978), thus any damage to the periderm will increase evaporation rate. Average water loss from mature undamaged tubers is approximately 0.14–0.17% of the tuber weight per week per mbar VPD which will increase to 0.5–0.8% per week in damaged tubers (Booth and Shaw, 1981). It has been reported that the removal of the tuber skin resulted in an immediate 300–500-fold increase in evaporation (Burton, 1989). Stage of maturity and sprouting also influences the evaporation rate. Freshly harvested immature tubers lose water more rapidly than mature tubers since the immature skin is more permeable to water vapor. The increase in evaporation loss of water during sprouting is due to the sprout surface being 100–150 times more permeable to water vapor than the periderm of the tuber per unit area and time (van Es and Hartmans,

1987b). Varietal differences in water loss through evaporation are attributed to differences in maturity at harvest, rate of wound healing, thickness of periderm and sprouting characteristics of the variety (Booth and Shaw, 1981; Burton, 1989; Gottschalk and Ezhekiel, 2006; van Es and Hartmans, 1987b).

12.6.3 Sprouting

In general, potatoes remain dormant for a period after harvesting and generally the dormancy period ranges from 5–9 weeks. The length of dormancy in tubers is affected by cultivar, maturity of the tuber, soil, and weather conditions (Burton, 1978). Extreme cold wet weather increases dormancy whereas extreme dry warm weather shortens dormancy length. Under normal conditions, specific treatments are needed to break dormancy. Sprouting is known to break dormancy. A major component of successfully managing potato quality in storage is effective sprout inhibition. Sprouting causes tuber dehydration and weight losses by increased respiration and transpiration, increases levels of toxic glycoalkaloids, accelerates starch breakdown with concomitant accumulation of undesirable reducing sugars and decreases vitamin content, increases physiological aging and affects the appearance of the tuber which all affect the quality of the tuber. Sprouted tubers impede air movement through the potato pile. Various factors such as the physiological condition of the tubers, diseases, and storage conditions influence sprout growth during storage. Damaged and diseased tubers sprout earlier than healthy tubers. The main factors that influence the rate and form of sprout growth are variety, previous storage history, temperature, humidity, composition of the atmosphere, and degree of exposure to light (Booth and Shaw, 1981; Burton, 1978; Rastovski, 1987a; van Es and Hartmans, 1987d). Some varieties have considerably shorter dormancy than others. For example, Shepody has a shorter dormancy than Russet Burbank. Below 5°C, sprout growth is slow and increases up to an optimum temperature of 20°C, above which the growth rate decreases. Higher relative humidity favors sprout growth and this growth is more pronounced at higher temperature. Higher CO_2 concentration also favors sprout growth. A storage atmosphere with a CO_2 content of 2.2–9.1% stimulated sprouting in potatoes irrespective of stage of dormancy (Burton, 1958). The optimum CO_2 content was found to be 2–4% and the stimulatory effect declined at higher concentrations, the result at 7–10% being the same as with normal air storage (Burton, 1978). The O_2 content required for optimum sprout growth depends on the physiological age of the potatoes. At the beginning of the season, the optimum content of O_2 required for sprouting was 4–5% at a storage temperature of 10–20°C, while it increased to 17–20% around June (Burton, 1968).

Various methods are available to control sprouting during storage. These include chemical, non-chemical, and organic methods (Buitelaar, 1987; Kleinkopf et al., 2003; Mehta Ashiv 2002; Rastovski, 1987a; van Es and Hartmans, 1987d). Even though chemical treatments are widely used for sprout control, the increased awareness of the environmental and health concerns have spurred an increase in the use of alternative non-chemical or organic methods for sprout control which are discussed briefly below.

12.6.3.1 Non-chemical methods for sprout control

Non-chemical methods to control sprouting include: (1) developing varieties having longer dormancy by breeding; (2) low-temperature storage; (3) controlled atmosphere conditions; and (4) irradiation (Buitelaar, 1987; Kleinkopf et al., 2003). Breeding for longer dormancy is the best natural control method for sprouting. However, it is a lengthy process. In addition there is a need to preserve other agronomic and quality characters and the varieties differ in the length of dormancy. Storing tubers at low temperature is an effective method of controlling sprouting since tubers do not sprout at temperatures below 4°C. However, low temperature will also result in other disadvantages depending upon the end use of the potato. For example, in potatoes destined for processing, a condition called low-temperature sweetening (LTS) will result depending on the cultivar resistance. For table stock and seed potatoes, LTS does not pose a problem. Other advantages of low temperature include the natural control of fungal and bacterial losses and avoidance of chemical use. Another non-chemical method for sprout control is controlled atmosphere storage, where a particular ratio of reduced O_2 and increased CO_2 concentration is used (Thompson, 1998). The major disadvantage with controlled atmosphere storage is its requirement for airtight rooms, which increases the cost of storage. Although in general, a very low O_2 concentration of 1% results in strong inhibition of sprouting at 6°C, the darkening of the fries, and increased tuber disorders and diseases may occur. The optimal concentration of O_2 for promoting sprout growth has been found to be 3–5% (Hartmans, 1993). However increased CO_2 concentration ranging from 3–9% can either increase or decrease sprouting depending on the storage temperature and physiological age of the tuber, and similar to very low O_2 concentrations, may affect the color of French fries, and increase the disease incidence (Schouten, 1994). It has been reported that controlled atmosphere storage of potato tubers at a specific atmosphere of 3% O_2 and 12% CO_2 decreased tuber respiration, minimized tuber weight loss, and inhibited or delayed sprout growth (Gubb and Moorby, 1995). Olsen et al. (2003/4) reported that controlled atmosphere conditions of 2% O_2 and 10% CO_2 at 7°C altered tissue mechanical properties such as lower tissue toughness and failure strain (deformation/length) and had the highest total sugar concentration (glucose, fructose, and sucrose), while tubers stored at 9°C had the lowest total sugar content. The physiochemical characteristics of tubers of controlled atmosphere storage were similar to those stored at 3°C.

Another effective non-chemical method for sprout inhibition is irradiation (Buitelaar, 1987; Kleinkopf et al., 2003). The principle of irradiation is the prevention of cell division. It is believed that low-dose ionizing irradiation may become a viable option due to the increasingly higher operating costs of low storage temperature and the possible phase-out of chemical suppressants. Usually exposure to 15–200 Gy ionizing radiation for 10–2 minutes (for a total of 150–400 Gy) is sufficient to control sprouting, however it may vary with variety. The sprout-inhibiting effect of ionizing radiation is outstanding and surpasses the chemical sprout inhibitors currently in use. There are some precautions to be considered before irradiating tubers. Bruising may result during harvesting and collection of potatoes. Irradiation delays curing of the bruises on potatoes,

which may result in putrefaction during storage. Hence, irradiation can be carried out only after the bruises have been completely cured, which takes about two to three weeks after harvesting, depending upon the curing conditions. In addition, irradiation should be carried out during the dormancy period of the tuber which varies among varieties. It is also necessary to consider the maturity and storage temperature for the irradiation treatment. Irradiation of tubers at an immature state sometimes causes the internal tissue to turn black (Kameyama and Ito, 2000). Storage temperature, humidity, and ventilation of the irradiated tubers should be controlled in order to provide suitable conditions to maintain the quality of the tubers. For the storage of irradiated potatoes, enough oxygen must be supplied to compensate the increase in respiration which occurs in storage during two weeks after irradiation. The most desirable temperature for storage is 5–10°C. Storing irradiated potatoes at a high temperature was found to result in the loss of freshness and the possibility of blackheart (Kameyama and Ito, 2000). A study was undertaken to examine the effect of different levels of gamma irradiation to control sprouting and increase the length of storage time of potatoes in relation to chlorophyll and glycoalkaloid synthesis using seven potato cultivars. Results showed significant genotype differences among cultivars in response to gamma irradiation levels, with some cultivars exhibiting dramatically reduced levels of glycoalkaloid synthesis as compared to others. Averaged over all cultivars, irradiation significantly reduced the accumulation of glycoalkaloids, but a significant interaction was found between cultivars and irradiation. The same trend was observed with chlorophyll synthesis after light exposure (Dale et al., 1997). Some of the disadvantages of irradiation are increased reducing sugars, gray discoloration of potatoes after cooking, increased *Fusarium* attack, gray discoloration of prefried French fries, darkened chip color, consumer resistance to nuclear technology, and cost (Buitelaar, 1987). In a cooperative project, the University of Idaho, Idaho State University Accelerator Center and Milliennial Technology evaluated the effects of high-energy electron treatment of potatoes for sprout control and found that treatment of potatoes with high-energy electrons produced with small linear accelerators (5–10 MeV capacity) effectively controlled potato sprout with minimum losses in tuber quality including fry color (Kleinkopf et al., 2003). Irradiation has been registered for more than 40 countries in use (Daniels-Lake and Barnes, 2007).

12.6.3.2 Chemical methods for sprout control

A large number of chemicals have been shown to have sprout-inhibiting effects such as ethylene, nonanol, chlorpropham, maleic hydrazide, carvone, abscisic acid, indol acetic acid, clove oil, mint oils, hydrogen peroxide, and 1, 4-dimethylnaphthalene (Buitelaar, 1987; Kleinkopf et al., 2003; Rastovski, 1987a; van Es and Hartmans, 1987d). However, the principal sprout inhibitors used worldwide are isopropyl N-phenylcarbamate (IPC, propham) and isopropyl N-(3-chlorophenyl) carbamate (CIPC, chloro-IPC, chloropropham). Both compounds stop cell division and the effect is irreversible and hence cannot be used in seed tubers. The effectiveness of CIPC depends on the storage conditions, application technology, and variety characteristics (Buitelaar, 1987; Kleinkopf et al., 2003). IPC and CIPC are available in powders and liquids.

CIPC is generally vaporized into piles of stored potatoes or applied in a water-based application to tubers being packed for grocery stores. The advantage of using powders is that they can be applied in a single operation which does not require excessive effort. However, the use of powder form results in irritation of the skin in potatoes if the potatoes are insufficiently suberized, resulting in the loss of natural color of potatoes (Buitelaar, 1987). The disadvantages of powder use are dust nuisance, natural color loss, and skin irritation, which can be reduced by using liquid form as the application is done in three treatments. The disadvantage of liquid form is it should be applied at an early stage, the first dose must be applied two or three weeks after the harvest (Buitelaar, 1987). Studies involving two chipping cultivars, Snowden and Monona, with or without Chlorpropham (CIPC) application, and stored in darkness at 10/12°C, showed no effect of CIPC on chip color quality or tuber concentrations of protein, dry matter, sucrose, reducing sugars, or the assayed enzymes and metabolites of glycolysis. Respiration, as measured by CO_2 production, was significantly suppressed in CIPC-treated tubers. Concentrations of ethanol and lactate, products of anaerobic respiration, were significantly higher in the CIPC-treated Snowden tubers, relative to the untreated tubers (Blenkinsop et al., 2002). Maleic hydrazide is often used as a preharvest foliar spray and is popular and effective (Buitelaar, 1987). Although it can control sprouting for 3–4 months, a subsequent treatment with CIPC is required during later storage. A recent Environmental Protection Agency mandate (1996) within the requirements of the Food Quality Protection Act (FQPA) resulted in a reduction in allowable CIPC residue on fresh potatoes in the USA from 50 ppm to 30 ppm. This mandate coincides with tolerance reductions or restrictions for use of CIPC in other parts of the world (Kleinkopf et al., 2003). Hence there is a need for alternative compounds for sprout suppression. Naturally occurring volatile compounds such as mono-, di-, and trimethylnapthalenes, benzothiophene (DMN), 2, 6-diisopropylnapthalene (DIPN), carvone and ethylene have been found effective at lower temperatures (7–10°C) and at higher concentrations as compared to CIPC (Kalt et al., 1999; Lewis et al., 1997). Strong sprout-suppressing effect of carvone was also observed at higher storage temperatures (24 ± 2°C; Mehta Ashiv 2002). A concentration of 300 μl/l completely inhibited sprouting for 110 days of storage with no rotting up to 80 days. Due to its high volatility, repeated applications are required which may cost up to 10 times the cost of CIPC treatment. The advantage of using carvone is that it does not affect the fry color, its effect is reversible so that it can be used for treating seeds, and it has been shown to have fungicidal activity against silver scurf, some *Fusarium*, and *Rhizoctonia* (Mehta Ashiv, 2002). In a study to compare the sprout inhibition effect of various suppressants and its effect on fry color and sugar content during 25 weeks of storage at 9°C, it was observed that maximum suppression of sprouting was observed with CIPC followed by carvone, ethylene, and dimethylnapthalene (Kalt et al., 1999). Another report (Vokou et al., 1993) where the sprout inhibition and antimicrobial effect of various aromatic plants were studied, showed that essential oils of *Lavandula angustifolia* (lavender), *Mentha pulegium* (mint), *Mentha spicata* (spearmint), *Rosmarinus officinalis (rosemary),* and *Salvia fruticosa (sage)* suppressed sprout growth and had antimicrobial activity against *Erwinia carotovora* strains and bacteria isolated from the surface of potato tubers.

The sprout inhibition was reversible, thus it can be used for seed tuber storage (Vokou et al., 1993).

Ethylene is a very effective sprout inhibitor, however, its use may result in darkening of fry color (Daniels-Lake et al., 2007). Like carvone, its effect is reversible and can be used for seed storage. In a study carried out to minimize the effect of ethylene on fry color, it was observed that continuous ethylene treatments inhibited sprout growth as effectively as CIPC, except at 13°C storage. Interruptions of 18 h and 2 or more days reduced sprout inhibition. It was also observed that an early start of ethylene exposure before the end of suberization using either a concentration or time-increment gradient had the least effect on fry color while maintaining good sprout inhibition (Daniels-Lake et al., 2007). Other emerging potent natural sprout inhibitors are salicylaldehyde and menthol which can be used under higher-temperature storage conditions (Mehta and Ezekiel, 2006).

12.6.4 Incidence of pests and diseases

Stored potatoes may be affected by microorganisms such as fungus or bacteria. These microbial infections may have occurred in the field prior to harvesting or due to infection following harvest. The infection may spread extensively under favorable conditions in the storage (Booth and Shaw, 1981; Meijers, 1987a). Rotting that occurs in the field is immediate and continues during post-harvest storage, e.g., late blight and pink-rot. Mechanical injury is the main reason for dry rot, watery wound rot, and for many of the post-harvest infections. Qualitative pathogenic diseases such as common scab, powdery scab, black scurf, and silver scurf affect the appearance of potato and thus affect its market value. Quantitative loss results from diseases such as blight, pink rot, dry rot, and bacterial soft rots. Tuber soft rot is one of the most common causes of storage losses (Figure 12.3) and can be avoided by storing tubers under dry, cool conditions,

Figure 12.3: Soft rot of potatoes developed in storage due to storage of wet tubers, condensations and anaerobic conditions in the storage.

avoiding storing wet tubers, preventing condensation and development of anaerobic conditions. Wherever soft rot is expected, it is advisable to avoid the curing period as conditions which promote curing are also favorable for soft rot development (Booth and Shaw, 1981; Meijers, 1987a). Seed tubers are affected by skin spot and Rhizoctonia which kill the potato eyes (for specific details on diseases and pests, refer to Banks, 2004; Booth and Shaw, 1981; Rastovski et al., 1987). There are various measures that can be taken to control storage diseases starting from site selection for growing potatoes, site preparation, selecting varieties resistant to pests and diseases, careful harvesting, and improved handling methods to avoid mechanical damage, good sanitation and cleanliness of implements, containers and stores, proper drying and curing of tubers, good phytosanitary practices such as eliminating infested or infected tubers, and when necessary the use of pesticides in the field appropriate for the conditions/uses as per the manufacturer's recommendations. Avoiding favorable conditions for the growth of microorganisms in the storage such as temperature and excess humidity is also important (Booth and Shaw, 1981; Meijers, 1987a).

12.6.5 Temperature

One of the key factors controlling storage quality is temperature through its various effects on various biological processes such as respiration, evaporation, sprouting, low-temperature sweetening, freezing effect, curing, mechanical injury, and incidence of pests and diseases (Booth and Shaw, 1981; Brook et al., 1995; Burton, 1978; Gottschalk and Ezhekiel, 2006; Rastovski et al., 1987), which have been discussed elsewhere in this chapter.

12.6.6 Low-temperature sweetening

The most important aspect of quality for processors and consumers is color. Potato tubers stored at temperatures below 9–10°C result in high concentrations of reducing sugars such as glucose and fructose known as low-temperature sweetening (LTS; Burton, 1978; van Es and Hartmans, 1987c). These reducing sugars participate in the Maillard browning reaction with free amino acids during frying resulting in dark-brown-colored fries and chips. These darkened chips and fries are unacceptable to consumers and also may result in greater amounts of acrylamide production which has been linked to many cancers (Chuda et al., 2003; Hogervorst et al., 2007). Tubers containing 0.1% reducing sugars are ideal for processing and unacceptable when over 0.33% (Dale and Mackay, 1994). LTS is a major challenge faced by the potato industry. It has been reported that the level and availability of sucrose at harvest is the critical factor that affects the initial rate of formation of reducing sugars, and thus, the processing quality of potatoes following storage (Sowokinos, 1978). A study by Copp et al. (2000) observed tuber respiration rate as a more reliable method for predicting chip color than either sucrose or reducing sugar content during long-term storage. In contrast, Herman et al. (1996) observed high negative correlations between chip quality and low reducing sugars for tubers stored at 4°C over six months. A physiological-based mathematical model describing the storage behavior of potato tubers was examined to determine its ability to explain the changing storage behavior as a

function of harvest time for ten different cultivars and confirmed the concept that the maturity at time of harvest determines the storage behavior through the initial amount of the enzyme (or enzyme system) responsible for cold-induced sweetening (Hertog et al., 1997). In order to minimize the reducing sugar production, growers need to select varieties which are resistant to LTS and ensure that tubers reach full physiological maturity prior to harvest. One way to reduce the reducing sugar content is pre-conditioning in conjunction with curing at the beginning of the storage season and re-conditioning at the end of the season prior to delivery (Brook et al., 1995). Usually potatoes destined for French fries are held at 12.7–15.5°C for as long as 6 weeks for both curing and pre-conditioning and may subsequently be re-conditioned at 20–30°C and 85–90% relative humidity for 5–6 weeks at the end of the storage season. However, varietal difference has been noted in their response to re-conditioning. For example, certain varieties such as Russet Burbank will condition in some years but not others. The disadvantages of re-conditioning are the risk of senescent sweetening and the conducive conditions for the growth of microorganisms on/in potato tubers (Walsh, 1995). Varieties such as Premier Russet released in 2006 by the USDA-ARS and the Agricultural Experiment Stations of Idaho, Oregon, and Washington, is a product of the Northwest Potato Variety (Tri-State) Development Program and is notable for its resistance to the accumulation of reducing sugars in its tubers. This characteristic allows tubers of Premier Russet to be stored at temperatures as low as 5.6°C for 250 days without the need for re-conditioning prior to processing (Novy et al., 2008). Varieties such as Golden Wonder that were common throughout the UK crisp industry 30 years ago have been replaced by varieties with lower reducing sugars such as Lady Claire (Foot et al., 2007). Recently progress has been made in identifying the pathways and genes responsible for low-temperature sweetening. Such information has been used for the development of cultivars resistant to low-temperature sweetening (Pinhero et al., 2007; Sowokinos, 2001a, b).

Genetic engineering can be used successfully to improve the LTS-tolerance in potato tubers. It has been reported that silencing the potato tuber-expressed phosphorylase-L gene (*PhL*) resulted in lowered *PhL* gene expression and reduced glucose accumulation in cold-stored tubers (Rommens et al., 2006; Yan et al., 2006). Besides lowering the glucose accumulation as a result of lowering the expression of starch-associated *R1* or *PhL* genes, French fries derived from the modified tubers of Ranger Russet displayed an improved visual appearance and aroma while accumulating much lower levels of acrylamide (Rommens et al., 2006).

12.7 Storage Preparations and Conditions

12.7.1 Storage methods

The choice of storage method depends upon the production system such as frequency of harvest, demand, and end use. In countries where more than one crop is feasible, the storage period need not be long, e.g., 3–4 months. Another factor is to store the surplus in response to market demand at the time of harvest. Countries where only one crop is feasible, storage becomes

imperative to meet the demand for the entire year. Thus information on production pattern, marketing system, and tuber demand whether it is for fresh market, seed purpose, or processing or export will influence the duration of the storage period and the magnitude of the facilities required. The storage methods may be either field storage or storage buildings (Booth and Shaw, 1981; Schouten, 1987; Sparenberg, 1987). Field storage may involve delayed harvest or in ground storage or variable types of clamps or pits covered with straw and sometimes soil. In Holland, about 200 000 tons of potatoes for processing purposes are stored in pits for longer or shorter periods (Schouten, 1987). Storage buildings are either multipurpose or purposely built for potato stores for long-term storage of large quantities of potatoes which are ventilated (Shaw and Booth, 1981; Sparenberg, 1987). In Canada and USA, most potatoes are stored in 'bulk', where large buildings often hold several million kg of potatoes individually. In Europe, storage of potatoes in moveable pallet boxes, each holding 700–1000 kg of potatoes, are stored in storage buildings (personal communication, Dr. Coffin).

12.7.1.1 Non-refrigerated storage

For short-term storage involving 3–4 months, non-refrigerated on-farm storage methods such as clamps are cheap alternatives. They have been used by small growers in the Andes, by experienced farmers in Europe, and large growers in Argentina. In clamping, the potatoes are placed in a pile 1–3 m wide at the base and as high as the natural angle of repose of the tubers permits. The pile is made as long as necessary and is made of straw. Clamps can be used in areas where the temperatures are low enough, e.g., in the mountains or on high plateaux in the tropics or in winter in the subtropics. If the tubers are to be stored beyond the period of their natural dormancy, chemical sprout inhibitors will be required (Booth and Shaw, 1981; Sparenberg, 1987). On-farm storage in heaps (unventilated clamps) and pits is common in some parts of India and Holland. Potatoes stored in this manner often contain low levels of reducing sugars and hence can be used for processing (Gottschalk and Ezhekiel, 2006; Schouten, 1987; Sparenberg, 1987).

12.7.1.2 Refrigerated storage

The optimum temperature required for storage primarily depends on the end use. High-quality tubers can be stored from 2–12 months depending upon the quality of tubers at harvest, quality of storage facilities, good storage management, and variety and whether or not sprout inhibitors are used (for details on sprout suppression refer to Section 12.6.3). Respiration rate of tubers is lowest at 2–3°C which will reduce the weight loss. However, storage at 0–2°C increases the risk of freezing or chilling injury. Usually potatoes that are chilled look sound when removed from low temperature. However, symptoms of chilling become evident in a few days at warmer temperatures (Chourasia and Goswami, 2001). Sprouting increases at storage temperatures above 4–5°C. Hence at temperatures above 4°C tubers needs to be treated for sprout suppression. Tubers for fresh consumption are stored at 7–10°C to minimize formation of reducing sugars such as glucose and fructose from starch which results darkening during cooking. Many

chipping cultivars accumulate excessive amounts of reducing sugars if stored at below 9–10°C, a process known as low-temperature sweetening (LTS, for a detailed description see Section 12.6.6). In many countries, tubers destined for processing are stored at temperatures of 10–12°C where accumulation of sugars is less and the resulting chips and fries are light in color. The chipping varieties which are resistant to LTS can be stored at temperatures as low as 5°C without being treated with sprout inhibitors. Sugar formation at low temperatures differs from that resulting from senescence. Sugar formed during senescence cannot be decreased by reconditioning. Potatoes used for French fries are usually stored at 8–10°C depending on the cultivar characteristics (Booth and Shaw, 1981; Brecht, 2003; Burton, 1978; Gottschalk and Ezhekiel, 2006; Sparenberg, 1987).

12.7.1.3 Controlled atmosphere storage

Controlled atmosphere storage (CA) refers to the constant monitoring and adjustment of the CO_2 and O_2 levels within gas-tight stores or containers. CA is most effective when combined with temperature control. There has been great interest in using CA storage on potatoes for fresh, processing, and seed potatoes (Butchbaker et al., 1967; Khanbari and Thompson, 1994; van Es and Hartmans, 1987c). Fellows (1988) recommended a maximum of 10% CO_2 and a minimum of 10% O_2 as the optimum controlled atmosphere storage for potatoes. The amount of O_2 and CO_2 in the atmosphere of the potato store can affect the sprouting of tubers, rotting, physiological disorders, respiration rate, sugar content, and processing quality (Table 12.1). Storing tubers in anaerobic conditions of total nitrogen can prevent accumulation of sugars at low temperatures but may result in undesirable side effects such as acid or bitter flavor (Harkett, 1971). Varying concentrations of CO_2 and O_2 in controlled atmosphere affected sprouting (Schouten, 1992, 1994) with 3–6% CO_2-stimulated sprouting while 9% O_2 completely inhibited sprouting. From the results, it was concluded that controlled atmosphere storage at 6°C was not an alternative to chemical control of sprout growth. Khanbari and Thompson (1994) showed that high CO_2 with low O_2 combinations during storage completely inhibited sprout growth and resulted in the darkest colored crisp (i.e., chips) which was reversed by reconditioning. In a comparative study on the effects of normal and CA combinations at 9 ± 1°C for 6 months using the tubers of Agria and Russet Burbank on tuber components responsible for acrylamide, it was found that there was only a limited increase in the concentrations of sugars under normal atmosphere conditions. Low-dose irradiation (50, 200 Gy) slightly decreased the rate of sweetening in tubers. The potential to form acrylamide remained almost the same in both storage conditions. CA storage, in which O_2 was decreased to levels below that required for respiration, increased sugars and thus the potential for acrylamide formation upon frying at 170°C for 10 min (Gokmen et al., 2007).

12.7.1.4 Seed storage methods

The seed potato market is very unique in that quality must be high not only when delivered to the grower but when planted by the user. Seed quality is dependent on freedom from pests and

Table 12.1: Sugars (g per 100 g of dry weight) in tubers of three potato cultivars stored for 25 weeks under different controlled atmospheres at 5 and 10°C and reconditioned for 2 weeks at 20°C (reproduced with permission from Thompson A.K. 1998)

Cultivars	Gas combinations		5°C			10°C		
	CO_2 (%)	O_2 (%)	Sucrose	RS	TS	Sucrose	RS	TS
Record	9.4	3.6	0.757	0.216	0.973	0.910	0.490	1.400
	6.4	3.6	0.761	0.348	1.109	1.385	1.138	2.523
	3.6	3.6	0.622	0.534	1.156	1.600	0.749	2.349
	0.4	3.6	0.789	0.510	1.299	0.652	0.523	1.175
	0.5	21.0	0.323	0.730	1.053	0.998	0.634	1.632
			0.650	**0.488**	**1.138**	**1.109**	**0.707**	**1.816**
Saturna	9.4	3.6	0.897	0.324	1.221	0.685	0.233	0.918
	6.4	3.6	0.327	0.612	0.993	0.643	0.240	0.883
	3.6	3.6	0.440	0.382	0.822	0.725	0.358	1.083
	0.4	3.6	0.291	0.220	0.511	0.803	0.117	0.920
	0.5	21.0	0.216	0.615	0.831	0.789	0.405	1.194
			0.434	**0.473**	**0.907**	**0.729**	**0.271**	**1.000**
Hermes	9.4	3.6	0.371	0.480	0.851	0.256	0.219	0.475
	6.4	3.6	0.215	0.332	0.547	1.364	0.472	1.836
	3.6	3.6	0.494	0.735	1.229	0.882	0.267	1.149
	0.4	3.6	0.287	0.428	0.715	0.585	0.303	0.888
	0.5	21.0	0.695	0.932	1.627	0.617	0.510	1.127
			0.412	**0.682**	**1.094**	**0.741**	**0.354**	**1.095**

RS, reducing sugars; TS, total sugars. Numbers in bold represent mean values.

diseases, defects, minimal sprouting, tuber vigor and a physiological age appropriate for the local growing season and end use. Hence, seed storage requires special attention to provide conditions that will preserve the seed quality and physiological conditions at planting time. Seed storage methods and management must provide the desired conditions for the optimal development of both the number and size of sprouts. Apical dominance can result if the tubers are stored at a temperature that results in short dormant period. Apical dominance is characterized by young buds at the apex that start growing while growth of the older buds is suppressed. The disadvantage of apical dominance is that it has few main stems that result in the formation of a smaller number of tubers which may grow too large for proper market size. Controlling apical dominance of seed tubers by proper storage temperature can result in proper number of main stems of 3–5, which can yield the maximum amount of tubers of suitable market size. There are different methods to control apical dominance in seed tubers. One such method is storage at 2–5°C to beyond the end of natural dormancy of the variety and until a few weeks before planting followed by storing at 15°C in light (natural or artificial) to develop multiple green sprouting (Booth and Shaw, 1981; Burton, 1978). The influence of a short tuber dormancy and the subsequent sprout

growth of the seed tubers during storage at 4°C on plant development and tuber formation studied in a diploid population, its crossing parents and various tetraploid varieties showed that sprout growth during storage at 4°C was positively correlated to the duration of the dormancy period at 18–22°C. The duration of the dormancy period and the sprouting of seed tubers during storage at low temperature did not have a determinant influence on plant development, tuber formation, or the duration of the plant cycle in this large and highly diverse population of potato (Celis-Gamboa et al., 2005). In another study (Love, 1987), where eight potato cultivars were stored at four temperatures ranging from 1.7°C to 10°C, results showed that ignoring cultivar differences, the best storage temperature was found to be 4.4°C, which resulted in a higher yield of US No.1 tubers, highest stand percentage and most rapid emergence. Individual cultivars demonstrated distinctly different reactions to storage temperature. The tendency for producing physiologically aged seed was cultivar dependent and appeared to be partly due to length of dormancy (Love, 1987). Situations where controlling storage temperature is impossible, apical dominance may be controlled manually by removing the apical sprouts produced by hand, which results in the sprouting of many other eyes but excessive weight loss during storage. To stimulate more uniform sprouting from all eyes in potato tubers, some growers precut tubers 3–4 weeks before planting and eyes which tends to result in more uniform growth. The latter two methods of controlling apical dominance will result in increased weight loss of the stored tubers and may cause spread of tuber-borne diseases. Storing seed tubers in diffused light will also control apical dominance. The advantages of using diffused light include: low-temperature storage is not required, control of sprout growth, reduction in apical dominance, increase in sprout number, and increased resistance to several pests and diseases due to tuber greening (Booth and Shaw, 1981). Studies carried out on six cultivars for seed potato three weeks after harvest, under two storage conditions either cold storage at 8°C, or simple diffused light for seven months, showed that best results in terms of tuber weight loss, marketable yield, tubers per plant, and mean tuber weight was obtained with cold storage, even though varietal differences were noted. Little difference was noted between cold storage and diffused light storage at normal temperature up to four months (Severian and Devinck, 1986).

12.8 Storage Process

For successful storage and quality management, various aspects of storage processes such as preharvest storage preparations, storages and equipment, filling, curing, cooling down and monitoring the pile, maintaining the desired temperature and relative humidity, warming the pile stack for unloading, and unloading the store need to be carefully monitored (for a detailed description of storage designs and management, refer to Brook et al., 1995).

12.8.1 Pre-harvest storage preparations

Prior to harvest, structural checks such as framing for decay and rot, doors with good seals, insulation for intact and dryness, and walls for cleanliness should be performed (Brook

et al., 1995; Lewis, 2007; Meijers, 1987b). All air equipment systems should be checked and repaired. Weight loss variations, sprouting problems or disease problems can often be traced to an improperly designed or unbalanced air flow system. It is also important that the storages and equipment used for storing should be checked, cleaned, and repaired before storing. The fans, ducts, and dampers should be examined. Similarly humidification systems (humidifiers for operation and water flow), ventilation systems, and insulation systems, fans and refrigeration systems should be inspected and corrective measures should be taken as well as making ensuring that temperature-recording equipment is functioning well (Brook et al., 1995; Lewis, 2007; Meijers, 1987b).

12.8.2 Filling the storage

Potatoes are stored either in bulk or in bins depending upon the need and convenience. Neither different varieties nor production from different farms should be mixed in bulk stores. Bulk storage is appropriate when only one variety is to be stored unless temporary storage dividers are erected. The storage height of bulk seed potatoes must be limited to 3–3.5 m, due to greater weight loss which will result in bruises at greater heights (Booth and Shaw, 1981; Gottschalk and Ezhekiel, 2006; Meijers, 1987b). Many commercial storages in Eastern Canada stack up to 5 meters high (Dr. Coffin, personal communication). Potatoes can be stacked with bin pilers in bulk up to a height of 4 m given good management, but this can cause bruises or pressure spots as the result of pressure exerted by the upper layer of potatoes on the lower layer. Storing potatoes in bin boxes up to a height of 1–1.5 m will help to avoid bruises or pressure spots. The advantages of using bin boxes are: (1) it helps to store potatoes of different varieties, for different purposes and of different origin separately; (2) facilitates drying and cooling easy; (3) effective application of sprout suppressants; and (4) unloading is easy (Gottschalk and Ezhekiel, 2006; Meijers, 1987b). The drawbacks of a bin system are the relative expensive, and the difficulty in maintaining uniform temperature and relative humidity in the stacks. Many improvements have been made with correct placement of air exchange ducts and higher-capacity fans to assure ‘bin balancing’ of air flow. Special care should be taken to store potatoes separately that are infected with *Phytophthora*. Given good management and if the affected lots can be blown dry quickly, lots containing 5% tubers affected with *Phytophthora* may be stored relatively well. In the case of soft rot and frozen tubers, the permissible limit is less than 1% and should be rapidly blown dry to store well (Meijers, 1987b).

12.8.3 Equalization and drying phase

Potatoes harvested under wet soil conditions should be dried immediately. Drying involves removal of water present on the outside of the potato or in the soil on the potatoes in order to eliminate conducive conditions for the multiplication of microorganisms and to prevent the spread of rot and other storage diseases (Sijbring, 1987). The ventilation fan should run continuously during this phase while the average potato pile temperature is allowed to settle

within 2°C of the average pulp temperature upon entry into storage. Ventilation should be at the maximum possible rate for the shortest time needed. Excessive ventilation after removal of the surface moisture can dehydrate and soften the stored crop. Careful control of the relative humidity is also important. Hence frequent inspection of the potatoes during the drying period is important (Brook et al., 1995; Sijbring, 1987).

12.8.4 Wound healing/curing

Injury to tubers may occur during mechanical harvest, lifting, transportation, and even during grading. Weight losses and entry of microorganisms can occur through the injured skin causing diseases and rot during storage. Lignification, suberization, and periderm formation help the tuber to recover from the damage incurred during mechanical injury (Booth and Shaw, 1981; Brook et al., 1995; Meijers, 1987c). Curing, a prestorage treatment, is very important to limit weight losses and prevent the penetration of microorganisms. Under favorable conditions, tuber tissue forms the protective wound periderm over the damaged area. Many factors such as temperature, atmospheric humidity, oxygen and carbon dioxide concentration, cultivar, physiological age of tuber, and use of sprout inhibitors affect the curing process. However, the main determinants of the rate of curing are temperature and relative humidity. The usual recommendation of curing for potato tubers is exposure to a temperature of 12–16°C and a relative humidity of 90–95% for two weeks where the tuber tissue forms a protective layer (wound periderm) over the damaged area (Booth and Shaw, 1981; Burton et al., 1992; Meijers, 1987c). Potatoes should be ventilated enough during the curing period to avoid a rise in temperature and humidity and to keep the oxygen concentration at approximately 20%. A thin layer of suberized (corked) cells is first deposited over the damaged area, followed by deposition of cork cambium (phellogen) under the sealing layer giving rise from the inside to the outside to a dense network of new cells without intercellular spaces, which set rapidly. This wound periderm seems to be even more impermeable than the ordinary skin. Suberization is slow at lower temperatures and stops at 2°C. Conditions required for curing also favor several diseases, especially bacterial rot. To prevent additional respiration loss and conducive conditions for the spread of diseases, the temperature of the curing process should not go above 20°C and the temperature should be reduced to the necessary holding temperature as quickly as possible following curing (Burton, 1978; Gottschalk and Ezhekiel, 2006; Meijers, 1987c).

12.8.5 Preconditioning phase

Preconditioning is used commercially by chip-potato processors to compensate the unpredictable nature of reconditioning of process varieties and to achieve market flexibility (Brook et al., 1995). During this phase, the storage environment is maintained at conditions similar to the wound healing phase (12–16°C) with the pulp temperature actively controlled in order to eliminate pools of reducing sugars in process potatoes. The duration of this phase is dependent

on the process quality of the potatoes as measured by sugar content and chip color (Brook et al., 1995).

12.8.6 Cooling

Temperature is the principal factor that influences the quality and losses of potato during storage through respiration, sprouting, sweetening, and affecting the spread of diseases. As a general rule, the rate of cooling should be limited to 0.5–3°C per week. The rate of cooling varies depending upon the end use. For processing varieties, it should be 1°C per week, while for fresh market it should be as fast as possible by maintaining a 1–2°C pile differential from top to bottom. The main ventilation fan should run continuously during the cooling phase to maintain a uniform pile temperature. Cooling results in weight reduction due to moisture loss which can be limited by rapidly cooling the potatoes with very humid air (Booth and Shaw, 1981; Brook et al., 1995; Gottschalk and Ezhekiel, 2006; Lewis, 2007; Rastovski, 1987b).

12.8.7 Holding period

Choice of holding temperature is influenced by the duration of storage period, end use and the variety. Processing potatoes are generally stored between 6 and 10°C, whereas fresh market tubers may be stored between 4 and 10°C, while seed tubers are usually stored at 3 to 4°C. Once the required temperature has been attained, the ventilation should be reduced to a minimum in order to maintain a uniform pile temperature within one degree of the desired level, and to maintain the stack temperature differential from top to bottom of the pile as low as possible. Relative humidity should be maintained as high as 90% during the holding period to minimize the loss of moisture due to evaporation (Booth and Shaw, 1981; Brook et al., 1995).

12.8.8 Conditioning

Conditioning may be required if the tubers are intended for processing after storage at low temperatures and subjected to low-temperature sweetening. It will also reduce the mechanical damage during unloading as potatoes are more susceptible to mechanical damage at low temperature. Another reason for conditioning is to stimulate sprouting of seed potatoes which is done by slow warming thereby allowing the generated heat to remain in the storage to a temperature of up to 12–15°C for 2–3 weeks. During this period free sugars will be converted to starch thereby decreasing the free sugar content. However, reconditioning is rarely complete and uneven. Senescent sweetening cannot be reversed (Booth and Shaw, 1981; Brook et al., 1995; Burton, 1978; Gottschalk and Ezhekiel, 2006). In order to avoid condensation during the reconditioning phase, ventilation air with a lower than normal relative humidity is recommended. Other precautions to prevent condensation are: (1) stop ventilation when the dew point is reached; (2) seal the store to prevent the entry of warm air; (3) mix inlet air with store air to avoid large temperature differences; and (4) use of forced air ventilation or refrigeration if sprouts start to elongate (Booth and Shaw, 1981; Brook et al., 1995; Burton, 1978;

Gottschalk and Ezhekiel, 2006; Pringle, 1996). Before unloading the potatoes, it is important to heat the potatoes stored at low temperature up to 12–15°C, to minimize the likelihood of damage (de Haan, 1987b; Gottschalk and Ezhekiel, 2006).

12.9 Management of Storage Environment

Critical for good storage environment are the successful management of temperature, relative humidity, CO_2 level of the store, and air exchange system. Daily monitoring of these factors is crucial for maintaining good quality (Booth and Shaw, 1987; Burton, 1978; Kleinkopf, 1995). The temperature within the storage facility and of the outside ambient air can be measured with a simple minimum and maximum thermometer situated away from external influences, particularly direct sunlight. Air temperatures surrounding the potatoes can be measured by direct reading instruments or remote station indicators. The hottest part of the potato stack is 400–500 mm below the top surface which should be monitored. Temperature from several levels is useful to determine stack temperature gradients and to localize the hot spots. A marked rise in temperature can be indicative of bacterial soft rot, which if identified early suitable measures can be taken to prevent the spread of rotting by increasing ventilation or removal of the tubers from the storage (Booth and Shaw, 1987; Figure 12.3). Relative humidity should be monitored by using a wet and dry bulb thermometer in a sling or battery-operated psychrometer. Portable CO_2 meters are useful tools for growers when monitoring potato storages. Although great advances have been made in automated monitoring of storages, improved success is guaranteed if the grower visits potato storages daily to verify temperatures, detect moisture on tubers, fruit flies and/or 'early warning' signals such as the smell of ammonia (Dr Coffin, personal communication).

12.10 Effect of post-harvest storage on processing and nutritional quality of potatoes

Maintenance of post-harvest quality is critical for both growers and processors. French fries and potato chips constitute the two major processed potato products in the food industry. Production of frozen French fries is the largest sector of the processing industry. Other processed products are dehydrated flakes or granules and canned potatoes in various forms. Numerous factors affect potato quality and many of them relate to the chemical composition of the tuber which is influenced by the environment during growth and storage (Mazza, 1983a; Salunkhe et al., 1989). The extent of biochemical changes occurring in tubers during storage, prior to processing provides a major influence responsible for finished product discoloration such as low-temperature sweetening (see Section 12.6.6). Post-harvest quality losses in stored potatoes can also occur through both physiological and disease-related processes. Two of the most important physiological processes affecting potato storage and market quality are dormancy/sprouting and wound-healing/skin set. The external qualities, such as extent of surface blemishes due to

diseases and pests, sprouting, and superficial damage and internal qualities, such as nutritional quality, color of the cooked product (after-cooking blackening, enzymatic and non-enzymatic browning), eating quality (texture, feel, crispness) determine the final tuber quality and consumer acceptability. The effects of diseases and pests and sprouting have been discussed elsewhere in this chapter (see Sections 12.5 and 12.6.3).

Potatoes are rich in carbohydrate and provide significant quantities of proteins, minerals (iron) and vitamins (B complex and vitamin C), dietary fiber, and antioxidants which vary with variety, storage conditions, growing season, soil type, and pre-harvest nutrition. Carbohydrates constitute about 75% of the total solids and consist mainly of starch. There are three major types of sugars in potatoes, the disaccharide sucrose, and the two monosaccharides, the D-glucose and D-fructose (Tarn et al., 2006). The sugar content of potatoes varies from 0.2 to 6% and it is a genotypic characteristic which varies with cultivars (Dale and Mackay, 1994; Tai and Coleman, 1999). The quality of potatoes is dynamic and continues to change as a result of physiological activity owing to accumulation of reducing sugars and depletion of starch (Nourian et al., 2003). Therefore, sugar and starch are the main components affected by post-harvest metabolism in potato tubers, which ultimately affects potatoes' cooking, sensory, and processing characteristics. Recently the concept of resistant starch has sparked new interest in the bioavailability of starch and its use as a source of dietary fiber, particularly in adults (Sajilata et al., 2006). The beneficial physiological effects of resistant starch include prevention of colon cancer, hypoglycemic effects providing improved metabolic control in type II diabetes, as a prebiotic, reduction of gall stone formation, hypocholesterolemic effects, inhibition of fat accumulation, and absorption of minerals. Studies on the effect of genotype, weight, and storage at 6°C, on in vitro availability of starch in heat-treated potatoes showed that the hydrolysis indices (HI) and predicted glycemic indices of all 19 potato products were high and fell within narrow ranges. No correlation between average weight of the potato tuber and HI was found. Furthermore, there was no difference in HI between potatoes stored for 1–3 or 8–10 months, nor between varieties of new potato and winter potato (Leeman et al., 2005). Another study reported to evaluate the effect of post-harvest storage at 4°C for 6 months after harvest on starch physico-chemical and functional characteristics of potato varieties showed significant difference from cultivar to cultivar (Singh et al., 2008). Decrease in starch swelling power, solubility, and light transmittance was observed during tuber storage, while a slight increase was noted in starch amylose content. The starch granule size distribution shifted to smaller granule size during tuber storage and showed degradation/erosion and pitting on the surfaces of many of the starch granules isolated from stored tubers. Transition temperatures and enthalpies of gelatinization of the starches increased somewhat during tuber storage, suggesting that changes in the stability of starch crystalline structures had occurred. Specific gravity of the tubers, which is a measure of the dry matter content of potato, substantially influences the processing quality of tubers. Starch being the largest component of potato, the dry matter content of potato is very often equated with its starch content (Salunkhe et al., 1989). Differences in size of

starch granules are associated with differences in amylose and amylopectin content (Salunkhe et al., 1989). Small granules contain less amylose and gel at higher temperatures than the larger starch granules. Storage temperatures markedly influence the size distribution of starch granules with storage temperatures above 21.1°C, the average length of starch granules increases as the numbers of smaller starch granules reduced concomitantly. This shift in distribution has been attributed to the rapid digestion of smaller granules and compositional differences between large and small granules (Salunkhe et al., 1989). A direct relationship was noted between specific gravity and starch size in a number of potato varieties (Barrios et al., 1961, 1963; Unrau et al., 1957a, 1957b). Greater percentage of large starch granules was found to be associated with high specific gravity and mealiness of potato tubers. It was also observed that higher amylose content is significantly correlated with mealiness (Unrau et al., 1957a, 1957b). Differences in properties of starch between tubers of LTS-resistant and -susceptible cultivars may influence the granular susceptibility to enzyme hydrolysis and partially determine the extent to which starch is converted to sugar. It was reported that starch isolated from LTS-tolerant ND 860-2 had higher amylose and lower amylopectin as well as higher crystallinity and greater resistance to α-amylase attack as compared to the starch from LTS-susceptible Norchip (Barichello et al., 1990). Hence it is assumed that starch structure could change as a result of low-temperature sweetening and will be highly dependent upon the LTS-tolerance of the cultivar.

Pasting, viscoelastic and texture profile analysis characteristics of starch gels were found to have been influenced by tuber storage time for all the cultivars, but to the greatest extent for Nadine and Huakaroro. Gels made from starches from the stored tubers had a reduced tendency towards retrogradation as evidenced by the decrease in syneresis during gel storage. Varieties with increased tuber solids and decreased glucose content are suitable for processing (Salunkhe et al., 1989) with 0.1% is ideal for processing and unacceptable when over 0.33% (Dale and Mackay, 1994). Maintaining the reducing sugars around 0.1% on fresh weight basis throughout the storage period results in light golden color chips (Storey and Davis, 1992). Low-temperature sweetening which results from storage of tubers at temperature below 9–10°C with a concomitant increase in reducing sugars such as glucose and fructose has been described elsewhere in this chapter (see Section 12.6.6). Ascorbic acid (vitamin C) is the major vitamin present in potato. Losses in storage were highly correlated with initial levels. Barker and Mapson (1950) reported that both temperature and length of period of storage significantly influenced the vitamin C content and the rate of loss in storage and the final level of ascorbic acid depended on the crop maturity and to some extent on the variety. It has been reported that a 24-chromosome Phureja-haploid Tuberosum hybrid retained twofold higher ascorbic acid content after storage at 5°C (Davis et al., 2002). Ascorbic acid also appears to play a role in unfavorable browning reactions (Mazza, 1983a). Nutritional quality changes studied in two potato chippers Kennebec and Norchip and a French fry cultivar Russet Burbank during growth and long-term storage at low temperature showed that ascorbic acid content increased with growth and maturity but steadily decreased by storage (Mazza, 1983a). True and crude proteins increased only slightly

with maturity and storage. Highest dry matter and lowest crude protein were observed in Russet Burbank. Correlation analysis showed that dry matter, reducing sugars, and sucrose had significant effect in determining the chip color of the freshly harvested potatoes while reducing sugars, tuber temperature, and sucrose influenced the chip color of stored tubers (Mazza, 1983a, 1983b). The relative importance of dry matter, sucrose, reducing sugars, ascorbic acid, protein, and storage temperature varied with age of the tubers, cultivar, and growth period (Mazza, 1983a, 1983b). It was reported that storage of tubers at 3°C caused dramatic increases in total fatty acid unsaturation, membrane permeability, and sugar content compared to tubers stored at 9°C over a period of 40 weeks. Cultivars with higher levels of fatty acid unsaturation had lower rates of membrane electrolyte leakage and lower sugar contents which shows that high initial levels or high induced levels of membrane lipid unsaturation mitigate increases in tuber membrane permeability during storage, thus positively influencing the processing quality of stored potato tubers (Spychalla and Desborough, 1990).

Potato tubers contain several antioxidants such as anthocyanins, carotenoids, and organic acids which can be significant sources of antioxidants in human nutrition. Carotenoids such as violaxanthin and lutein are primarily present in yellow flesh pigmentation in tetraploid and diploid cultivated potatoes (Lu et al., 2001; Nesterenko and Sink, 2003). Total carotenoid levels are at a maximum early in tuber development and decrease as dry weight increases. During storage, total carotenoid levels decrease only slightly while there is a shift in the relative amounts of individual carotenoids (Morris et al., 2004). A study on the effect of seasons and post-harvest storage at 4°C and 10°C on *Solanum phureja* potato tubers over three years showed that post-harvest storage significantly reduced the carotenoid content of the tubers and that reducing storage temperature further lowered the carotenoid content. Lutein was the most stable form while beta-carotene levels reduced significantly at both storage temperatures (Griffiths et al., 2007).

The undesirable quality aspects resulting from the use of stored tubers for processing are glyckalkaloid and acyrlamide production. Greening of tubers occurs when they are exposed to light intensities as low as 3–11 $W\,m^{-2}$ for a short period of as low as 24 h and is influenced by variety, stage of maturity, and temperature (Salunkhe et al., 1989). At a temperature of 5°C, no greening was noticed, whereas it was extensive at 20°C (Salunkhe et al., 1989). Greening affects the nutritional quality of tubers. Greening in potatoes occurs by the synthesis of chlorophyll in the peridermal layers of tubers exposed to light and is very often associated with the formation of glycoalkaloid (Salunkhe et al., 1989). Glycoalkaloids are mainly glycosides of the aglycone, solanidine. Solanidine can cause off-flavors on cooking at concentrations of 15–20 mg per 100g. Since glycoalkaloids impart a bitter taste and can be toxic above threshold levels, in many countries an official guideline of less than 200 mg kg^{-1}fresh weight level has been recommended (Friedman and McDonald, 1997). A study carried out on the relationship of solanidine and chlorophyll synthesis as affected by light and specific chemicals showed that

the chlorophyll synthesis increased up to the light intensity of 100-foot candles, slowed and degraded gradually up to 150-foot candles and rapidly at 200-foot candles during 5 days of light exposure (Patel et al., 1971). No significant differences were noted in the high glycoalkaloid contents after exposure to four light intensities of 50-, 100-, 150-, and 200-foot candles and this was attributed to the prolonged storage of tubers at low temperature. Studies carried out on six cultivars selected on the basis of their glycoalkaloid formation on exposure to light and stored under various storage temperature showed that glycoalkaloid accumulation was independent of the level found at harvest and significant interactions were found between cultivar and temperature. Cultivars which did not accumulate glycoalkaloids rapidly in response to light exposure were the most stable and least sensitive to storage temperature. Tubers transferred to colder conditions 9 weeks after storage at 10°C did not accumulate glycoalkaloids at a similar rate to those placed in similar conditions soon after harvest (Griffiths et al., 1997). It has been reported that the increase in glycoalkaloid content during storage is lower at 10°C than at 4°C (Cieslik and Praznik, 1998). Nitithamyong et al. (1999) found that temperature had a larger influence on glycoalkaloid levels than light intensity, day length, carbon dioxide concentration, or humidity. There are various ways to control greening of tubers and the glycoalkaloid content in storage, such as chemical control methods, controlled and modified atmospheric conditions, and ionizing irradiation which has been discussed under sprout control or storage methods in this chapter. Genetic improvements to reduce the glycoalkaloid contents in tubers has been reported by breeding *S. tuberosum* and *S. chacoense* (Sanford et al., 1995) and using antisense technology with the gene for solanidine UDP-glucose glucosyltransferase in Lenape and Desiree lines (Moehs et al., 1997).

Acrylamide is formed in processed potato products such as chips (crisps) and French fries (Tareke et al., 2002) by Maillard browning reactions of reducing sugars with the amino acid, asparagine, at temperatures above 120°C. Acrylamide is known to cause cancer in animals and has been classified by the WHO's International Agency on Cancer as probably carcinogenic to humans. Since the discovery of acrylamide in potato processed products, a large number of studies have been conducted on potato products and acrylamide formation (Chuda et al., 2003; Foot et al., 2007; Mestdagh et al., 2008; Meulenaer et al., 2008). A study conducted to determine the effect of potato varieties (French fry varieties Bintje and Ramos and crisp varieties Lady Rosetta and Saturna) and season on acrylamide production over a period of 9 months storage showed a significant impact of variable climatological conditions on reducing sugar, dry matter, total free amino acid, and free asparagine contents of tubers. Lower reducing sugar was observed on exceptionally warm summers which resulted in lower acrylamide production (Meulenaer et al., 2008). It should be noted that in a separate study, it was found that the content of reducing sugars determined the level of acrylamide formed in potato crisp (Wicklund et al., 2006). Chuda et al. (2003) reported that potato chips made from tubers stored at 2°C contained ten times more acrylamide than the chips from 20°C and is highly correlated with glucose and fructose levels in the tubers. All these studies show a strong correlation between

acrylamide formation and reducing sugar concentrations. Thus substantial improvements can be made in reducing acrylamide formation in chips and French fries by developing varieties which are resistant to LTS either by breeding or genetic engineering. For a more detailed study on acrylamide reduction see the review by Foot et al. (2007).

For additional reading on potato post-harvest storage, the reader is referred to Booth and Shaw (1981), Rastovski et al. (1987), Banks (2004), and Gottschalk and Ezhekiel (2006).

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CHAPTER 13

Nutritional Value of Potatoes: Digestibility, Glycemic Index, and Glycemic Impact

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13.1 Introduction

Potatoes have been valued around the world for centuries because they are an easily cultivated, easily prepared and readily assimilated source of carbohydrate energy. The ease with which potatoes are digested has, however, become a double-edged sword, as the interrelated epidemics of metabolic syndrome and type 2 diabetes become global crises linked to rising obesity. At the same time as carbohydrates have been blamed for obesity, glucose intolerance has become recognized as a core feature of the metabolic syndrome and type 2 diabetes (Seidell, 2000).

Glucose intolerance is a condition in which the capacity of cells to remove glucose from the blood is reduced, so that after a carbohydrate meal the blood becomes overloaded with glucose. This so-called hyperglycemic state of elevated blood glucose concentrations and high insulin demand is largely unfelt and its effects are insidious, leading gradually to widespread tissue damage, and eventually contributing to conditions typical of long-term diabetes – circulatory, kidney, and eye diseases to mention a few (Brownlee, 2001; Riccardi et al., 2008).

Linking such gloomy prospects with carbohydrate foods of high glycemic index (GI) has in recent years led the potato to be maligned. As the misconception grew in the public mind and in dietetics that GI was a measure of a food's effect on blood glucose, and as several studies concluded that potatoes generally have a high GI, a popular nutritional recommendation appeared that potatoes be replaced in the diet by more benign, low-GI foods, such as pastas and whole grains (Brand Miller et al., 1996).

Potatoes should not, however, be disadvantaged because of glycemic indices measured in initial studies. More critical analysis is required of the meaning of glycemic index, of the roles that potatoes may play in the diet, and of the validity of generalizing from a limited number of studies to potatoes and potato products as a whole. There is a need to appreciate that many layers of variables determine the relationship between potato consumption and glycemic response in any

particular instance, and that the digestibility of potatoes creates as many opportunities as it does difficulties for the potato industry and for human nutrition.

In this chapter we review the relationship between potato consumption and blood glucose responses, and explore causes and variations in the glycemic impact of potatoes, and the opportunities that they provide for the potato industry.

13.2 Potato as a Nutrient Source

The potato tuber is a subterranean swollen stem evolved to be an overwintering energy storage organ. In it, the form of energy storage is almost entirely starch, which is almost totally digestible by humans when potatoes are consumed freshly cooked, so is classed as 'available carbohydrate.' However, as the potato tuber must resist decay and support vigorous sprouting in spring, it also contains a range of other bioactive components, many of them of nutritional value to humans, as discussed elsewhere in this book.

To place the available carbohydrate of potato, which is the main focus of this chapter, into a nutritional context, the nutrient composition of a typical commercial potato variety is shown in Table 13.1. An important aspect of the nutrient composition of potato tubers is that they are predominantly water (about 80%), and on a dry weight basis they consist mostly of available carbohydrate in the form of starch, with virtually no lipid.

The influence of the water content of potatoes extends to all nutrients and to energy density. Thus, on an equal weight (100 g) basis, potato has an intrinsically low energy density compared with many other carbohydrate staple foods (Table 13.2). In a world in which obesity and energy excess is a growing problem, potatoes consumed in forms unadulterated by fat can, therefore, be valued for their potential role in obesity management, and indirectly in blood glucose management, since obesity predisposes to glucose intolerance.

For many years, and still, controlling available carbohydrate intake has been a cornerstone of diabetes management. However, in many foods 'available carbohydrate,' measured as carbohydrate available in food analysis, is not quite the same as carbohydrate that is available in the gut in food as normally consumed. Glycemic response depends not only on the amount of potentially available carbohydrate consumed, but also on how rapidly it is digested, absorbed, and disposed of in the body, and that depends on a myriad of factors including food structure and the influence of other food components that vary in importance from food to food.

The glycemic index was, therefore, introduced as an *adjunct* to available carbohydrate values in diabetes management, to indicate the glycemic potency of available carbohydrate in foods relative to glucose (Jenkins et al., 1981). In recent years GI has developed its own momentum, helped by vigorous promotion, and despite continued discussion about the value it adds to

Table 13.1: Nutrient composition of potato (*Solanum tuberosum*), white, flesh and skin, raw per 100 g[1] (USDA National Nutrient Database, No: 11365)

Nutrient	Units	Value per 100 g	Nutrient	Units	Value per 100 g
Proximates			**Vitamins**		
Water	g	81.6	Vitamin C	mg	19.7
Energy	kj	288	Thiamin	mg	0.071
Protein	g	1.68	Riboflavin	mg	0.034
Total lipid (fat)	g	0.1	Niacin	mg	1.066
Ash	g	0.94	Pantothenic acid	mg	0.281
Carbohydrate, by diff.	g	15.7	Vitamin B-6	mg	0.203
Fiber, total dietary	g	2.4	Folate, total	mcg	18
Sugars, total	g	1.15	Folic acid	mcg	0
Sucrose	g	0.28	Folate, food	mcg	18
Glucose (dextrose)	g	0.53	Folate, DFE	mcg_DFE	18
Fructose	g	0.34	Choline, total	mg	11
Lactose	g	0	Betaine	mg	0.2
Maltose	g	0	Vitamin B-12	mcg	0
Galactose	g	0	Vitamin A, IU	IU	8
Starch	g	13.5	Vitamin A, RAE	mcg_RAE	0
Available carbohydrate[2]	g	14.65			
Minerals					
Calcium, Ca	mg	9			
Iron, Fe	mg	0.52			
Magnesium, Mg	mg	21			
Phosphorus, P	mg	62			
Potassium, K	mg	407			
Sodium, Na	mg	6			
Zinc, Zn	mg	0.29			
Copper, Cu	mg	0.116			
Manganese, Mn	mg	0.145			
Selenium, Se	mcg	0.3			

[1] *One potato: small, 170 g; medium, 213 g; large 360 g. One cup, diced, 150 g.*

[2] *Starch + sugars.*

simple available carbohydrate values in terms of glycemic response and health (Livesey et al., 2008).

13.3 The Measurement and Meaning of Glycemic Index and Glycemic Impact

13.3.1 Clinical measures of glycemic potency

Different ways of expressing the capacity of foods to affect blood glucose concentrations – glycemic index (GI) and relative glycemic impact (RGI) (or glycemic load) – may give very

Table 13.2: Glycemic properties and other components of potatoes and other carbohydrate foods[1]

Food	(CSM)	Wgt (g)	RGI (gge)	RGP (gge)	GI	Energy (kJ)	Avail. CHO (g)	Tot.fat (g)	Water (g)
Potato, Rua,		100		**10**	**56**	341	18	0.2	77
flesh, boiled	1 cup	164	**17**[2]		**56**	559	30	0.3	126
Potato, mashed		100		**11**	**74**	394	15	3.3	79
+butter, salt	1 cup	209	**22**		**74**	823	30	6.9	166
Potato, Rua,		100		**17**	**79**	394	21	0.1	72
microwaved	1 potato	90	**15**		**79**	355	19	0.1	65
Potatoes fried		100		**21**	**75**	723	28	5.3	58
In oil	1 cup	60	**13**		**75**	434	17	3.2	35
Potato crisps		100		**26**	**54**	2100	48	32.1	3
	1 sm pack	50	**13**		**54**	1050	24	16.1	1
Banana, cooking,		100		**18**	**70**	453	26	0.2	69
boiled	1 whole	140	**26**		**70**	634	37	0.3	96
Spaghetti,		100		**10**	**42**	499	24	0.5	64
boiled	1 cup	148	**15**		**42**	739	35	0.7	94
Rice, white,		100		**10**	**56**	338	18	0.3	76
polished, boiled	1 cup	144	**14**		**56**	487	25	0.4	109
Beans, Haricot,		100		**6**	**38**	380	15	0.5	70
boiled, drained	1 cup	180	**10**		**38**	684	27	0.9	125
Bread,		100		**37**	**70**	1090	53	1.6	34
white	1 roll	77.2	**29**		**70**	841	41	1.2	26
Bread, pita,		100		**38**	**86**	864	45	0.7	44
white	1 lg. pocket	84	**32**		**86**	726	37	0.6	37
Muffin,		100		**24**	**60**	1070	41	8.1	33
bran	1 medium	105	**26**		**60**	1124	43	8.5	34
Corn flakes,		100		**69**	**81**	1540	85	0.2	3
Kellogg's	1 cup	30	**21**		**81**	462	25	0.1	1
Muesli, natural,		100		**28**	**49**	1260	57	4.4	11
Sanitarium	1 cup	107	**30**		**49**	1348	60	4.7	12

[1] *From Tables of Glycemic Glucose Equivalents in New Zealand Foods (Monro et al., 2004). CSM = common standard measure. 1 GGE = fractional effect equivalent to that per 1.0 g of dietary glucose at an intake of 50 g glucose.*

[2] *Example: Effect would be equivalent to that of 17 g glucose. RGP = relative glycemic potency = calculated GGE per 100 g food. GI = glycemic index = calculated GGE per 100 g available carbohydrate in food.*

different impressions of the glycemic potency of potatoes. Both GI and RGI are determined from clinical measurements of the effect of consuming foods, and are expressed relative to the effect of a glucose reference, but with GI the effect is attributed to the available carbohydrate component of the food, while with RGI the effect is attributed to the whole food. So in a food such as potato, which contains about 80% water, GI is very much higher than GL (Figure 13.1).

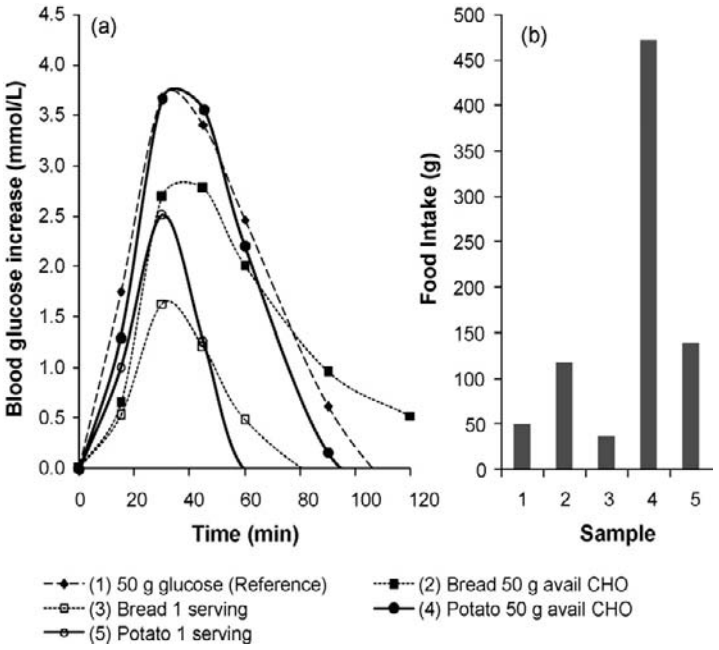

Figure 13.1: (a) Blood glucose responses to 50 g available carbohydrate portions of potatoes, bread, and glucose (reference) and to a serving of bread and potato (Monro et al., 2008). (b) Food intakes that gave the responses shown in (a).

However, as people eat foods, not simply the carbohydrate in them, RGI is a more helpful way of communicating the glycemic impact of foods.

GI was originally invented to compare foods already grouped into *equal available carbohydrate* categories, to facilitate food exchanges in diabetes management, because in many foods 'available' carbohydrate did not accurately predict glycemic responses (Jenkins et al., 1981). But generally, GI is not very useful in comparing the effect of a serving of one food with a serving of another, because in most common foods servings do not contain the same amount of available carbohydrates (Monro, 2002), so it is not simple for consumers to use.

When writers refer to the 'glycemic index of potato' they should really say the glycemic index of *available carbohydrate* in potato (Monro, 2003), which is very different from the relative glycemic impact of potato per se. And because the glycemic effect of a food depends on all of the amount of food consumed, the amount of carbohydrate in the food and on the GI of the carbohydrate in it, a high GI is not necessarily synonymous with a large glycemic effect either on an equal weight, or standard amount of food consumed basis, and certainly not for potatoes (Figure 13.2).

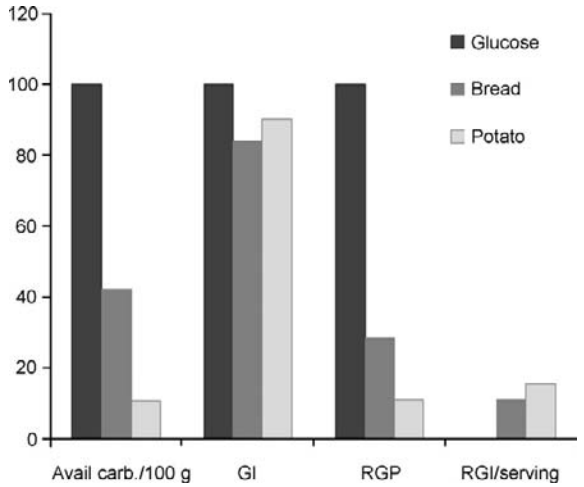

Figure 13.2: Different ways of expressing glycemic potency give different impressions of the glycemic impacts of foods. GI (GGE/100 g available carbohydrate) which is based on available carbohydrate alone is high, but the relative glycemic potency (GGE/100 g food) and the relative glycemic impact (RGI) per serving (GGE/serving) are quite low for potato. Serving sizes: bread 37 g, potato 140 g. Glucose reference GI is set at 100, and as glucose is 100% available carbohydrate, its relative glycemic potency (RGP = GGE /100 g) is 100.

Relative glycemic impact (RGI) and its close relation, glycemic load (GL), in contrast to GI, refers to the glycemic effect of an entire food (not only its carbohydrate) relative to the effect of glucose, and has been defined as '. . . the weight of glucose that would induce a glycemic response equivalent to that induced by a given amount of food' (Miller Jones, 2007). It may therefore be expressed as grams of glucose equivalents (Livesey, 2005) or because of the context, glycemic glucose equivalents (Monro, 2002; Monro and Shaw, 2008), in both cases abbreviated as GGE.

Relative glycemic potency (RGP) is a calculated RGI of 100 g of food, that is, GGE/100 g food (Monro, 1999). It allows foods to be compared on an equal (100 g) weight basis. If a true glycemic index of a food (rather than of a food carbohydrate) was wanted, RGP would be it. For potato it is much lower than the available carbohydrate-based GI (Figure 13.2). Together, RGI and RGP allow the glycemic impact of potato to be expressed per serving, and per 100 g, as if it were a food component with weight units (Table 13.2). Saying that a serving of potato has an RGI (or GL) of 20 GGE is simply saying that it would have about the same effect as consuming 20 g of glucose. It is a very simple way of comparing foods directly in terms of their glycemic potential (Monro, 2004).

The terminology surrounding glycemic responses, with respect to potatoes, is summarized in Table 13.3, and the different perspectives of the glycemic properties of potatoes given by different terms are summarized in Figure 13.2.

Table 13.3: Clinical measures of the glycemic potency of potatoes

Term: **Glycemic index** (GI) (%)
Definition: The increase in area under the blood glucose response curve (Figure 13.1a), after consuming a food portion containing 50 g available carbohydrate, as a percentage of the response to a 50 g dose of glucose. Thus, if enough potato is consumed to provide 50 g of available carbohydrate, and it induces a blood glucose response 80% that of a 50 g reference dose of glucose, it has a GI of 80.
Relevance to potatoes: GI gives an inflated idea of the glycemic impact of entire potatoes (Table 13.2) because it refers to the available carbohydrate alone, yet potatoes contain only about 20% available carbohydrate. Because it is an index, GI does not directly indicate how glycemic impact is affected by the quantity of food consumed, unlike RGI.
Term: **Relative glycemic impact** (RGI) (g)
Definition: The weight of glucose that would induce the same glycemic response as a given amount of food (Monro, 2002; Brookes et al., 2003).
Relevance to potatoes: Unlike GI, RGI refers to the relative glycemic effect of the entire food, and depends on food quantity consumed (Table 13.2), so it allows a direct comparison of the relative glycemic impact of any amount of potato, such as a serving or 100 g, with a serving or any other amount of another food (Table 13.2). For potato RGI is much lower than GI because fresh potatoes contain only about 20% available carbohydrate.
Term: **Glycemic glucose equivalent** (GGE) (g)
Definition: A single unit of RGI. One GGE unit is equivalent in glycemic effect to one gram of glucose. For example, one microwaved potato (Table 13.2) with a relative glycemic impact (RGI) of 15 GGE would induce the same glycemic response as 15 g glucose.
Relevance to potatoes: Because it has weight units GGE can be used as if it were a food component, but representing glycemic impact. It allows the relative effect of a food to be shown concurrently with nutrient values for the food in food composition tables (e.g. Table 13.2), so has been termed a Virtual Food Component (Monro, 2004).
Term: **Relative glycemic potency** (RGP) (g)
Definition: The calculated glycemic glucose equivalent (GGE value) of 100 g of food, that is, the weight of glucose that would induce the same glycemic response as 100 g of food.
Relevance to potatoes: RGP is a way of comparing foods on an equal (100 g) food weight basis to allow the relative glycemic potency of different foods to be seen immediately (Table 13.2). As it is a measure of the relative glycemic potency of the whole potato it is much less than GI and shows that potato is not highly glycemic on an equal weight basis.
Term: **Glycemic load** (GL)
Definition: The product of GI and the available carbohydrate content of a food.
Relevance to potatoes: GL is similar to but not quite the same as RGI. The differences are discussed elsewhere (Monro and Shaw, 2008). For foods such as potatoes, in which most carbohydrate is in the form of moderate to highly digestible starch, the values of GL and RGI, when applied to a single food intake, are similar enough to be interchangeable (Venn et al., 2006).

Clinical measures of glycemic response are obtained by measuring the increase in the area under the blood glucose response curve for 2–3 h after ingesting food, compared with the increase caused by consuming a reference, usually glucose (Figure 13.1a). They are, therefore, all relative measures that tell how glycemic one food or food carbohydrate is compared with another, but do not say what the glycemic effect is in any individual.

13.3.2 In vitro measures of glycemic potency

In vitro laboratory-scale digestion of carbohydrate foods using simulated gastrointestinal conditions has been used by a number of authors to measure starch digestibility as a predictor of glycemic response in a range of carbohydrate foods, and specifically in potatoes (García-Alonso and Goñi, 2000; Kingston and Englyst, 1994; Mishra et al., 2008). The big advantage of in vitro analysis is that it is very precise, because it avoids the enormous intersubject variabilty of clinical trials, and it has been shown to significantly predict relative glycemic responses in humans (e.g. Brighenti et al., 1995; Englyst et al., 1999).

Methods and measurements used in in vitro glycemic analysis have not been standardized. However, the division of starch fractions into rapidly digested (RDS), slowly digested (SDS) and resistant (RS) starch has proved useful and is consistent with physiological processes underlying the glycemic response. The glycemic response occurs when the rate of glucose loading into the blood exceeds the rate at which it is able to be disposed of by the body, so there is a temporary net excess of glucose and blood glucose concentrations rise. RDS provides a measure of the starch that is hydrolysed quickly enough to overwhelm glucose disposal until homeostasis returns blood glucose to baseline. The term 'rapidly' does not specify a time, but 20 min from the onset of digestion is often used, and is compatible with the blood glucose response curves in Figure 13.1, which show that at 20 minutes the rate of increase in blood glucose concentrations has reached a maximum and the blood glucose levels start to decrease between 30 and 40 minutes irrespective of carbohydrate dose. Glucose released by 20 minutes digestion in vitro has been shown to be a valid predictor of glycemic impact (Englyst et al., 1999; Monro et al., 2007).

In vitro digestive analysis has proved useful not only for measuring the relative glycemic potency of foods, but by measuring the formation of RS and SDS it also provides an explanation for changes in glycemic impact as a result of retrogradation of amylase and partial retrogradation of amylopectin respectively. Because it is much more precise and cheaper than clinical analysis, in vitro digestive analysis is likely to play an increasing role in developing foods for glycemic control.

13.4 Differences between Potatoes in Glycemic Impact

In the best-selling 'The GI Factor' (Brand Miller et al., 1996) there is a bolded statement that reads 'Bread and potatoes have high GI factors (70 to 80),' which is qualified less visibly with 'Of course, some types of bread and potatoes have a lower GI factor than others . . ..' Such statements soon gave potatoes a reputation as being high GI, yet, the acknowledgment that some have a lower GI than others begs the questions, 'Why?' and 'Can the differences be exploited?'

Continued research that has broadened the context of potatoes and the blood glucose response suggests that not only do potatoes show intrinsic differences in GI, but the ways that they are

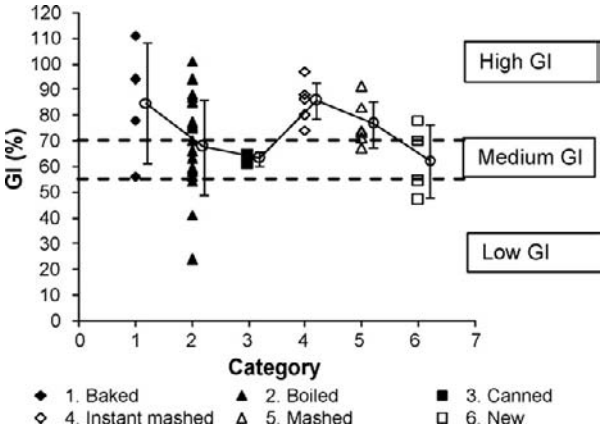

Figure 13.3: The range of GI values in potatoes prepared in a number of ways, with means and standard deviations shown for each category. Values are from the International Table of Glycemic Index and Glycemic Load (Foster-Powell et al., 2002), and from Henry et al. (2005).

prepared and consumed can also significantly influence their glycemic impact. However, even when published mean GI values for potatoes are grouped according to preparation method there are large within-group differences in GI (Figure 13.3). Given the susceptibility of potato starch digestibility to processing (Mishra et al., 2008), the poor inter-laboratory reliability in GI determinations (Wolever et al., 2003), and the many variables affecting clinical glycemic analysis (Venn and Green, 2008), the values in Figure 13.3 are likely to have been influenced by many different factors, because they were taken from many different studies. Within each GI category in Figure 13.3 – low, medium, high – the standard deviation of the means is very large, and is often larger than the differences between GI categories, even when, as in group 1, all of the values in the category 'Baked' were from the same potato variety, Russet Burbank. The results show that generalizations about the glycemic impact of potatoes, based on GI values, should be tempered with an appreciation of the large amount of uncertainty associated with the values.

Buyken and Kroke (2005) have summarized some reasons why a conclusion that all potatoes have a high GI is unjustified, pointing out the sometimes tenuous evidence for varietal differences, effects of maturity, and effects of processing as well as the effects of food consumption habits, all of which are discussed below.

Apart from the lack of research, one of the biggest problems in making GI comparisons between potato samples is the large error associated with clinical blood glucose response measurements. In an inter-laboratory study (Wolever et al., 2003), seven laboratories tested the GI of one potato preparation (instant potato) provided from a single source and obtained a mean GI value of 65.2, with a range of 44.6–98.5! Such findings suggest that highly precise in vitro determinations of

glycemic impact as a food property may be more useful than clinical values in developing new potato cultivars and products, and in gaining insights into factors that might influence the glycemic properties of potatoes.

13.5 Factors Determining the Glycemic Impact of Potatoes

The factors that may determine differences in glycemic impacts of potatoes and potato products include those that are intrinsic to the potato, that is, present in the potato at harvest, and those that are extrinsic, that is, the result of external influences grouped generally as processing effects. Apart from factors that determine glycemic impact in the sense of the loading of available glycemic carbohydrate in a food intake, there is, at a higher level, a cluster of factors that determine how an individual responds to the glycemic impact.

Despite the widespread cultivation and use of potatoes, very little is known about the influence of some factors on their GI and RGI, whereas some obvious factors, such as starch structure and processing, have been subjected to a limited amount of research.

13.5.1 Intrinsic factors

Intrinsic factors that may influence potato digestibility include the structure of the starch molecules, the structure of starch granules, the composition of the potato, the presence of phosphate in starch granules, tissue structure, and factors that may affect these, including agronomic and growth history, storage, maturity, and variety.

13.5.1.1 Starch structure: amylose/amylopectin ratio

Amylose tends to be digested more slowly and to a lesser extent than amylopectin. Blood glucose responses to amylose have been shown to be less than to a similar amount of amylopectin both in biscuits containing cornstarch (Behall et al., 1988) and in rice (Goddard et al., 1984). There is some evidence that the amylase/amylopectin ratio also affects the digestibility of starch in potatoes as in other starchy foods. Potatoes were genetically modified to increase or decrease starch branching, providing lines with starch containing 64% amylose and 1% amylose, compared with the parent cultivar starch which contained 23% amylose (Leeman et al., 2006). After cooking and digesting in vitro, the high amylose line contained 26% resistant starch (RS) and the low amylose line 0.1 % RS, and the predicted GIs were 94 versus 68. The results suggest that increasing the amylose content of potatoes may be a strategy for lowering their glycemic impact. However, there was little difference between the parent cultivar (23% amylose) and the high (64%) amylose line in starch digestibility or predicted GI, and screenings of non-genetically modified cultivars have shown amylose ranges of 24–31% (Cottrell et al., 1995) and 25–32% (Haase and Plate, 1996), so the potential for conventional plant breeding to reduce glycemic impact through increased amylose content is uncertain.

As well as the amylase/amylopectin ratio the size of the amylopectin side chains may affect digestibility, especially under conditions when potato starch gelatinized during cooking is allowed to retrograde (Jane et al., 1999). Linearization of amylopectin with debranching enzymes followed by controlled retrogradation has been shown to promote the formation of slowly digested starch (SDS) in rice (Guraya et al., 2001). Presumably the longer the amylopectin branch chains the less the structural impediment to their alignment, so the greater the tendency to form slowly digested starch, and therefore the lower the GI of the starch. Although the relationship between amylose chain length and SDS formation has not been studied in detail in potatoes, the propensity to form SDS after cooking has been shown to vary greatly amongst potato genotypes (Monro et al., 2008). The structural basis of this variation demands attention.

The relationship between starch chain length and digestibility may not be simple, as some recent work has shown that amylopectin with a high density of branching digests at a slower rate than amylopectin with longer internal chains, but as chain length increases the inhibition of digestion again increases (Hamaker et al., 2008). It has been suggested that the high density of branching inhibits digestive enzyme access to the starch, but as chain length increases digestibility increases until the length of the starch chains become sufficient for chain alignment, when the inhibitory effects of retrogradation on digestion become apparent.

13.5.1.2 Granule structure

The highly ordered, tightly packed structure of native potato starch granules confers on them a high degree of resistance to attack by amylases. Raw potato starch is virtually resistant to the enzyme activity that it would be exposed to in the gut, but as soon as it has been gelatinized it is rapidly digested (Figure 13.4). Thus, in its native ungelatinized state potato starch has almost

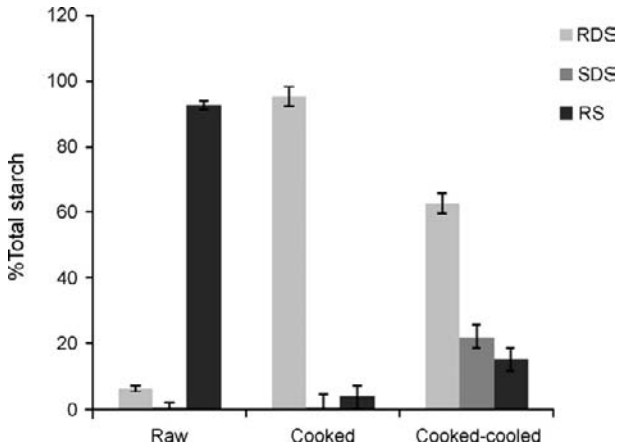

Figure 13.4: Effects of cooking and cooking plus cooling on rapidly digested (RDS), slowly digested (SDS) and resistant (RS) starch in pasted potato measured by in vitro digestion of the potato cultivar Frisia (Mishra et al., 2008).

no effect on blood glucose (Raben et al., 1994), whereas, when gelatinized it may have a high GI, although its glycemic impact (RGI) in a food will depend on its concentration in the food and on the amount of the food consumed.

As resistant starch is classified as a form of dietary fiber, ungelatinized potato starch could be used to augment the dietary fiber content of products in which gelatinization would be limited. Under such circumstances it may be valuable as an effective promoter of colonic health, as it has been shown to produce a significantly higher proportion of butyric acid in the cecal short-chain fatty acids of rats than a commercially accepted resistant starch ingredient already promoted as a prebiotic (Henningsson et al., 2003). Butyric acid is an important energy source for the colonic epithelium and is thought to reduce the risk of colorectal cancer (Bird and Topping, 2001).

13.5.1.3 Potato composition

Composition is important to glycemic impact because glycemic impact depends not only on the glycemic potency of the carbohydrate in potato (its GI) but on its concentration in the potato, and on the amount of potato consumed. Thus, even if potato carbohydrate has a high GI, the water component of potato is so high that on an equal weight basis the available carbohydrate content and glycemic potency of potato per se is relatively moderate (Tables 13.1 and 13.2, Figure 13.2). This is somewhat counteracted by the fact that the high water content is usually compensated for by a relatively large weight of a standard serving of potato.

The relationship between food composition and the relative glycemic potency of potato carbohydrate (GI), of potato (GGE/100 g = RGP), and of a serving of potato (Table 13.3), shows why it can be misleading to judge the glycemic quality of a food in terms of GI alone, and why GI values have created an excessively negative view of potatoes. When the comparison is based on common standard measures and servings, potatoes do not have a high glycemic impact compared with other starchy foods (Table 13.2). Boiled potato contains 77% water compared with 34% in bread, 33% in muffin, and 11% in muesli, for instance, so that even if the GI of carbohydrate in a food is less than that of potato carbohydrate, the glycemic impact of the food may be higher because the amount of carbohydrate in that food is greater.

The value of potatoes in nutritional management may depend on other food components which contribute to the composition of the form of potato consumed. For instance, fried potatoes in the form of potato crisps (Table 13.2) contain a high proportion of fat, and very little water, so on an equal weight basis they are about six times more energy-dense than potatoes alone. However, that has little to do with potatoes, but is more a result of the way they are treated.

13.5.1.4 Starch granule phosphate

Potato starch differs from cereal starches in that it contains a high level of phosphate. The phosphate content of starch granules is thought to encourage larger granule size and an increased susceptibility to gelatinization, and is important in potato starch rheology (Bergthaller, 2004). In so far as gelatinization confers susceptibility to digestion, phosphate content could play a role in developing glycemic potency in food systems containing potato starch in which gelatinization is limited. However the authors are not aware of studies in which digestibility/glycemic impact of potato has been studied as a function of starch granule phosphate content.

13.5.1.5 Tissue structure

The encapsulating cellular structure of the tissue in particles, and the geometry of particles in its own right, can reduce the digestibility and glycemic impact of starchy foods, such as whole-grain cereals and pastas. The effect of tissue structure on the glycemic impact of potatoes has not been investigated in depth. The thin parenchymatous cells of the potato tuber, and the absence of seed coats and of the gluten-like proteins that give cereal products such as pasta their resilience, suggest that structure will be less important in modulating glycemic impact in potato than in some other foods. The differences between the means of GIs from potatoes cooked by different methods (Figure 13.3) suggest that the physical form of the potato may have some influence, as there is a difference of about 20 GI points between the means. Finely divided potato preparations such as instant and mashed gave higher mean GIs than the forms that use more resilient tubers that remain intact, namely boiled, canned and new, perhaps because access of digestive enzymes to starch is most rapid in finely divided potato. However, the comparisons in Table 13.4 do not suggest that tissue disruption, at least as it occurs in mashing, has any significant influence on

Table 13.4: Effects of different cooking methods on measures of the glycemic potency of potatoes

Ref.	Boiled	Mashed	Baked	Fried	Microwaved	Canned	Roasted
1	96*	99*	95*	88*	96*	–	
2	88 ± 9*	91 ± 9*	93 ± 11*	–	79 ± 9*	65 ± 9	
3	99.6*	107.5*	67.8*	56.6*	–	–	–
4	89.4 ± 7.2	87.7 ± 8.0	72.8 ± 4.5	63.6 ± 5.5	–	–	72.3 ± 8.2
5	104 ± 39*	106 ± 42*	–	–	–	–	–
6	111 ± 14*	77 ± 10*	–	–	–	–	–

References:

1. *Kingman and Englyst (1994); In vitro SDRI (Starch digestion rate index = RDS/total starch)*
2. *Soh and Brand-Miller (1999); GI (Mean ± S.E.M.)*
3. *García-Alonso and Goñi (2000); GI estimated from in vitro data. Variety not stated.*
4. *Fernandes et al. (2005); GI (Mean ± S.E.M.)*
5. *Tahvonen et al. (2006); GI ± SD*
6. *Leeman et al. (2008); GI ± S.E.M.; GI based on a white bread reference (GI = 100)*

**Same variety within a row.*

GI. In a comparison of coarsely minced and finely pasted freshly cooked potato Mishra et al. (2008) showed only a slightly and non-significantly greater RDS content in the finely pasted potato than in the coarsely minced potato.

Henry et al. (2006) found that floury potatoes tend to have a higher GI than waxy potatoes, perhaps because flouriness involves tissue disintegration. Baked potatoes are typically floury in texture, so when swallowed may be similar in form to mashed potatoes whereas varieties used for boiling and canning maintain their tissue integrity relatively well during wet cooking, and are less disintegrated during consumption.

In a comparison of RDS, SDS, and RS formation in cooked-cooled potatoes that were intact, coarsely minced, pasted or finely dry-milled the extent of tissue disruption appeared to have little influence on the formation of SDS and RS after cooking (Mishra et al., 2008). The formation of SDS and RS is likely to be more a manifestation of the structure of the starch molecules involved without any constraints imposed by the starch-containing tissue.

13.5.1.6 Agronomy/growth history

There has been little systematic evaluation of the effects of growth conditions and other agronomic factors on the glycemic impact of potatoes. Starch levels measured during development of potato tubers in two varieties remained quite constant, even though sugar and enzyme levels changed (Lewis et al., 1994). In potatoes that had been genetically modified for a high amylose content the harvest yield and degree of starch branching were stable over three consecutive years (Hofvander et al., 2004).

Agronomic factors reportedly influence the rheology of potato starch (Bergthaller, 2004) but whether or not these effects have any relevance to glycemic impact has not been investigated.

13.5.1.7 Maturity

The effects of maturity on the glycemic impact of potatoes, if any, may be mediated through changes in starch structure. The quantity of amylose is reported to not change greatly, while the amylopectin branching is reported to increase significantly with maturation of potato, which is speculated to increase gelatinization and therefore digestibility (unpublished results of Brunt and Zinsmeester quoted in Soh and Brand-Miller, 1999). Increased branching with maturation may also decrease the tendency to form SDS in cooked-cooled potatoes if it reduces the possibility of amylopectin side chains becoming aligned. Whether or not SDS formation played a role in the results of Soh and Brand-Miller (1999) is uncertain as the time between cooking and food consumption during glycemic response measurement was not specified. Buyken (2008) has pointed out that most GI values for potatoes are from varieties which are used in the USA for baking, mashing, roasting, and frying and which are more mature and contain more starch than the varieties consumed as boiled potatoes in Europe, which tend to have lower

GI values. The relationship between maturity, starch structure, and glycemic impact requires further research.

13.5.1.8 Storage

The effect of storage of potatoes on their glycemic impact has not been studied in detail. Cold-induced sweetening is a well-known and troublesome phenomenon in potato storage, but whether or not it has any relevance to glycemic impact is uncertain. The presence of free sugars will not necessarily raise the GI of potatoes if the GI value is already high, because there is such an excess of amylase in the gut that consumed starch is almost immediately converted to glucose for absorption. Both the onset of the blood glucose response and the time to peak blood glucose concentration (about 30 minutes) were similar for potato and free glucose, and were influenced little by the intake of potato (Figure 13.1). Except in genetically modified potatoes (Karlsson et al., 2007), the amount of free sugar that accumulates during cold-induced sweetening is usually only a few percent so it is unlikely that substantial differences in GI would occur, or could be detected with a method as imprecise as clinical GI determination.

During in vitro glycemic analysis of freshly boiled minced potatoes of a range of varieties nearly all of the starch was almost immediately digested by pancreatic amylase. Not surprisingly, therefore, in vitro analysis has not revealed any effects of storage time in potatoes stored for 1–3 months or 8–10 months (Lehman et al., 2005).

However, if cold-induced sweetening is accompanied by changes in amylopectin structure, such as shortening of side chains, or debranching an impact on GI in products in which SDS formation is favored may occur. This is another area requiring further research.

13.5.1.9 Cultivar

There have been relatively few studies specifically on the dependence of glycemic impact on variety of potato, often because the focus has been on processing effects using varieties suited to particular processing methods (e.g. Fernandes et al., 2005), or a single variety has been used (e.g. Tahvonen et al., 2006), while in others the cultivar has not been specified (e.g. García-Alonso and Goñi, 2000). However, two studies have compared varieties prepared by the same method. Soh and Brand-Miller (1999) compared three varieties, all boiled, and obtained GI values (mean $\pm$ S.E.M.) of 87 ± 7, 101 ± 15, and 88 ± 9, which were not statistically significantly different. Henry et al. (2005) made a larger comparison of eight varieties of boiled potatoes and obtained GI values ranging from 56 ± 3 to 94 ± 16, but again, because of the variability intrinsic to clinical measurements could not demonstrate statistically significant differences. The inability to show varietal differences in clinical trials reflects the logistical problems in using enough subjects to give a clinical trial sufficient power to detect statistically significant differences, rather than showing that differences that would be nutritionally important on a population scale do not exist.

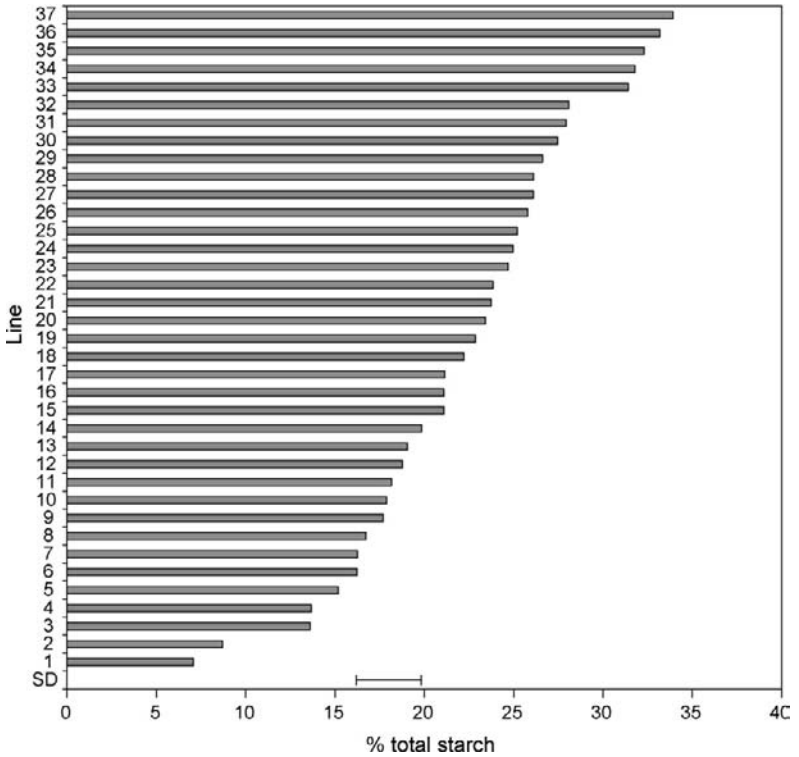

Figure 13.5: Differences between 37 potato lines in SDS formation after cooking and cold treating suggests that there is potential for traditional breeding to enhance SDS formation for low GI potato products (Monro et al., 2008).

Nutritionally valid in vitro tests of high precision have shown that there can be considerable and statistically significant differences between potato lines and cultivars in starch fractions defined in terms of digestibility (Figure 13.5; Mishra et al., 2008; Monro et al., 2008). In developing new cultivars of reduced glycemic impact in vitro glycemic analysis will have an important role to play by providing the precision necessary to measure real differences between lines in respect of digestibility and glycemic impact.

13.5.2 Extrinsic factors: processing

The effects of intrinsic factors that influence glycemic impact of potatoes are further modulated by a number of external or processing factors that act through their effects on the digestibility of starch in the potatoes and/or on the glycemic responsiveness of those who eat them. In general, starch digestibility is limited by lack of access of digestive enzymes to the starch, so it is increased by gelatinization/cooking processes that disperse starch, that break enzyme-restricting associations between starch molecules, and which disrupt any protective effects of

potato tissues, cell walls, and food matrices. Conversely, processes subsequent to cooking that restrict enzyme access, such as retrogradation, in which aggregation or annealing of starch molecules occurs, will reverse the increases in digestibility brought about by gelatinization, to various degrees. And at a higher level, properties of food matrices and of foods and ingredients that are combined with potato may superimpose their own glycemic properties on the potato component.

Digestibility patterns associated with the above processes are summarized in Figure 13.4. Generally, in the raw state in which starch molecules are tightly organized into starch granules, potato starch is virtually indigestible (Kingman and Englyst, 1994; Mishra et al., 2008). When the potatoes have been cooked and the starch chains hydrated and disorganized during gelatinization the starch becomes almost totally digested by amylase within 20 minutes (rapidly digested starch; RDS). However, when the cooked potato is then cooled, and the starch chains reaggregate, a proportion of the starch becomes indigestible (resistant starch; RS), and an even greater proportion becomes slowly digested starch (SDS), that is, digested between 20 and 120 minutes in vitro (Mishra et al., 2008).

13.5.2.1 Effects of cooking on potato digestibility

Although there are many GI values for potatoes cooked in different ways (Figure 13.3), because of the variability in GI determinations and varietal differences a comparison of the effects of cooking methods requires that the methods be compared using a single variety in a single experiment. There are a few clinical studies in which the GI of potatoes has been measured as a function of cooking with a range of methods, and a few in vitro studies (Table 13.4). The differences are not consistent, although in two studies fried potatoes gave lower GI values, perhaps because fat reduces the glycemic response to starchy foods. Resistant starch formation was measured in the studies of Kingston and Englyst (1994) and García-Alonso and Goñi (2000) and remained a minor component irrespective of processing method, when the potatoes were consumed without a cold treatment after cooking.

13.5.2.2 Effects of cooling after cooking

When cooked potatoes are subjected to a period of cooling after cooking, the in vitro digestibility of the starch in them decreases (Mishra et al., 2008), and the glycemic effect that they have is also significantly reduced (Leeman et al., 2005; Tahvonen et al., 2006). The reduction in starch digestibility is apparent as a decrease in RDS accompanied by an increase in both SDS and RS, with the increase in SDS contributing more to the decrease in rapid (RDS) digestibility than RS formation (Mishra et al., 2008). The reduction in digestibility of potatoes during cool storage after cooking has been attributed to partial starch retrogradation (Karlson et al., 2007). It has been suggested that starch chain alignment that normally leads to retrogradation of amylose occurs sufficiently in the disorganized amylopectin of gelatinized potato starch, to impede, but not prevent, digestion (Fredriksson et al., 2000).

From a food-labeling and nutritional perspective it is important that cooling after cooking can lead to a substantial reduction in glycemic potency more by increasing SDS, than by increasing RS, because it would mean that the energy availability from potato would be largely maintained, while both the glycemic impact per given weight, and the glycemic index would be reduced. If RS rather than SDS had been formed, glycemic impact and energy availability would both have been reduced, but the GI would have remained high because GI is, by definition, based on available carbohydrate, so any RS should be excluded from the measurement and calculation of GI (Monro, 2003). SDS, on the other hand is available, so any reduction in glycemic effect that it causes will translate to a lower glycemic response per unit of available carbohydrate, that is, a lower GI.

In a comparison of nine supermarket varieties, on average 96% of the available starch (RDS + SDS) in freshly cooked potato was RDS, and as a result of cool treatment the proportion of available carbohydrate that was RDS decreased to 64% (Monro, 2008). If a corresponding proportional decrease in GI occurred, potato would move from a high GI (>70) category in the freshly cooked state to a low GI (GI < 55) category in the cooked-cooled state, a reduction in GI confirmed in recent studies (Leeman et al., 2005; Tahvonen et al., 2006). Assuming that RDS is a valid indicator of glycemic impact, the cool treatment would lead to a reduced intake of glucose from 13.5 to 8.6 g per 100 g serving (equivalent to about one potato). As an intake of 10 g GGE is considered the border between low and medium glycemic loading per serving (Brand-Miller et al., 2003), cool treatment could lead to potato being classed as a low to medium glycemic impact food product, because, although predominantly carbohydrate, it has a high water content and therefore a low density of carbohydrate.

Not only is the glycemic potency of the potato likely to be reduced by cool treatment after cooking, but the increase in RS that occurs will increase the dietary fiber content, because RS is classed as a component of dietary fiber (Table 13.5). In the above study of nine supermarket cultivars, RS doubled as a result of cool treatment to 7.4% on a dry weight basis (Monro et al., 2008). Thus dry cooked-cooled potato could be classed, in terms of food regulations, as high in dietary fiber (>6%), so that if used as an ingredient it would not reduce the ability to make a claim of ‘high in dietary fiber.’ And if prebiotic effects similar to those induced by cereal RS (Bird and Topping, 2001) can be demonstrated for potato RS, claims for colonic benefits of cooked cool-treated potatoes or products containing them may be justified.

SDS formed by cool-treating cooked potato is largely reversed by heat treatment whereas RS is more stable. Where a role for SDS can be found in food products of low glycemic impact potato has the potential to make a valuable contribution.

Table 13.5: In vitro measures of the glycemic potency of potatoes

Term: **Available carbohydrate** (CHOAVL) (g)
Definition: Carbohydrate absorbed and metabolised by the body.
Meaning: Is analytically measured as sugars soluble after amylase digestion of finely ground samples, so consists of food sugars and digested starch. But it represents potential availability rather than true availability because fine grinding removes the constraints of food structure.
Relevance to potatoes: Available carbohydrate is often not a good indicator of glycemic response because its value does not include a component for the rate of digestion of carbohydrate, or account for a range of other factors that may alter absorption rate.
Term: **Rapidly digested starch** (RDS) (g); Rapidly available glucose (RAG); Rapidly available carbohydrate (RAC).
Definition: Free sugars released within 20–30 min of in vitro digestion by amylase.
Relevance to potatoes: The precision of in vitro glycemic analysis is important in potato research aimed at determining relative digestibility of potatoes and potato products, identifying changes in digestibility that arise from food processing, and in identifying lines with particular digestion characteristics, and so on.
Term: **Slowly digested starch** (SDS) (g)
Definition: Starch digested after 20 min of in vitro digestion by amylase.
Relevance to potatoes: Slow starch digestion leads to a rate of blood glucose loading that does not greatly exceed the rate glucose disposal, so leads to a small glycemic response. SDS is therefore available but not highly glycemic carbohydrate. It contributes to a low GI because GI is calculated on the basis of available carbohydrate. A high SDS content means sustained but not highly glycemic energy. Some potato lines have a large capacity to form SDS after cooking and cooling (Figure 13.5).
Term: **Resistance starch** (RS) (g)
Definition: Starch that is not digested by prolonged amylase treatment.
Relevance to potatoes: RS includes raw and retrograded starch that is resistant to digestion because of the way it is organized, and digestible starch that is inaccessible to digestive enzymes. Retrograded RS develops in cooked cooled potatoes leading to a reduced glycemic impact, as it is unavailable as carbohydrate. However, it is fermented in the colon so may contribute to colonic health. Because RS is included in the definition of dietary fiber increasing it improves the nutritional profile of potatoes.
Term: **Non-starch polysaccharide** (NSP) (g)
Definition: Mainly cell wall polysaccharides and the principal component of dietary fiber.
Relevance to potatoes: Potatoes have thin cell walls so are not rich in NSPs. They are a valuable source of carbohydrate energy, but would not on their own provide enough dietary fiber to meet daily requirements. Increasing RS levels partially compensates for the low dietary fiber content of potatoes.
Term: **Dietary fiber** (NSP + RS)
Definition: Polysaccharides resistant to human digestive enzymes.
Relevance to potatoes: Dietary fiber includes cell wall NSP and RS, as both are digestion-resistant polysaccharides and both enter the colon where they may contribute to colonic health. NSP is low in potatoes but with RS formation NSP + RS (= dietary fiber) in potatoes can reach levels that allow the nutrient claim 'high in dietary fiber.'

In vitro measurement of the release of free sugars from foods during enzymic digestion, with timed sampling that allows rapidly available carbohydrate components to be determined, provides a measure of glycemic potency because a glycemic response occurs when rapidly available carbohydrate absorption exceeds the rate at which the body is able to remove glucose from the blood, so there is a temporary accumulation of glucose in the blood.

13.6 Effects of Food Combinations

'The GI is a property of carbohydrate rich foods consumed alone' and '. . . it is naïve to believe that the GI is the only or even the major determinant of the postprandial glycemic response' are encouraging words from one of the founders of the GI concept (Wolever, 2006). They mean that even if potatoes have a high GI, because they are usually consumed with other foods as part of a meal, any glycemic impact that they have will be modulated by the other foods. Their glycemic effect may therefore be much less than one would expect from a food with a high GI. The ability of various food constituents to modify the glycemic response to carbohydrate foods has been demonstrated many times, and two recent papers have shown that the effect occurs strongly with potatoes.

Henry et al. (2006) investigated the impact of adding various toppings to commonly consumed carbohydrate foods, including potato, on their glycemic effect. Potato alone had a GI of 93 ± 8 (mean $\pm$ S.E.M.). The GI of potato plus cheddar cheese was 39 ± 5. The quantity of potato plus cheddar required to deliver 50 g of available carbohydrate for GI determination was 410 g compared with 290.7 g for potato alone, so one can calculate that the RGP values would have been: Glucose (reference), 100 GGE/100 g; potato alone, 16.0 GGE/100 g; potato plus topping 4.75 GGE/100 g. That is, 100 g of the potato plus cheddar was equivalent in its glycemic impact to 4.75 g, about one teaspoon (5 g = 5 GGE) of glucose. The GI of the potato was reduced 58% by adding cheddar, and the RGP, which tells us the glycemic potency of the whole food, was reduced by 70%.

Precisely what component of the cheddar was responsible for depressing the glycemic response was not determined, but all of the fat, organic acids, and protein could have contributed.

Several studies have shown that that adding organic acids to starchy foods can suppress the glycemic response. Most research has used bread as the starchy food base (summarized in Leeman et al., 2005), but recently the effect of a vinegar dressing on the postprandial response to boiled potato was measured (Leeman et al., 2005). Adding vinegar reduced the GI of the boiled potato by 31% from 168 to 96 (based on a bread reference).

Considering both the tendency of accompanying foods in a meal to reduce the glycemic impact of the potatoes, and the fact that relative to other foods the relative glycemic potency of potatoes is not high (RGP, Table 13.2), it seems safe to conclude that even if potatoes have a high GI it is unlikely to be a threat to health in the context of a mixed diet with a healthy balance of nutrients. Measures of the glycemic potency of foods, including GI, RGI, GGE, and RGP were never intended to be used separately from other dimensions of diet and health, and should not be used in isolation to tarnish the reputation of potatoes. They apply only to glycemic response, and should always be used as part of a multifaceted approach to health.

13.7 Potato as an Ingredient

If potato, or potato starch, is used as an ingredient in a product, a large number of properties of the product will modulate any contribution of the potato to the product's glycemic impact. Together, the properties of the potato component and of the other ingredients provide an opportunity to design a range of products with specified glycemic and other functional properties. The glycemic impact of potato-containing products may be manipulated through effects on potato starch digestibility, by controlling the degree of gelatinization of the raw starch and/or by controlling and preserving the formation of SDS and RS by retrogradation. Beyond the potato component the functionality of the product may be modified by including hydrating non-starch polysaccharides (dietary fibers) to delay gastric emptying and slow glucose absorption, and by using appropriate lipids, organic acids, and proteins to achieve functional and nutritional aims. Physical attributes of the product such as porosity may also play a role in reducing the rate of digestion, and as glycemic impact depends on food intake, portion size may have an important role in determining glycemic impact and energy intake.

13.8 Conclusion

In the preceding discussion we have seen that potatoes are not an intrinsically unhealthy component of the diet despite the common incorrect inference from their high GI values that they are highly glycemic. They have an important role as a benign source of moderate-density carbohydrate energy that fits comfortably into a range of balanced diets, and can help put into practice the recommendation of the World Health Organization (1997), that carbohydrates should be the main energy source in a healthy diet. However, they are a victim of the human predilection for fatty salty foods such as potato chips, and of the willingness of food manufacturer's to profit from that predilection. Table 13.3 showed that when a healthy boiled potato with an energy density of 3.4 kJ/g is converted into potato chips the energy density rises to 21 kJ/g! And if potatoes are considered unsuitable for modern populations, the fault lies not with the potatoes, but with human behaviors and lifestyles. In a population that eats well and lives and exercises well, the potato has a valuable role to play as a versatile source of low-fat, moderate-density, carbohydrate energy.

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CHAPTER 14

Nutritional Value of Potatoes: Vitamin, Phytonutrient, and Mineral Content

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14.1 Introduction

Potatoes were domesticated between 7000–10 000 years ago, likely around Lake Titicaca, an alpine lake at 12 500 feet in the Andes between Peru and Bolivia (Spooner et al., 2005). Potatoes may contain more genetic diversity than any other crop and this may reflect the ability of potatoes to grow in remarkably divergent environments, from arid alpine highlands to tropical rainforests to permafrost soils just below the Arctic Circle and even on tree branches (Hawkes, 1990). This genetic diversity is a valuable resource towards further improving tuber nutritional content, especially when taking into account that modern cultivars are estimated to contain less than 1% of the available genetic diversity of wild species. About 200 wild potato species exist, in addition to thousands of primitive varieties. Potatoes are the fourth most grown crop in the world, after the cereals rice, wheat, and maize and are the only major food crop that is a tuber.

Potato tubers are highly specialized organs evolved to improve a plant's chances of survival and to allow vegetative reproduction. Tubers are not derived from roots, but are modified stems, originating on stolons from axillary buds on the underground part of the stem (Ewing and Struik, 1992; Fernie and Willmitzer, 2001; Jackson, 1999). The fact that tubers are modified stems influences tuber characteristics and chemical composition. For example, the greening of tubers that occurs on exposure to light in which amyloplasts in the tuber parenchyma redifferentiate into chloroplasts (Deng and Gruissem, 1988), reflects the stem origin of the tuber. Tubers are metabolically active, contain an abundant amount of plastids and synthesize numerous compounds derived from plastidic biosynthetic pathways. Indeed, the complex metabolite composition of tubers belies their misperception as simple organs containing starch and not much else.

In fact, tubers contain plentiful amounts of small molecules and secondary metabolites, which have roles in an array of key tuber processes from regulating tuber organogenesis to mediating

responses to the environment. Moreover, many of these compounds have positive effects on human health and are highly desirable in the diet (Flamini, 2003; Katan and De Roos, 2004).

14.2 The Dietary Importance of Potatoes

Potatoes are uniquely positioned to be a valuable source of dietary vitamins, minerals, and phytonutrients because of their per capita consumption. In most of the developed world, potatoes are by far the most eaten vegetable (Figure 14.1). Because of this high consumption the vitamin and phytonutrient content of potato will have much more dietary relevance and impact than foods eaten in sparse quantities. Moreover, in the developing world, potato consumption is increasing at about 5% a year and in 2005 the developing world for the first time produced more potatoes than the developed world. China and India produce about one third of the world's potatoes.

Potatoes yield more calories per acre than any other major crop, a criterion that becomes even more important in light of the planet's ever-increasing population, food shortages, price spikes, and the recent competition for farmland by biofuel crops. Collectively, these facts emphasize the impact potatoes can have on global nutrition.

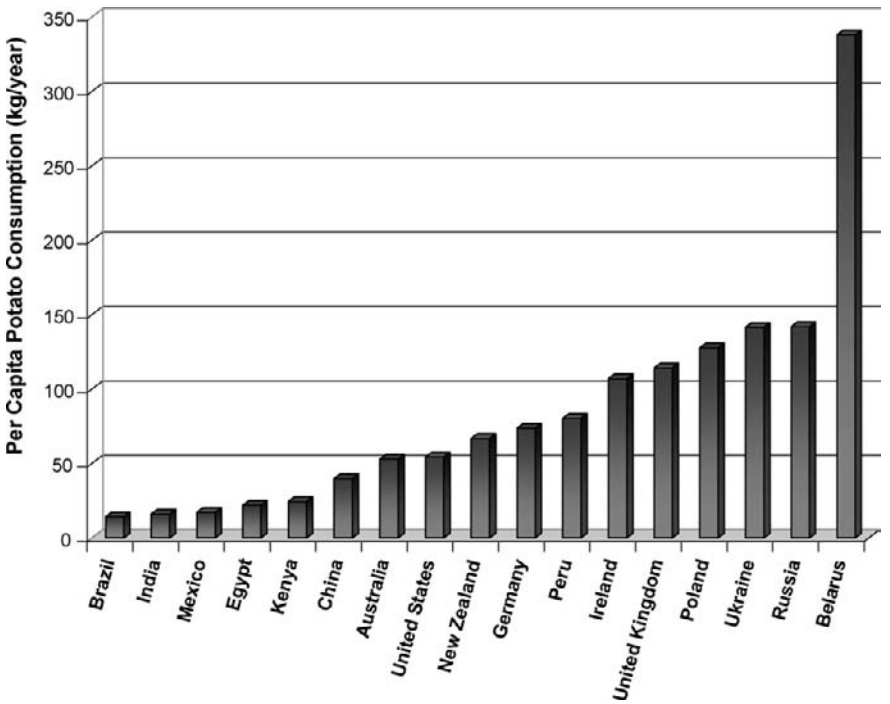


Figure 14.1: Per capita consumption of potatoes in 17 countries in 2005. Based on numbers reported by the Food and Agricultural Organization of the United Nations.

14.3 Popular Diets and Potato Consumption

Unlike during most of its long gastronomic history, the nutritional qualities of potatoes are perhaps currently under-appreciated due to negative publicity from various sources, such as low-carbohydrate diet advocates. The recent popularity of low-carbohydrate diets has impacted consumption of traditional staples such as potatoes, rice, bread, and pasta. Million dollar marketing campaigns have been launched in recent years to promote the nutritional advantages of potato, including the British Potato Council's 'Fab not Fad' marketing campaign that aggressively criticized 'fad diets.'

Recent years have shown that consumer perception about the nutritional value of potatoes impacts sales and strongly suggests that perceived nutritional value is a very important trait for any vegetable, especially potatoes given the recent negative publicity. A growing number of consumers appear increasingly interested in the medicinal benefits of foods and the relationship between diet and health. It has been estimated that one third of Americans take a daily vitamin or dietary supplement and annual supplement sales in the US top 18 billion dollars. Furthermore, in the years ahead, medical research will discover much more about which plant nutrients have positive effects on health, drawing more attention to the diet–health link.

Generally, crops have been bred and selected primarily for traits such as yield, disease resistance, and appearance. Historically, little effort has been directed towards increasing the nutritive value of any crop for reasons including that there were more pressing issues, plus the daunting technical difficulty of such an undertaking. With most crops, including potatoes, nutrient profiles are available only for a few varieties. Thus, surprisingly little is known about just what vitamins and nutrients are in potatoes. Which varieties have the most? Can new varieties be developed that have even more? Answering these types of questions has been made easier due to recent technological advances, like high-throughput assays, affordable and powerful mass spectrometers, and myriad molecular biological tools.

14.4 Tuber Composition

Potatoes are approximately 80% water and 20% solids, although this can vary by several percentage points depending on the cultivar. Of the 20 grams of solids in a 100 gram tuber, about 18 grams are carbohydrate and 2 grams protein. The primary storage proteins in tubers are patatins, which account for 40% of the soluble protein content (Prat, 1990). Potatoes are a good source of many vitamins and minerals; if one compares percentage of recommended daily allowance (RDA) of calories in a given portion size versus the percentage of the RDA of vitamins and minerals in that same portion, many vitamins and minerals exceed the percentage of calories. For example, according to the USDA nutrient database, 100 grams (3.5 ounces) of potatoes contains 4% of the RDA calorie intake, 33% of the RDA of vitamin C, the most abundant vitamin in potatoes and 12% of the RDA for potassium.

In addition to vitamins and minerals, tubers contain a complex assortment of other small molecules, many of which are phytonutrients. These include polyphenols, flavonols, anthocyanins, phenolic acids, carotenoids, polyamines, glycoalkaloids, tocopherols, calystegines, and sesquiterpenes.

14.5 Vitamin C

Potatoes are a well-known source of vitamin C, with a medium red-skinned potato (173 grams) providing about 36% of the RDA according to the USDA databases. Vitamin C has a major role in detoxifying reactive oxygen species in plants, which are the primary source of vitamin C in the human diet. Leafs and chloroplasts can contain 5 to 25 mM L-ascorbate, respectively (Wheeler et al., 1998). Plants may have multiple vitamin C biosynthetic pathways, with all of the enzymes of the L-galactose pathway recently characterized (Laing et al., 2007; Wolucka and Montagu, 2007). Vitamin C is a cofactor for numerous enzymes, functioning as an electron donor. The best known symptom of vitamin C deficiency is scurvy, which in severe cases is typified by loss of teeth, liver spots, and bleeding.

One study examined tuber vitamin C content in 75 genotypes and found concentrations ranging from 11.5 to 29.8 mg/100 g FW (Love et al., 2004). This study also reported that some genotypes had more consistent concentrations of vitamin C than others across multiple years or when grown in different locations and suggests that the year may have a bigger effect than location. A British study measured vitamin C in 33 cultivars grown in three locations around Europe (Dale et al., 2003). If these author's results in dry weight are converted to fresh weight assuming potatoes are 80% water, a range of 13–30.8 mg vitamin C per 100 grams FW is obtained, which is consistent with the Love et al. report.

Numerous studies have shown that vitamin C levels decrease rapidly during cold storage of potatoes and losses can approach a 60% decrease (Keijbets and Ebbenhorst-Seller, 1990). After placing 33 genotypes in cold storage for 15–17 weeks, Dale et al. found substantial decreases in vitamin C compared to pre-storage (Dale et al., 2003). Vitamin C decreases ranged from 20–60% depending on the genotype. The authors make the important point that breeding efforts to increase vitamin C should focus on post-storage content and that in most cases this is more relevant than fresh-harvest concentrations. This will be truer for countries that place a majority of the potato harvest in cold storage than for developing countries that make limited use of cold storage and for which post-harvest loses consequently should be less.

A Turkish study examined the effect of freeze-storing peeled, blanched then fried potatoes and found a 10% loss of vitamin C after 6 months of storage at -18°C (Tosun and Yücecan, 2008). However, a 51% loss was caused by the pre-freezing operations, which sounds a cautionary note about the importance of how potatoes are handled during processing. Some of our own

cooking studies with skin-on potatoes using microwaving, steaming, baking, and boiling have shown a negligible loss of vitamin C. Thus, in the absence of cultivars with stable vitamin C levels during cold storage, one solution that may help to minimize post-harvest loss of vitamin C for some commercial products would be minimally destructive cooking of tubers shortly after harvest, followed by flash-freezing of the product.

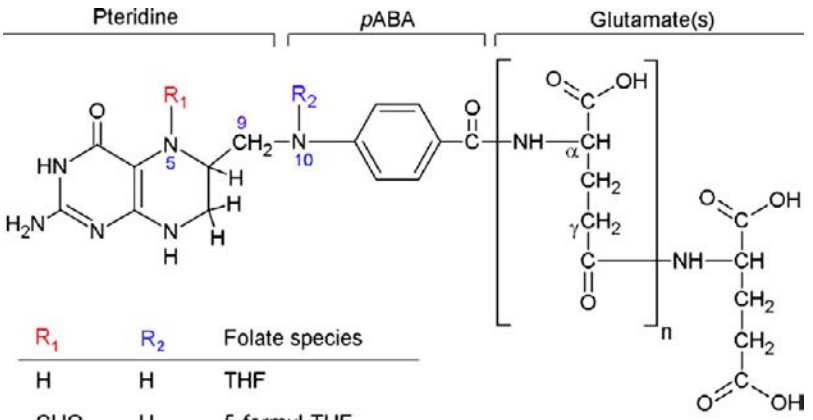
Wounding can substantially increase vitamin C levels. A study examined changes in vitamin C after storing potatoes for 2 days following slicing or bruising, and measured a 400% increase in vitamin C in sliced tubers, but a 347% decrease in bruised tubers (Mondy and Leja, 1986). Vitamin C levels in fresh-cut potatoes stored in air were found to increase, whereas levels decreased in those stored frozen or under a modified atmosphere (Tudela et al., 2002b). These and similar results suggest that wounding of potatoes can be used to substantially increase vitamin C in commercial products. However before this is likely to be widely adopted as a strategy to increase vitamin C, a method must be found to decrease the browning that can occur in cut tissue and which consumers find undesirable.

14.6 Folate

Folates (vitamin B9) is a generic name commonly used to design tetrahydrofolate (THF) and its one-carbon (C1) unit derivatives (Figure 14.2). Folates are important cofactors involved in C1 unit transfer reactions. Two crucial pathways occurring in mammalian and plant cells that involve C1 unit transfer reactions are DNA biosynthesis and the 'methylation cycle' (Scott, 1999). Folates are essential micronutrients in the human diet. Indeed, while plants and microorganisms can synthesize folates, humans lack this ability and require a dietary supply. Plants represent the major source of folate in the diet.

14.6.1 Importance of potato folate in the diet

Potato is a well-known significant source of folates in the diet due to its high level of consumption more so than for its endogenous content. In the Netherlands, Brussaard et al. (1997) reported that potatoes, among vegetables, were the most important source of folate in the diet, supplying 10% of the total folate intake. Potatoes were the third most important overall source of folate in the Dutch diet, providing 7% of the total folate intake (Konings et al., 2001). Potatoes provided 9–12% of the total folate intake in a Norwegian study (Brevik et al., 2005). In Finland, potatoes were among the best source of folate in the diet (Vahteristo et al., 1997) providing ~10% of the total folate intake (Alfthan et al., 2003). In a Spanish subpopulation, potatoes provided 3.6% of the total folate intake (Plannels et al., 2003). Hatzis et al. (2006) examined the association between serum folate status and food consumption in a Greek population and showed that increased consumption of potatoes was associated with decreased risk for low serum folate.



R_1	R_2	Folate species
H	H	THF
CHO	H	5-formyl-THF
H	CHO	10-formyl-THF
CH_3	H	5-methyl-THF
=CH-		5,10-methenyl-THF
$-CH_2-$		5,10-methylene-THF

Figure 14.2: Chemical structure of folates. Folate molecules consist of pteridine, para-aminobenzoate (pABA), and glutamate moieties. Plants usually contain polyglutamylated forms of folates that are made by the addition of up to about six glutamate residues (which form the γ-glutamate tail) attached to the first glutamate, each linked by amide bonds to the preceding molecule of glutamate through the γ-carboxyl of the latter. C1 units at various levels of oxidation can be attached to N5 and/or N10, as indicated by R_1 and R_2.

14.6.2 Folate concentrations in potato and other crops

Several studies reported folate concentrations in potatoes of usually unspecified genotypes and the reported values can vary substantially depending on the analytical method used (Konings et al., 2001). Values for folate concentrations in mature raw potato vary between 12 and 37 μg/100 g FW (Holland et al., 1996; Konings et al., 2001; Vahteristo et al., 1997) except in a study by McKillop et al. (2002) who reported an exceptionally high folate concentration (125 μg/100 g FW). The USDA National Nutrient Database for Standard Reference (SR20) gives values of 14 and 18 μg/100 g FW for raw potatoes. Recently, we determined total folate concentrations of potato tubers from >70 cultivars, advanced breeding lines, and wild species and found values ranging from 0.46 to 1.37 μg/g DW or 11 to 35 μg/100 g FW (Goyer and Navarre, 2007 and unpublished). Seven of the top ten varieties were yellow-fleshed, two were red-fleshed and one was white-fleshed. Yellow color was not always associated with high folate content since yellow-fleshed cultivars covered the whole range of folate concentrations. Cultivars Winema and Ranger Russet had the highest amounts of folate among white-fleshed cultivars (0.95 and 1.04 μg/g DW, respectively). Among the top yellow cultivars were Golden

Sunburst, Satina, and Carola (1.19, 1.25, and 1.37 μg/g DW, respectively). Colorado Rose was the top red-fleshed cultivar (1.03 μg/g DW). Also in the top 10 were breeding lines from Washington, Oregon, and Colorado. Genotypes *Solanum pinnatisectum* and Gayna had the highest folate concentrations among wild species and primitive germplasm (0.99 and 1.05 μg/g DW) and *S. pinnatisectum* also had the highest folate content of all genotypes analyzed on a fresh weight basis (0.35 μg/g FW). Despites the small number of wild species analyzed (12), an approximately two-fold difference between the lowest and the highest genotypes was found, suggesting that among the large number of existing indigenous potato species there might be some with even higher folate concentrations.

Potato is in the lower range of folate contents among plant foods (Table 14.1) as are other underground organs, ranking ahead of polished rice but behind green leafy vegetables and pulses. A number of factors may affect the bioavailability of folates from plant foods. These

Table 14.1: Folate content in various plant foods. All values are given for raw food. References for the lowest and the highest folate content values are indicated

Crop	Folate content (μg 100/g FW)	References
Rice (white unenriched)	6–9	1
Sweet potato	11	1
Onions	10–19	2, 1
Tomato	8–30	2, 1
Potato	11–37 (125? Ref. 5)	3, 4, 5
Banana	13–20	6, 1
Carrot	16–19	6, 1
Corn (yellow)	19	1
Orange (peeled)	18–30	2, 1
Cassava	27	1
Peas (green)	25–65	7, 1
Strawberry	13–96	8
Snap beans	37	1
Wheat (hard, white)	38	1
Lettuce (fresh)	38–43	1, 2
Corn (sweet, white or yellow)	46	1
Rye (grain)	60–78	1, 9
Wild rice	95	1
Broccoli	63–114	1, 6
Spinach	100–194	2, 1
Peanut	110–240	4, 1
Lentils	151–479	7, 1
Beans (navy, pinto, Great Northern)	143–525	7, 1

References: 1, The USDA National Nutrient Database for Standard Reference (SR20) http://www.ars.usda.gov/main/site_main.htm?modecode=12354500; 2, Konings et al., 2001; 3, Goyer and Navarre, 2007; 4, Holland et al., 1996; 5, McKillop et al., 2002; 6, Vahteristo et al., 1997; 7, Han and Tyler, 2003; 8, Tulipani et al., 2008; 9, Kariluoto et al., 2001.

include the instability of certain labile folate derivatives during digestion, the food matrix, the presence of food constituents that may enhance folate stability during digestion, and the efficiency of intestinal deconjugation of polyglutamylated folates to monoglutamates for normal absorption in the proximal small intestine.

14.6.3 Folate derivatives composition and glutamylation levels in potato tubers

All native reduced folate derivatives are very sensitive to oxidative cleavage at the C9 and N10 bond (Figure 14.2), however there are marked differences in stability of those species, 5-formyl-THF being the most stable natural folate, THF the least, and 5-methyl-THF intermediate (Forssén et al., 2000). Polyglutamates must be hydrolyzed to the respective monoglutamylated forms by folate deconjugase before absorption by the intestine, but it remains controversial whether polyglutamates are less bioavailable than monoglutamates (McNulty and Pentieva, 2004). Vahteristo et al. (1997) determined that raw potatoes contained 21 μg/100 g FW of 5-methyl-THF, 3 μg/100 g FW of THF, and traces of 10-formyl-folic acid, an oxidation product of 10-formyl-THF. Konings et al. (2001) showed that >95% of folates were present as a 5-methyl-THF derivative in potato tubers, the rest comprising 10-formyl-folic acid and folic acid, and that total folate derivatives were >90% polyglutamylated. Therefore, polyglutamylated forms of 5-methyl-THF seem to constitute most of the folate pool in potato tuber as is the case in most fruits and vegetables (de la Garza et al., 2004; Freisleben et al., 2003; Konings et al., 2001; Orsomando et al., 2005; Storozhenko et al., 2007; Vahteristo et al., 1997).

14.6.4 Food matrix

Folates can be covalently bound to macromolecules of the food matrix and entrapped folates must be released from plant cellular structure before absorption by the intestine. There is little information on how much matrix-bound folates are in potato. While Konings et al. (2001) reported that the addition of protease and amylase did not significantly increase folate content values in potato, we found that protease treatment followed by amylase and conjugase gave folate values ~20% higher than when protease treatment was performed last, indicating that a significant amount of protein-bound folates became accessible to conjugase after protease treatment (Goyer and Navarre, unpublished).

14.6.5 Stabilizers

Some dietary constituents may protect folate against degradation during digestion (as well as during processing and cooking). Binding of folates to folate-dependent proteins (e.g. T-protein of glycine decarboxylase) greatly improves their stability (Rébeillé et al., 1994). Antioxidants such as ascorbic acid or thiol compounds also protect folates against oxidative degradation (McNulty and Pentieva, 2004). It is noteworthy that potato is a good source of vitamin C.

14.6.6 Effect of storage, processing, and cooking on folates

A significant amount of literature exists regarding the effects of storage, processing, and cooking on folate retention in some vegetables, legumes, and cereals (Kariluoto et al., 2006; Melse-Boonstra et al., 2002; Scott et al., 2000; Strålsjö et al., 2003). In contrast, there is little information on the effects of storage and processing on folate contents in potato and most of the available information concerns the effect of cooking. McKillop et al. (2002) showed that boiling of whole potatoes for 60 minutes resulted in a less than 20% decrease in folate content whether or not skin was retained during boiling. Konings et al. (2001) reported folate concentrations for cooked French fries, boiled potatoes and fried potatoes that were similar to those in raw potatoes (16% increase, and 25 and 8% decreases, respectively).

Earlier reports by Vahteristo et al. (1997) showed 35 and 52% decrease in French fries and boiled potato compared to raw potatoes. Augustin et al. (1978a) examined the effect of four cooking methods on folate concentrations in four different potato cultivars and showed that overall retention of folate was >70%. However, retention was cultivar-dependent, Norchip and Pontiac having the lowest retention values (e.g. 46% for boiled, peeled, Pontiac samples) and Russet Burbank and Katahdin the highest. Boiled, peeled samples had consistently lower folate concentrations than boiled unpeeled samples. In addition to its positive effect on folate retention, skin has higher folate concentrations than flesh (Augustin et al., 1979; Goyer and Navarre, 2007). The highest retention values were always obtained from unpeeled boiled or microwaved samples, with a few cases where boiling or microwaving led to an increase in folate concentrations compared to raw tubers (maximum 111% increase). Oven-baked potatoes had the lowest overall retention values.

14.6.7 Strategies towards improving folate content and bioavailability in plant foods

Folate deficiency is associated with the increased risk of neural tube defects (spina bifida, anencephaly), cardiovascular diseases, megaloblastic anemia, and some cancers (Bailey et al., 2003; Finglas et al., 2006; Scott et al., 1999). Unfortunately, folate intake is suboptimal in most of the world's populations, even in developed countries (Scott et al., 2000). Therefore there is an urgent need to increase folate content and bioavailability in staple foods. Because of its large consumption worldwide, potato is an appealing target for enrichment.

Folate biosynthesis has been well delineated in recent years, enabling metabolic engineering of the pathway. Successes in enhancing folate production in tomato fruit and rice grain by over-expressing the first enzyme of both the para-aminobenzoic acid and the pteridine branches of the folate pathway were recently reported (Díaz de la Garza et al., 2007; Storozhenko et al., 2007), and the strategy is well in place to be implemented in other crops such as potato tubers which contain all the genes necessary for folate biosynthesis. Other possible strategies for metabolic engineering were described in detail in recent reviews (Basset et al., 2005;

Bekaert et al., 2007; Rébeillé et al., 2006) and include increasing the proportion of 5-formyl-THF, the most stable natural folate, sequestering folates into vacuoles, increasing folate salvage capacity or over-expressing folate-binding protein of plant (yet to be identified) or mammalian (Jones and Nixon, 2002) origin in plant cells.

Natural variation of folate concentration among germplasm within a species has been reported for a number of crops and could be exploited in breeding programs to increase folate concentrations in crops. We showed an approximately three-fold difference in folate values amongst >70 potato genotypes (Goyer and Navarre, 2007). An ~7.5-fold difference in folate values was reported amongst nine strawberry genotypes (Tulipani et al., 2008). Smaller variations were reported for pulses and rye (Han and Tyler, 2003; Kariluoto et al., 2001) but only very few genotypes were analyzed in each case.

Household strategies to improve the bioavailability of folates from foods have been suggested especially for developing countries where folic acid supplementation and food fortification remain far from accessible (Gibson et al., 2006). Thermal processing generally increases the digestibility of proteins and carbohydrates and therefore the release of folates from the food matrix. Combining ingestion of certain foods in the diet, for instance foods rich in antioxidants, may also improve the stability of folates. Various potato germplasm has markedly different antioxidant properties (Brown et al., 2005; Dale et al., 2003) and consumption of high antioxidant genotypes, independently of their endogenous folate contents, may provide larger amounts of bioavailable folates than those with lower antioxidants contents.

14.7 Vitamin B6

Like folate and vitamin C, vitamin B6 (pyroxidine) is water soluble and like folate has several vitamers. Vitamin B6 may be involved in more bodily functions than any other nutrient (Tambasco-Studart et al., 2005), is a cofactor for many enzymes, especially those involved in protein metabolism, and is also a cofactor for folate metabolism. Vitamin B6 has anticancer activity (Theodoratou et al., 2008), is a strong antioxidant (Denslow et al., 2005), is involved in hemoglobin biosynthesis, lipid and glucose metabolism and immune and nervous system function. Possible consequences of deficiency include anemia, impaired immune function, depression, confusion, and dermatitis (Spinneker et al., 2007). Vitamin B6 deficiency is generally not a problem in the developed world, but there could be as yet poorly defined consequences of suboptimal intake particularly for the elderly.

Potatoes are an important source of dietary vitamin B6 (Kant and Block, 1990) with a medium baked potato (173 grams) providing about 26% of the RDA (USDA National Nutrient Database SR20). Very little research has been conducted on this vitamin in potato, thus little is known about how much its concentrations vary among genotypes; ranges of 0.26–0.82 mg/200 g FW have been reported (Rogan et al., 2000). One study found that its concentration increased during

storage (Augustin et al., 1978) and that losses were less than 10% during cooking (Augustin et al., 1980).

Recently much has been learned about vitamin B6 synthesis in plants including identification of the key genes *PDX1* and *PDX2* (Tambasco-Studart et al., 2005). Such information should enable new approaches to further enhance vitamin B6 concentrations in potatoes.

14.8 Glycoalkaloids

Potentially toxic compounds called glycoalkaloids (GAs) are found in many members of the Solanaceae, including potatoes, eggplants, and tomatoes. GAs are secondary metabolites and their role in plants is to contribute to pest and pathogen resistance. From a dietary standpoint, GAs are regarded as anti-nutritive compounds capable of causing vomiting and other ill effects if ingested in high enough amounts (Hopkins, 1995; McMillan and Thompson, 1979). Another undesirable trait is that GAs can contribute a bitter taste at higher concentrations (Sinden et al., 1976). Newly developed potato varieties in the United States must contain less than 20 mg/100 gram fresh weight (FW) of total GAs (Wilson, 1959) but the guidelines established in other countries vary. However, as discussed below, the assumption that GAs are categorically undesirable in the diet is complicated by recent studies that show some GAs have health-promoting effects. Thus a more nuanced approached to tuber GA content may be in order.

14.8.1 Glycoalkaloid biosynthesis

Potato GAs are steroidal alkaloids comprised of a heterocyclic nitrogen, and a C27 steroid conjugated to a sugar moiety, most commonly a tri- or tetrasaccharide. The GA biosynthetic pathway is not fully delineated, even for solanine and chaconine, the major potato GAs. GAs are derived from the mevalonate pathway via cholesterol (Heftmann, 1983; Johnson et al., 1963), occur throughout the tuber, but are primarily synthesized in the phelloderm (Krits et al., 2007). The nitrogen is suggested to be derived from arginine (Kaneko et al., 1976). GAs are found in much higher concentrations in leaves, sprouts, and fruit than in tubers. GA concentrations approaching 18 grams/kg FW have been reported in sprouts (Valkonen et al., 1996).

Much remains to be elucidated about the genes and enzymology involved in conversion of cholesterol into the various GAs. Various glycosylation steps and several glycosyltransferases have been characterized or cloned (McCue et al., 2007; Moehs et al., 1997; Stapleton et al., 1991; Zimowski, 1991). Identification of these GA biosynthetic genes has enabled transgenic approaches to decrease potato GA content. Potatoes overexpressing a soybean sterol methyltransferase exhibited decreased amounts of GAs (Arnqvist et al., 2003), while antisense expression of several potato steroidal glycosyltransferases reduced GA levels (McCue et al., 2005, 2007).

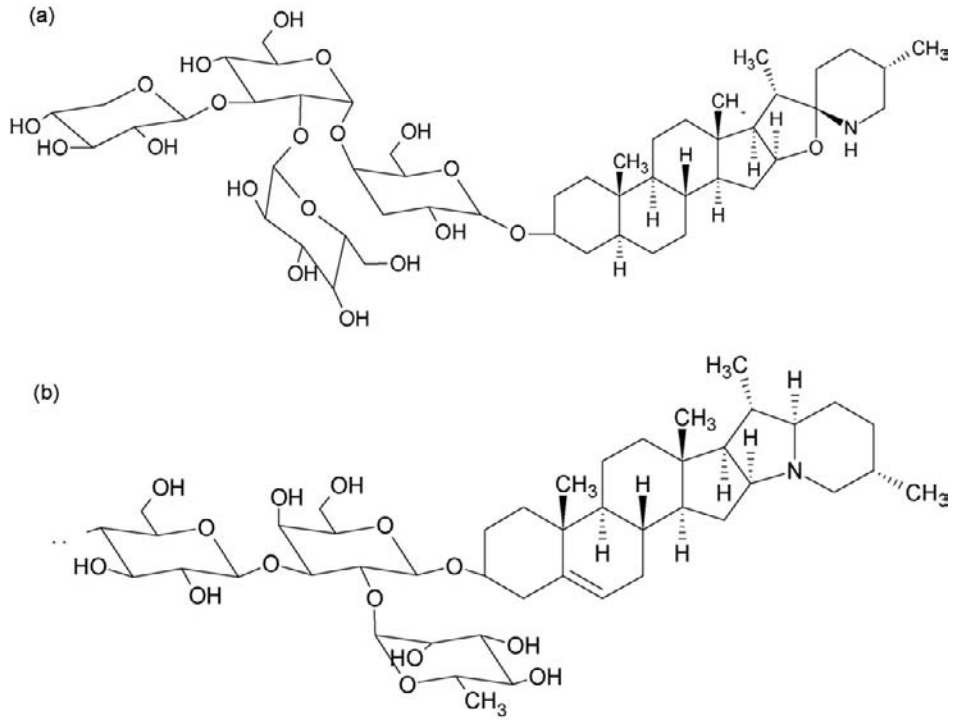


Figure 14.3: Potato glycoalkaloids. (a) is tomatine, a spirosolane and (b) solanine, a solanidane.

14.8.2 Types of potato GAs

Potato GAs usually belong to one of two structural types, either solanidanes or spirosolanes (Figure 14.3). Solanine and chaconine, both solanidanes, often comprise upwards of 90% of the total GA complement of domesticated potatoes, with chaconine often more abundant than solanine (Griffiths et al., 1997; Sotelo and Serrano, 2000).

Many in the potato industry may be familiar only with solanine and chaconine, but estimates have been made that the potato family, including wild species, may contain about 90 GAs (Friedman and McDonald, 1997). In the course of LCMS characterization of small molecule diversity in tubers from diverse potato germplasm, we observed that GAs constituted a major source of diversity.

Mass spectrometry is well suited to GA analysis and is much more selective and sensitive than many methods used to analyze GAs. In our study of tubers from four wild potato species and three cultivars, about 100 GAs were tentatively identified (Shakya and Navarre, unpublished results). This number of GAs was unexpected, especially when considering only seven genotypes were analyzed and that only tubers were used, which have much lower GA concentrations than leaves, sprouts, flowers, or leaves. Consequently, potatoes may have a much greater diversity of GAs

than previously realized. This GA diversity may offer opportunities for the production of future varieties with a more optimal GA compliment. The predominance of solanine and chaconine in modern Western cultivars may be due to the fact that only a tiny percentage of available potato germplasm was used in the breeding of these cultivars and reflects something of a bottleneck in the genetic diversity of commercial cultivars.

14.8.3 Toxic effects of GAs

The effects on humans of eating potatoes with high GA concentrations have been well documented (Friedman, 2006). Symptoms can include cramping, diarrhea, vomiting, sweating, rapid pulse, and coma. The physiological effects of GAs are mainly a consequence of their disruption of cell membranes and inhibition of cholinesterase activity. Estimates have varied about the amount of GAs needed to be ingested to have toxic effects, with 1–5 mg/kg of body weight one suggested range, which is roughly equivalent to that of strychnine (Mensinga et al., 2005; Morris and Lee, 1984). Doses as low as 5–6 mg/kg body weight may be lethal (Morris and Lee, 1984).

Chaconine is more toxic than solanine and these two GAs become less toxic with progressive loss of sugars, with the aglycone being the least toxic (Friedman and McDonald, 1997). An important determinant of GA cholinesterase inhibitory activity seems to be the E and F rings of the aglycone (Roddick et al., 2001). In general solanidanes seem to be more toxic than spirosolanes. Friedman has suggested replacing solanidine and chaconine in potatoes with the less toxic tomatine (Friedman, 2002), which also has health-promoting properties. Such a goal could perhaps be accomplished by transgenic approaches or by identifying potato genotypes with low solanidine/chaconine and high tomatine.

14.8.4 Health-promoting effects of GAs

Health-promoting effects of GAs have been reported for several decades, such as inhibition of mice sarcoma tumors by a solamarine (Kupchan et al., 1965). The spirosolane, solasodine, may protect against skin cancer (Cham, 1994). Recent studies have convincingly shown that some GAs have anticancer properties. GAs including tomatine, solanine, and chaconine were shown to inhibit growth of human colon and liver cancer cells in cell culture assays (Friedman et al., 2005; Lee et al., 2004) with a potency similar to the anticancer drug adriamycin. Anticancer effects were also seen in assays using cervical, lymphoma, and stomach cancer cells and treatments using two or more GAs suggested both synergistic and additive effects (Friedman et al., 2005).

A key question that cannot be answered using cell culture assays is whether dietary GAs can have similar effects. Importantly, evidence that dietary tomatine is effective against cancer was shown in a feeding study using rainbow trout, in which reduced tumor incidence was found in tomatine-fed trout (Friedman et al., 2007). Tomatidine has potential as a chemosensitizing

agent, increasing the effectiveness of cancer chemotherapy by inhibiting multidrug resistance in human cancer cells (Lavie et al., 2001). Lung cancer is the most frequent cause of cancer-related death, in part because of its propensity to metastasize before the cancer is diagnosed. Using a human lung cancer cell line, α-chaconine was shown to reduce metastasis and it was suggested this may allow new chemotherapeutic approaches (Shih et al., 2007).

A separate study showed that solamargine, a glycoalkaloid found in some potatoes, increased the susceptibility of two different types of human lung cancer cell lines to several anticancer drugs (Liang et al., 2008). Beyond potential anticancer efficacy, GAs have been shown to boost the immune response. Mice fed GAs were more resistance to infection by *Salmonella* (Gubarev et al., 1998) and tomatine was demonstrated to potentiate the mice immune response to vaccines (Rajananthanan et al., 1999). GAs are reported to inactivate several types of herpes viruses (Chataing et al., 1997).

Much more medical information about the bioavailability, dietary relevance and both positive and negative effects on health of individual GAs must be obtained before it will be possible to develop potatoes with an optimal GA compliment.

14.9 Potato Minerals

A wide range of mineral elements occurs in fruits and vegetables, which are a primary dietary source. Minerals can generally be classified as major minerals such as calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), cobalt (Co), manganese (Mn), nitrogen (N), chlorine (Cl), and nutritionally essential minor and trace minerals such as iron (Fe), copper (Cu), selenium (Se), Nickel (Ni), lead (Pb), sulfur (S), boron (B), iodine (I), silicon (Si), bromine (Br). The importance of optimal mineral intake to maintain good health is widely recognized (Avioli, 1998).

Potatoes are an important source of different dietary minerals. Potato is listed as providing 18% of the RDA of potassium, 6% of iron, phosphorus and magnesium, and 2% calcium and zinc. Retention of most minerals is high in boiled potatoes cooked with skin (True et al., 1979). Baking a potato with the skin is a good cooking method to retain minerals.

There are significant differences in major and trace mineral contents amongst different genotypes of potato (Randhawa et al., 1984; True et al., 1978). Potassium levels varied the most and manganese the least. In a study of 74 Andean landraces, the iron content ranged from 29.87 to 157.96 μg/DW, the zinc content from 12.6 to 28.83 μg/g DW, and the calcium content from 271.09 to 1092.93 μg/g DW (Andre et al., 2007).

Many factors affect the mineral composition of potatoes, for example location, stage of development, soil type, soil pH, soil organic matter, fertilization, irrigation, and weather. Genotypic

variation is also important. Cationic mineral content in *Arabidopsis* is genetically controlled and candidate genes identified are cation transporters (Vreugdenhil, 2004). The same genotypes grown in different locations may have different mineral concentration due to environmental interactions (Burgos et al., 2007).

Potassium, phosphorus, calcium, and magnesium concentrations changed with irrigation and fertilization in physiologically mature tubers (Ilin et al., 2002). The total concentration of iron, calcium, and zinc increased with application of fertilizers whereas the content of phosphorus and molybdenum was reduced (Bibak et al., 1999; Frossard et al., 2000). The wide range of mineral content reported in potatoes may not only be due to genotype and environmental factors, but also sampling issues.

14.9.1 Potassium

In terms of mineral content, potato is best known as an important source of dietary potassium, which plays a fundamental role in acid–base regulation and fluid balance and is required for optimal functioning of the heart, kidneys, muscles, nerves, and digestive systems. Health benefits of sufficient potassium intake include reduced risk of hypokalemia, osteoporosis, high blood pressure, stroke, inflammatory bowel disease (IBD), kidney stones, and asthma. A high intake of potassium and low intake of sodium have been hypothesized to reduce the risk of stroke (Larsson et al., 2008; Swain et al., 2008). However, most American women 31–50 years old consume no more than half of the recommended amount of potassium and men's intake is only moderately higher (IOM, 2004).

Potatoes qualify for a health claim approved by the U.S. Food and Drug Administration, which states: 'Diets containing foods that are good source of potassium and that are low in sodium may reduce the risk of high blood pressure and stroke.' Potatoes rank highest for potassium content among 20 most frequently consumed raw vegetables and fruits (source: US Potato Board; DHHS FDA). Potassium varies from 3550–8234 μg/g FW (Casañas et al., 2002; Rivero et al., 2003; Sánchez-Castillo, 1998). One report listed potassium as low as 5.6 μg/g FW (True et al., 1978). Potassium content increases during the entire growing season (Lisinka and Leszczynski, 1989). On average one baked potato (156 g) contains 610 mg potassium (USDA/HHS, 2005). This is an even higher amount than in banana, a food often recommended by dieticians to people who need to supplement potassium consumption. The dietary reference intake of potassium for adult men and women is 3000–6000 mg per day. The US National Academy of Sciences recently increased the recommended intake for potassium from 3500 mg to at least 4700 mg per day.

14.9.2 Phosphorus

Besides potassium, phosphorus is the main mineral present in the tubers. It has many roles in the human body and is a key player for healthy cells, teeth, and bones. Inadequate phosphorus

intake results in abnormally low serum phosphate levels, which affect loss of appetite, anemia, muscle weakness, bone pain, rickets osteomalacia, susceptibility to infection, numbness and tingling of the extremities, and difficulty walking. In potatoes phosphorus ranges from ~1300–6000 μg/g DW (Lisinka and Leszczynski, 1989; Randhawa, 1984; Sánchez-Castillo, 1998). Daily requirements are 800–1000 mg.

14.9.3 Calcium

Potatoes are a significant source of calcium, with a wide range reported. Two studies reported calcium content up to 130 mg/100 g DW and 455 mg/kg FW (Lisinka and Leszczynski, 1989; Randhawa, 1984). Among 74 Andean landraces, calcium ranged from 271–1093 μg/g DW (Andre et al., 2007a). Wild *Solanum* species vary in the ability to accumulate tuber calcium (Bamberg, 1998). High levels of tuber calcium are associated with resistance to pathogens (McGuire, 1986) and abiotic stress (Tawfik, 1996). Calcium is important for bone and tooth structure, blood clotting, and nerve transmission. Deficiencies are associated with skeletal malformations and blood pressure abnormalities. The RDA for calcium is set at levels to reduce osteoporosis (Bachrach, 2001; Bryant et al., 1999) and varies depending on age and gender, but for young adults is 1300 mg.

14.9.4 Magnesium

Potato magnesium levels range from 142 to 359 μg/g FW (Casañas et al., 2002; Rivero et al., 2003). Magnesium is required for normal functioning of muscles, heart, and immune system. Magnesium also helps maintain normal blood sugar levels and blood pressure. The RDA for magnesium is 400–600 mg.

14.9.5 Manganese

The range of potato manganese content has been reported from 0.73–3.62 μg/g FW (Rivero et al., 2003) to 9–13 μg/g DW (Orphanos, 1980). Manganese has a role in blood sugar regulation, metabolism, and thyroid hormone function. Recommended daily intake in the USA is 2–10 mg.

14.9.6 Iron

Iron deficiency affects more than 1.7 billion people worldwide and has been called the most widespread health problem in the world by the World Health Organization. Due to severe iron deficiency, more than 60 000 women die in pregnancy and childbirth each year, and almost 500 million women of childbearing age suffer from anemia. Dietary iron requirements depend on numerous factors, for example, age, sex, and diet composition. Recommended daily intake in the USA varies dependent on gender and age. Potato is a modest source of iron. A study of cultivated varieties showed 0.3–2.3 mg of Fe in a 100 g tuber (True et al., 1978). Ranges of iron content from 6 to 158 μg/g of DW have been reported (Andre et al., 2007; Wills et al.,

1984). Some Andean potatoes have iron content comparable to levels found in some cereals (rice, maize, and wheat; Scurrah et al., 2007). Potato iron should be quite bioavailable because it has very low levels of phytic acid, unlike the cereals.

14.9.7 Zinc

Significant differences in zinc content occur in potatoes. The zinc content ranges from 1.8 to 10.2 μg/g FW (Andre et al., 2007; Randhawa et al., 1984; Rivero et al., 2003). Yellow-fleshed potatoes from different cultivars contain zinc in 0.5–4.6 μg/g FW (Dugo et al., 2004). Zinc is needed for the body's immune system to properly work and is involved in cell division, cell growth, and wound healing. The US RDA is 15–20 mg.

14.9.8 Copper

Copper in potatoes varies from 0.23 to 11.9 mg/kg FW (Randhawa et al., 1984; Rivero et al., 2003). Like zinc, copper is also high in yellow-fleshed potatoes (Dugo et al., 2004). Copper is needed for synthesis of hemoglobin, proper iron metabolism, and maintenance of blood vessels. The US RDA is 1.5–3.0 mg.

14.10 Potato phenolics

Potatoes are an important source of dietary phenolics. Phenolics are a diverse group of tens of thousands of different compounds, some of which are effective against diseases or have other health-promoting qualities including effects on longevity, mental acuity, cardiovascular disease, and eye health (Manach et al., 2004; Parr and Bolwell, 2000; Scalbert et al., 2005). Phenolics are the most abundant antioxidants in the diet. Plant phenolics may contain a treasure trove of potential health-promoting compounds. For example, many of the reports in the popular press about positive health effects of green tea, coffee, or wine are due to phenolic content. The role of phenolics in health is an area of active ongoing medical research that is only beginning to be understood. Upon consumption, phenolics are metabolized by digestive and hepatic enzymes, by the intestinal microflora and have a wide range of bioavailability not yet thoroughly defined (Manach et al., 2004). Conducting a Google search using phenolics and health as keywords returned over 700 000 links in 2005 and 1.6 million in 2008, reflecting the rising interest in these phytonutrients.

A study of 74 Andean potato landraces found about an 11-fold variation in total phenolics and a high correlation between phenolics and total antioxidant capacity (Andre et al., 2007a). We screened tubers from hundreds of cultivars and wild potato species for phenolics and found over a 15-fold difference in the amount of phenolics in different potato genotypes. Many phenolics are colorless, and thus are relevant phytonutrients for white-fleshed cultivars, which are the consumer-preferred type of potato in many countries. Russet Norkotah has high amounts among the white-fleshed cultivars, about 4 mg/g DW. *S. Pinnatisectum*, a purple-fleshed wild species,

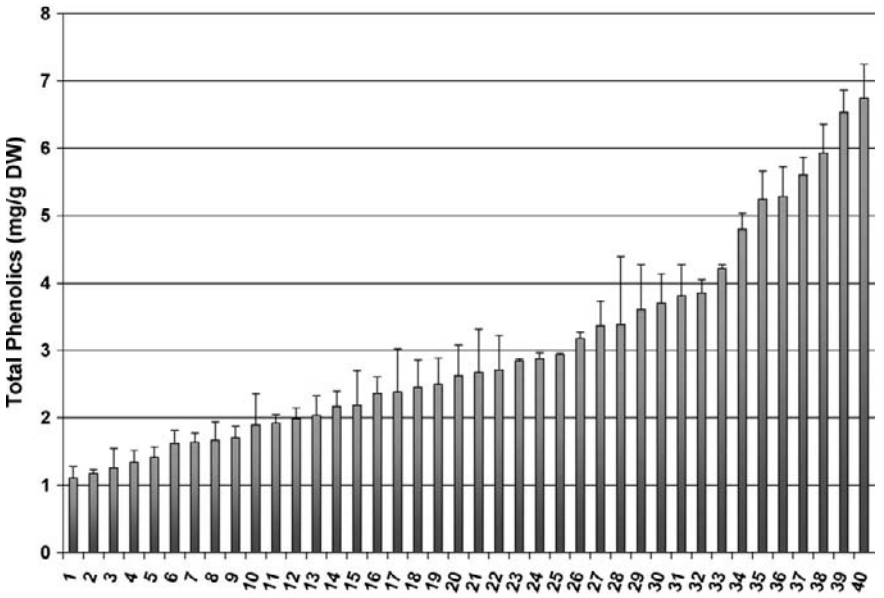


Figure 14.4: The wide range of total phenolics possible in potato tubers is evident in this analysis of 40 genotypes. LCMS analysis was used to obtain phenolic profiles from tuber extracts. Total phenolics of three independent replicates are shown with standard deviation.

has over 5 mg/g DW total phenolics. The potatoes with the highest total phenolics we have yet found are purple-fleshed lines, such as the two genotypes shown in Figure 14.4 with over 6.5 mg/g DW total phenolics.

If we compare high phenolic potatoes to some published reports of total phenolic amounts found in other plants, these potatoes have more phenolics than tomatoes, peas, onions, French beans, cucumbers, white cabbage, carrots, lettuce, or cucumbers (Figure 14.5). Furthermore, the amounts in these potatoes rival some reported phenolic amounts for broccoli, Brussels sprouts, and spinach. We have identified several potato genotypes that have over double the phenolic amounts of the potatoes listed in Figures 14.4 and 14.5. Thus, potatoes can be a substantial source of phenolics in the diet and compare very favorably to other vegetables. One study evaluated the contribution of 34 fruits and vegetables to phenolic intake in the American diet and concluded that potatoes were the third most important source after apples and oranges (Chun et al., 2005). The potatoes used in this study were an unspecified variety bought at a supermarket and almost certainly contained a small amount of phenolics relative to the high phenolic potatoes. The variation in phenolic content in potatoes is an excellent example of the potential to further increase its nutritional value by more fully utilizing existing germplasm.

Many potato nutrients differ in the amounts that accumulate in the skin versus the flesh. The majority of phenolic compounds are found in greater concentrations in the skin, but large

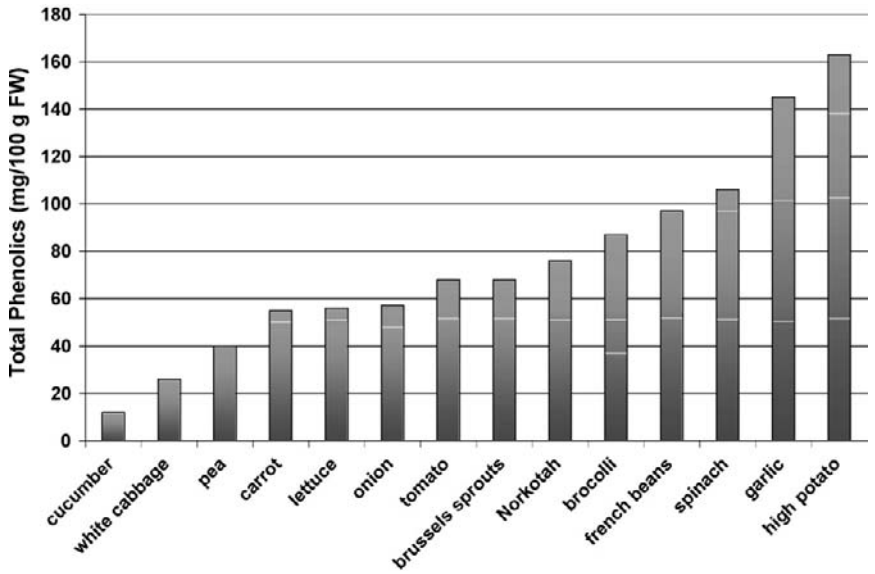


Figure 14.5: Total phenolic content of multiple vegetables is shown. Russet Norkotah and an advanced breeding line labeled 'high potato' are the two potato types used in this comparison.

quantities are also present in the flesh. Because a sizeable majority of the fresh weight of a mature potato is contributed by the flesh, overall the flesh will typically contain more phenolics than the skin on a per tuber basis. Potato skins are well known to be nutritious and consumers who realize this can choose recipes and products which include skins. Since potato skins are a rich source of phenolics (Nara et al., 2006), the phenolic content in the potato skins generated as waste during French fry processing might be easily recoverable and a potential 'value-added' product.

14.10.1 Chlorogenic acid

The most abundant phenolics in tubers are caffeoyl-esters. Typically chlorogenic acid (CGA; Figure 14.6) comprises over 90% of a tuber's total phenolics (Malmberg and Theander, 1985). Given the enormous contribution of CGA to the total phenolic content of potatoes, an interesting question is what changes in the tuber phenolic complement would occur if CGA formation was inhibited by antisense or RNAi methods. The biosynthetic pathway of chlorogenic acid in plants has been elucidated, creating new opportunities for engineering CGA biosynthesis in potatoes (Niggeweg et al., 2004).

There is evidence that chlorogenic acid has numerous health-promoting effects and CGA supplements are available in health stores. Dietary CGA is bioavailable in humans (Monteiro et al., 2007), is known to protect animals against degenerative, age-related diseases when added to

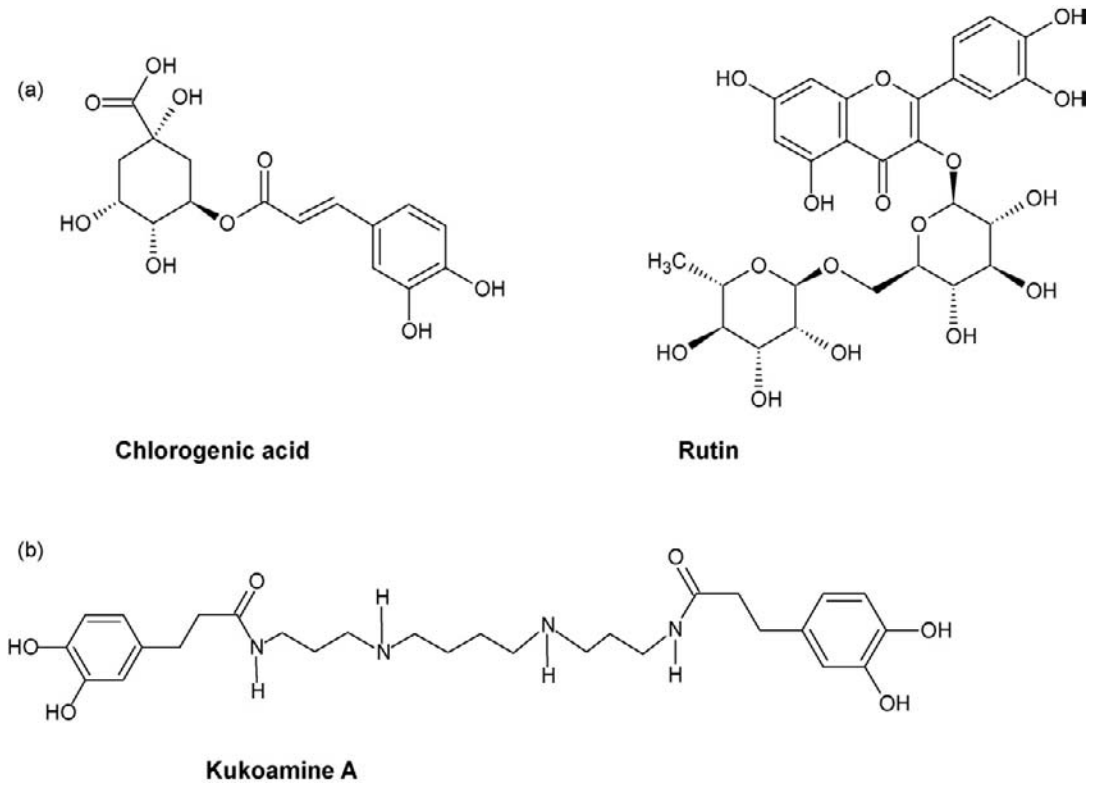


Figure 14.6: Structure of chlorogenic acid, rutin and kukoamine A.

their diet, and may reduce the risk of some cancers and heart disease (Nogueira and do Lago, 2007). CGA is also thought to be anti-hypertensive (Yamaguchi et al., 2007). CGA is reported to be anti-viral and anti-bacterial. Chlorogenic acid may decrease the risk of type 2 diabetes (Legrand and Scheen, 2007) and has been shown to slow the release of glucose into the blood-stream (Bassoli et al., 2008). This could be important towards lowering the glycemic index value of potatoes.

A concern about developing high phenolic potatoes is whether they would have unacceptable levels of browning or after cooking darkening, as suggested by the older literature. One more recent study showed that neither the amount of total phenolics, chlorogenic acid, or polyphenol oxidase correlated with the amount of browning observed in fresh-cut potatoes and that they were not rate-limiting in the development of browning (Cantos et al., 2002). Additionally, using a QTL approach, another group found no correlation between browning and chlorogenic acid (Werij et al., 2007).

14.11 Flavonols, anthocyanins, and kukoamines

Potatoes contain flavonols such as rutin (Figure 14.6), but have not been thought to be important sources of dietary flavonols, however little is known about qualitative and quantitative variation of flavonols in diverse germplasm. One group showed that flavonols increased in fresh-cut tubers, observing concentrations up to 14 mg/100 g FW and suggested that because of the large amount of potatoes consumed, they can be a valuable dietary source (Tudela et al., 2002a). In our screening of potato germplasm we have found over a 30-fold difference in flavonol content. Numerous studies suggest quercetin and related flavonols have multiple health-promoting effects, including reduced risk of heart disease, lowered risk of certain respiratory diseases, such as asthma, bronchitis, and emphysema, and reduced risk of some cancers including prostate and lung cancer.

Potatoes, particularly colored-fleshed cultivars, can contain substantial amounts of anthocyanins, compounds that can function as antioxidants and have other health-promoting effects. A gene encoding dihydroflavonol 4-reductase is required for production of pelargonidins in potato and other candidate genes have been identified (De Jong et al., 2003, 2004). A recent report found that an anthocyanin-enriched fraction from potatoes had anticancer properties (Reddivari et al., 2007). Lewis et al. (1998) screened 26 colored-fleshed cultivars for anthocyanin content and found up to 7 mg/g FW in the skin and 2 mg/g FW in the flesh. Another study evaluated 31 colored genotypes and found a range of 0.5 to 3 mg/g FW in the skin and up to 1 mg/g FW in the flesh (Jansen and Flamme, 2006). Brown et al. (2005) evaluated several genotypes for anthocyanins and found whole tubers that contained up to 4 mg/g FW and that anthocyanin concentration correlated with antioxidant value.

In June 2005, a British group reported the discovery of compounds called kukoamines in potatoes (Parr et al., 2005). These compounds are phenolic–polyamine conjugates (Figure 14.6) and had previously only been found in a Chinese medicinal plant, in which they were being studied because they lower blood pressure. It still needs to be established whether enough of these compounds survive cooking and are bioavailable enough to have any effect on humans. Nevertheless, the presence of these compounds is indicative of the complex chemical makeup of tubers. In our LCMS analysis of tubers, we have observed up to 30 putative polyamines in a single tuber. Roles for tuber polyamines include regulation of starch biosynthesis (Tanemura and Yoshino, 2006), calystegine synthesis (Stenzel et al., 2006), disease resistance (Matsuda et al., 2005), and sprouting (Kaur-Sawhney et al., 1982).

14.12 Carotenoids

Most of the compounds described to this point are hydrophilic. Potatoes also contain lipophilic compounds that are dietarily desirable, such as carotenoids. Carotenoids are synthesized in plastids from isoprenoids (Dellapenna and Pogson, 2006) and one role is coping with

photo- and oxidative stress. Over a 20-fold range in carotenoid concentrations has been reported in potato germplasm with much of the variation controlled at the transcriptional level (Morris et al., 2004).

Carotenoids have numerous health-promoting properties including provitamin A activity and decreased risk of several diseases (Fraser and Bramley, 2004). Because two of the most abundant potato carotenoids are lutein and zeaxanthin, potatoes may be particularly important for eye health and reduced risk of age-related macular degeneration (Chucair et al., 2007; Tan et al., 2008). The carotenoid complement varies by cultivar, but violaxanthin and lutein are usually the most abundant tuber carotenoids. The yellow/orange flesh color found in some potatoes is due to carotenoids. Orange coloration in potatoes is due to zeaxanthin (Brown et al., 1993) whereas the lutein concentration correlates well with the intensity of yellow coloration. White-fleshed potatoes usually contain less carotenoids than the yellow or orange cultivars. One study found white cultivars had 27–74 μg/100g FW of carotenoids (Iwanzik et al., 1983). Cultivated diploid potatoes derived from *S. stenotomum* and *S. phureja* were found to contain up to 2000 μg/100 g FW of zeaxanthin (Brown et al., 1993). A study of 74 Andean landraces found total carotenoids concentrations ranging from 3 to 36 μg/g DW (Andre et al., 2007a). A screen of 24 Andean cultivars identified genotypes with almost 18 μg/g DW each of lutein and zeaxanthin and just over 2 μg/g DW of β-carotene (Andre et al., 2007b).

Numerous groups recently have attempted to increase potato carotenoids using transgenic strategies. Overexpressing a bacterial phytoene synthase in tubers of the cultivar Desiree increased carotenoids from 5.6 to 35 μg/g DW and changed the ratios of individual carotenoids. β-carotene concentrations increased from trace amounts to 11 μg/g DW and lutein levels increased 19-fold (Ducreux et al., 2005). Carotenoids can be increased by approaches that do not directly involve use of carotenoid biosynthesis genes, as shown by overexpression of the cauliflower Or gene in Desiree resulting in a six-fold increase in tuber carotenoids to about 20–25 μg/g DW (Lu et al., 2006). A two-fold increase in carotenoids was observed in tubers overexpressing Or after 6 months of cold storage but no such increase was observed in wild-type or empty-vector transformed plants (Lopez et al., 2008). This is in contrast to what is seen with cultivars undergoing cold storage that undergo a decline in total carotenoids during storage (Griffiths et al., 2007; Morris et al., 2004).

An elegant approach using three bacterial genes overexpressed in Desiree achieved a 20-fold increase in total carotenoids to 114 μg/G DW and a 3600-fold increase in B-carotene to 47 μg/g DW (Diretto et al., 2007). A 250 g serving of these potatoes was estimated to provide 50% of the RDA of vitamin A. Potatoes engineered to have higher zeaxanthin levels were fed to human subjects and the zeaxanthin was found to be readily bioavailable (Bub et al., 2008).

14.13 Conclusion

Although this chapter is by no means a comprehensive listing of all health-promoting compounds present in tubers, it does give a good sense of the complexity of tuber chemistry and how the starch content of the tuber is only a small part of the story. The diverse milieu of tuber metabolites may be partly a reflection of the complexity of a tuber's primary task: to allow the plant to survive in varied environments.

Efforts to maximize the nutritional potential of potatoes are in their very early stages. Given the genetic diversity of wild-potato species available to be tapped into for nutritionally superior traits and the ever-increasing power of biotech approaches it is clear that future cultivars have the potential to be nutritional powerhouses.

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CHAPTER 15

Novel Applications and Non-Food Uses of Potato: Future Perspectives in Nanotechnology

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15.1 Introduction

Nanoscience is the fabrication, study, and modeling of principles of devices and structures for which at least one dimension is several 100 nanometers or smaller (Ladisch, 2004). The aim of nanotechnology is to understand and apply atomic- or molecular-level manipulation to design novel molecular goods with improved properties. The potential advantages of nanotechnology have been identified in many areas, such as in microelectronics, aerospace, and pharmaceutical industries. These achievements and discoveries have begun to impact many aspects of the food and agriculture systems such as food security, disease-treatment delivery methods, the protection of the environment; and the molecular synthesis of new food products and ingredients, etc. (Weiss et al., 2006). The development of novel coatings, barriers, release devices, and novel packaging materials are some of the areas where food nanotechnology can play its part. Novel barriers are being developed in the synthetic polymer field through the use of composite structures produced from successive molecular layers of different polymers; this approach can be applied to the food area (Morris, 2003). The development of bio-compatible surfaces for medical or pharmaceutical use may lead to novel surfaces or coatings that repel or combat bacterial adhesion and biofilm formation. There are new opportunities for colloid scientists in the design and production of nanocrystals and nanoparticles, which are finding new applications as non-viral gene vectors and as molecular delivery systems (Morris, 2003).

Polymers from renewable sources have received great attention over many years, predominantly due to the environmental concerns. Potato starch is a promising biopolymer for various food, pharmaceutical, and biomedical applications because of its higher water solubility that raises its degradability and speed of degradation; non-toxicity, easy availability, and abundancy. The role of starch for tissue engineering of bone, bone fixation, carrier for the controlled release

of drugs and hormones; and as hydrogels has already been documented (Chakraborty et al., 2005; Lenaerts et al., 1998; Mano and Reis, 2004; Pal et al., 2006; Pereira et al., 1998; Won et al., 1997). Starch-based biodegradable bone cements may provide for immediate structural support; and in addition, as they degrade from the site of application allow the in-growth of new bone for complete healing of bone fracture (Domb et al., 1996; Pereira et al., 1998). Starch nanoparticles, nanospheres, and nanogels have also been used as base materials for nanoscale construction of sensors, tissues, mechanical devices, and drug delivery systems (Chakraborty et al., 2005).

Biodegradable plastics are seen as one of many strategies to minimize the environmental impact of plastics and to develop sustainable plastics (McGlashan and Halley, 2003). The use of starch-based protective coatings and biodegradable packaging materials may extend the shelf life of food products along with solving global environmental problem (Sorrentino et al., 2007). However, because of poor barrier and mechanical properties of starches, their use in biodegradable food packaging has been limited. These limitations can be overcome by either blending of the starches with synthetic polymers or by chemical modification of the starches. Starch-based biodegradable plastics materials can be prepared by various methods: embedding starch in synthetic polymeric matrices such as polyethylene, polypropylene, polystyrene, poly vinyl chloride; blending with hydrophilic polymer such as poly vinyl alcohol (PVA); using the modified starch by substitution, copolymerization, oxidation, and hydrolysis; foaming of starch within the extruder; and preparing thermoplastic starch by melting under high pressure and temperature (Kim et al., 2003). Recently, the use of hybrid starch–nanoclay composites for the preparation of packaging films and other packaging materials has been reported (Park et al., 2002, 2003). The incorporation of clay nano-particles markedly increases the physical properties of the polymers such as shear resistance, heat resistance, hydrophobicity, and strength of the packaging materials. However, many features of starch-nano-particle systems are still to be explored.

In this chapter, we have discussed the potential role of potato and its starch in nanotechnology, with an emphasis on some of their novel applications in food and non-food areas.

15.2 Biodegradable packaging

Potato producers are currently aiming at three major areas of biodegradable polymers: food and non-food packaging; personal and health care items; and other disposables. The use of these biodegradable materials may slow down the emission of fossil-fuel-derived carbon dioxide into the air (Stearns et al., 1994). Potato-starch-based polymers have been used to make packaging for peanuts, candle cups for churches, and golf tees during the past decade and are currently exploited for food packaging and many other non-food uses. These products decompose in sewage treatment plants or in soil composts.

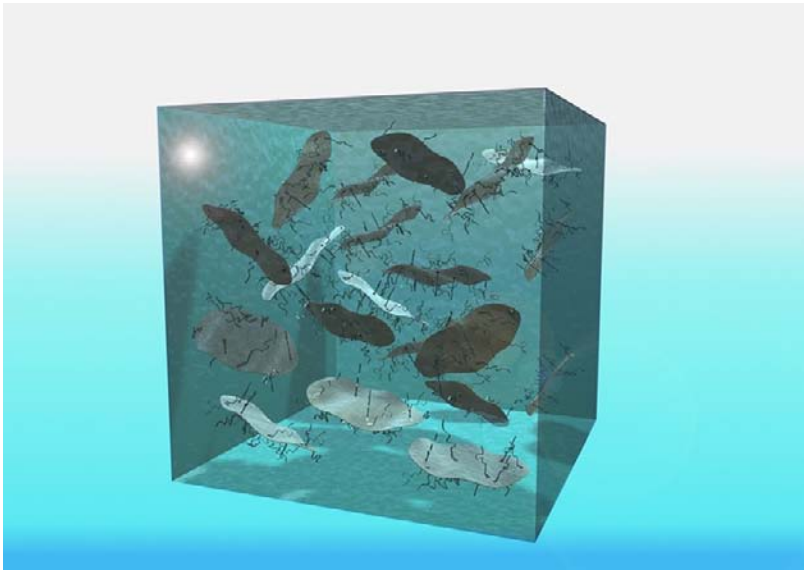

Figure 15.1: Schematic picture of a polymer–clay nanocomposite material with completely exfoliated (molecular dispersed) clay sheets within the polymer matrix material.
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Polymer–clay nanocomposites (PCN) are a class of hybrid materials composed of organic polymer matrices and organophilic clay fillers, introduced in late 1980s by the researchers of Toyota (Kawasumi, 2004). They observed an increase in mechanical and thermal properties of nylons with the addition of a small amount of nano-sized clays. This new and emerging class of polymers has found several applications in the food and non-food sectors, such as in construction, automobiles, aerospace, military, electronics, food packaging and coatings, because of its superior mechanical strength, heat and flame resistance and improved barrier properties (Ray et al., 2006).

The structure and properties of PCN can be controlled depending on their end use by manipulating the polymer–clay interactions during their manufacturing. The most commonly used nanoscale clay particles in the preparation of PCN are montmorillonite (MMT), hydrated alumina-silicate-layered clay with layer thickness in nanometer dimensions (Weiss et al., 2006). The layered silicates are of great interest because of their low cost, abundance, and high aspect ratio, which give greater possibility of energy transfer from one phase to another (Pandey et al., 2005). Solid layered dispersion in polymers involves two major steps: intercalation and exfoliation (Figure 15.1). In intercalation, the spacing between the clay layers increases as the extended polymer chains diffuse into the clay galleries, but the inorganic layers remain parallel to each other. On the other hand, in exfoliation, the silicate layers are completely separated from each other and dispersed in a continuous polymer matrix with no inter-clay particle interactions. The

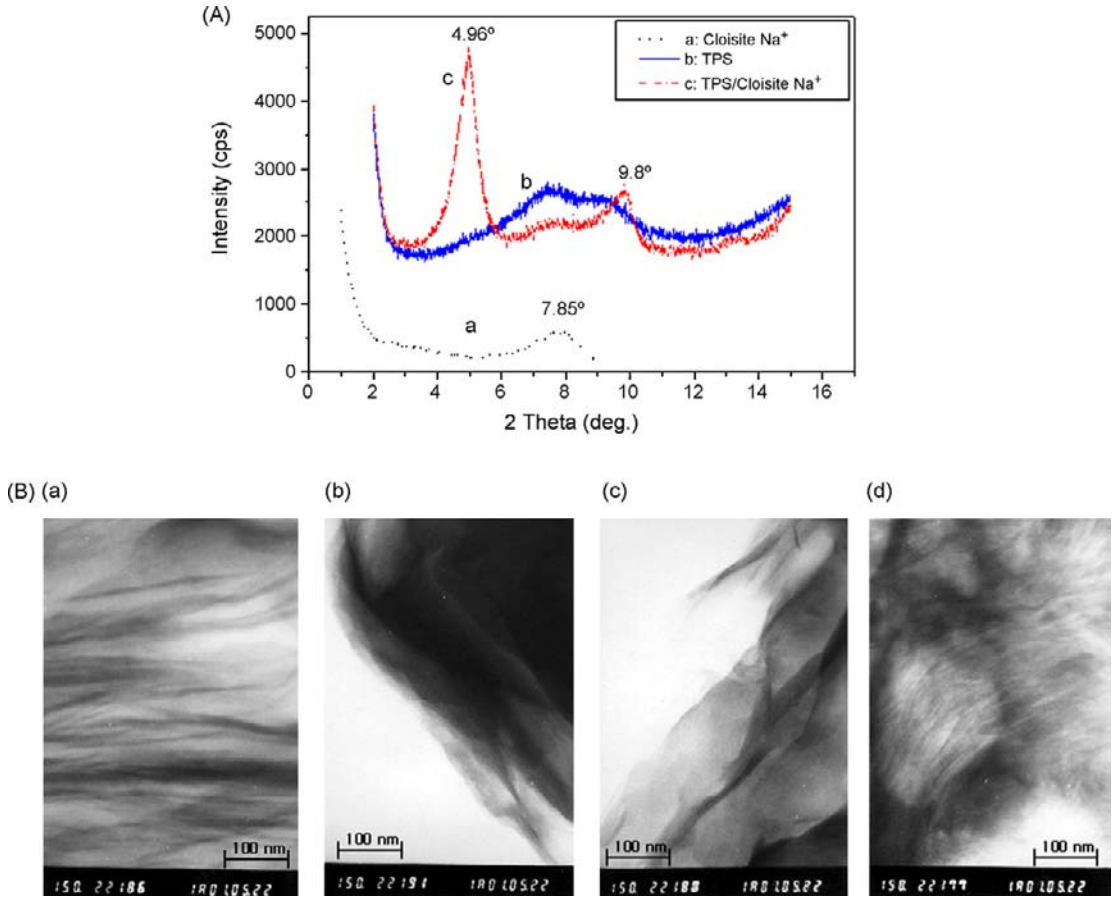

Figure 15.2: (A) XRD patterns of Cloisite Na^+ (a), Thermoplastic starch TPS (b), and the TPS/Cloisite Na^+ hybrid (c). (Source: Park et al., 2002). (B) TEM images of TPS/clay hybrids of different kinds of clay. (a) TPS95/Cloisite Na^+, (b) TPS95/Cloisite 6A, (c) TPS95/Cloisite 10A, (d) TPS95/Cloisite 30B. (Reproduced with kind permission from: Park et al., 2002, ©Wiley-VCH Verlag GmbH & Co. KGaA).

level of intercalation and exfoliation is generally confirmed through techniques such as X-ray diffraction and transmission electron microscopy (Figure 15.2A, B). The intercalation or exfoliation of a clay–polymer mixture depends on the characteristics of the polymer matrix and the organic modifiers. These characteristics include the nature of the polymer; and type, packing density, and size of the organic modifiers on the inorganic surface (Alexandre and Dubois, 2000; Pantoustier et al., 2001; Sorrentino et al., 2007).

The manufacturing process of PCN involves different methods: (1) Solution method: in which clay and polymer are dissolved in a polar organic solvent to avoid coiling of the polymer in the

intergallery space. After solvent evaporation, generally intercalated nanocomposites may result. (2) In situ method or interlamellar polymerization: in which nano-filler is swollen within the liquid monomer and after a complete dispersion in the monomer solvent, curing agent is added and this generally results in exfoliated nanocomposites. (3) Melt intercalation method: in which the layered inorganic is mixed with polymer in molten state; this may result in either exfoliated or intercalated nanocomposites. However this process is quite temperature sensitive as the temperature should not exceed the decomposition temperature of the clay modifier (Pandey et al., 2005). A new method for the preparation of nanocomposites has been reported recently, which involves a solid-state mixing at room temperature using milling tools (ball milling; Mangiacapra et al., 2005; Sorrentino et al., 2005). This method has the advantage of not requiring the use of high temperatures or solvent treatments (Sorrentino et al., 2007).

Biodegradable polymers have been referred to as polymeric materials in which at least one step in the degradation is through metabolism in the presence of naturally occurring organisms (Sorrentino et al., 2007). These can be classified into different categories according to their source: (1) Polymers, such as polysaccharides (starch, cellulose), proteins, polypeptides or polynucleotides, which are directly extracted from biomass. (2) Polymers, such as polylactic acid or bio-polyester, which are produced by chemical synthesis using renewable sources. (3) Polymers such as bacterial cellulose, xanthan, or pullan, which are produced by microorganisms (Sorrentino et al., 2007). Starch-based biodegradable materials come under the first category. Potato starch increases the biodegradability of a non-biodegradable plastic and also can be used together with a completely biodegradable synthetic plastic producing biodegradable plastic of low cost (Park et al., 2003). Starch-based absorbent pads may also be a potential alternative to conventional absorbent pads for meat exudation (Smith et al., 1995). And also it can be used to make packaging films for fruits and vegetables, snacks, and other dry products. However the major drawback of granular potato starch is its limited processability, due to the large granule sizes (5–100 μm). Therefore, it is difficult to make blown thin films for package applications, which require efficient mechanical and barrier properties. Chemical modification of starch or plasticization of starch may help in improving the mechanical strength of the final product.

Thermoplastic starch (TPS) is the most widely used bioplastic, accounting for 50% of the bioplastics market and is commonly derived from potatoes or corn (Robertson, 2006). TPS has been developed by gelatinizing starch with 6–10% moisture with heat and pressure (along with a plasticizer), which gives superior product properties (George et al., 1994; Usuki et al., 1993). Common plasticizers for the preparation of TPS are glycerol and other low-molecular-weight polyhydroxy compounds, polyethers, urea and water, which are added to reduce intra-molecular hydrogen bonds and to provide stability to product properties (Sorrentino et al., 2007). However TPS still needs blending with other polymers, such as poly (ethylene-co-vinyl alchohol) because of the low strength and poor water resistance of the final product (Park et al., 2003). The addition of clay, as potential filler, has also been reported to improve the properties of TPS in different

Figure 15.3: Starch–clay nanocomposite bag made by film blowing technology. This bag has been stored filled with water and did not show any release nor failure after 3 weeks. (Reprinted from: Fischer, 2003, with permission from Elsevier).

applications (Chen and Evans, 2005; De Carvalho et al., 2001; McGlashan and Halley, 2003; Park et al., 2002, 2003; Sorrentino et al., 2007; Wilhelm et al., 2003; Yoon and Deng, 2006; Figure 15.3). However, the extent of improvement in the mechanical and thermal properties depends on the type of the starch and nanoclay used along with the extent of dispersion of the filler in the polymer matrix (as discussed above). Park et al. (2003) investigated the effect of two different clays – one organically modified Cloisite 30B with ammonium cations located in the silicon gallery and one unmodified Cloisite Na^+ – on the tensile, mechanical, and thermal properties of potato TPS–clay nanocomposites using a melt intercalation method. It was found that TPS-Cloisite Na^+ nanocomposites had higher tensile strength, mechanical stability and better barrier properties to water vapor than TPS-Cloisite 30 B nanocomposites (Figures 15.4 and 15.5, Table 15.1). Also, the addition of a small amount of nanoclay (less than 5%) increased the decomposition temperature and decreased the relative water vapor diffusion coefficient of TPS.

Table 15.1: Tensile properties of TPS/clay hybrids of 5wt.% clay contents

Clay type	Tensile strength (MPa)	Elongation at break (%)
Cloisite Na^+	3.32	57.2
Cloisite 30B	2.80	44.5
Cloisite 10A	2.14	34.9
Cloisite 6A	2.51	38.0
Blank (0%)	2.61	47.0

(Source: Park et al., 2002)

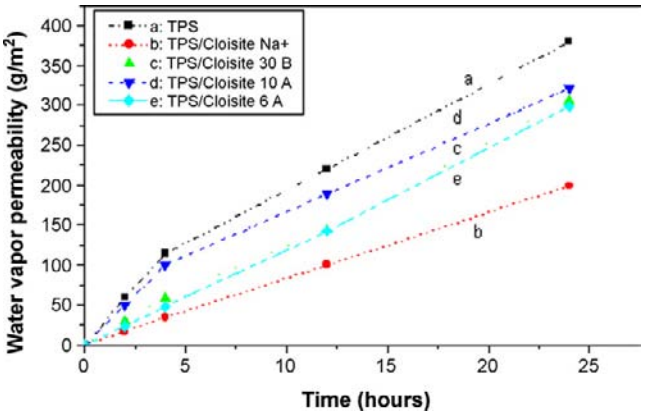

Figure 15.4: Water vapor permeability behavior of TPS/clay hybrids of different kinds of clays at 24°C. (Reproduced with permission from: Park et al., 2002, ©Wiley-VCH Verlag GmbH & Co. KGaA).

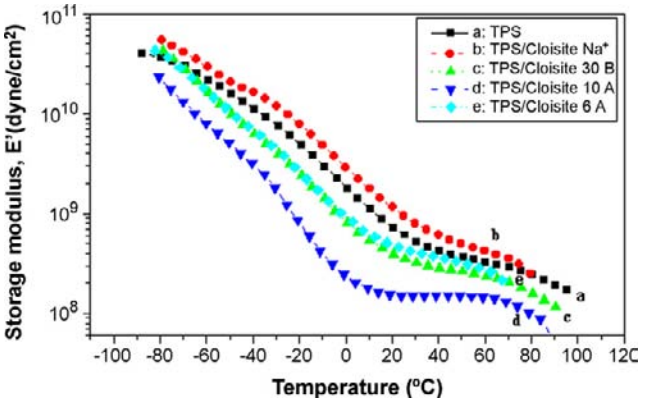

Figure 15.5: Typical storage modulus behavior of TPS/clay hybrids of different kinds of clays. (Reproduced with permission from: Park et al., 2002, ©Wiley-VCH Verlag GmbH & Co. KGaA).

Light weight and bulky nature of the conventional foam plastic packaging presents a major disposal problem for companies because of its high handling and transportation costs (Nabar et al., 2006). Novel TPS–clay nanocomposite foams with potentially enhanced mechanical and barrier properties have been prepared by melt-processing using urea as a plasticizer (Chen et al., 2005; Figure 15.6A). The use of urea as a plasticizer has been reported to avoid the cracking of TPS during storage and enhance the dispersion of ammonium-treated clay in TPS. Extruded starch foams with PVA have been prepared by some researchers, which perform similar to conventional packaging foams and can be safely disposed of in soil or in composting operations (Lacourse and Altieri, 1991; Roesser et al., 2000). Chemically modified starch, such as hydroxypropylated and acetylated starch, has also been used in the preparation of these foam

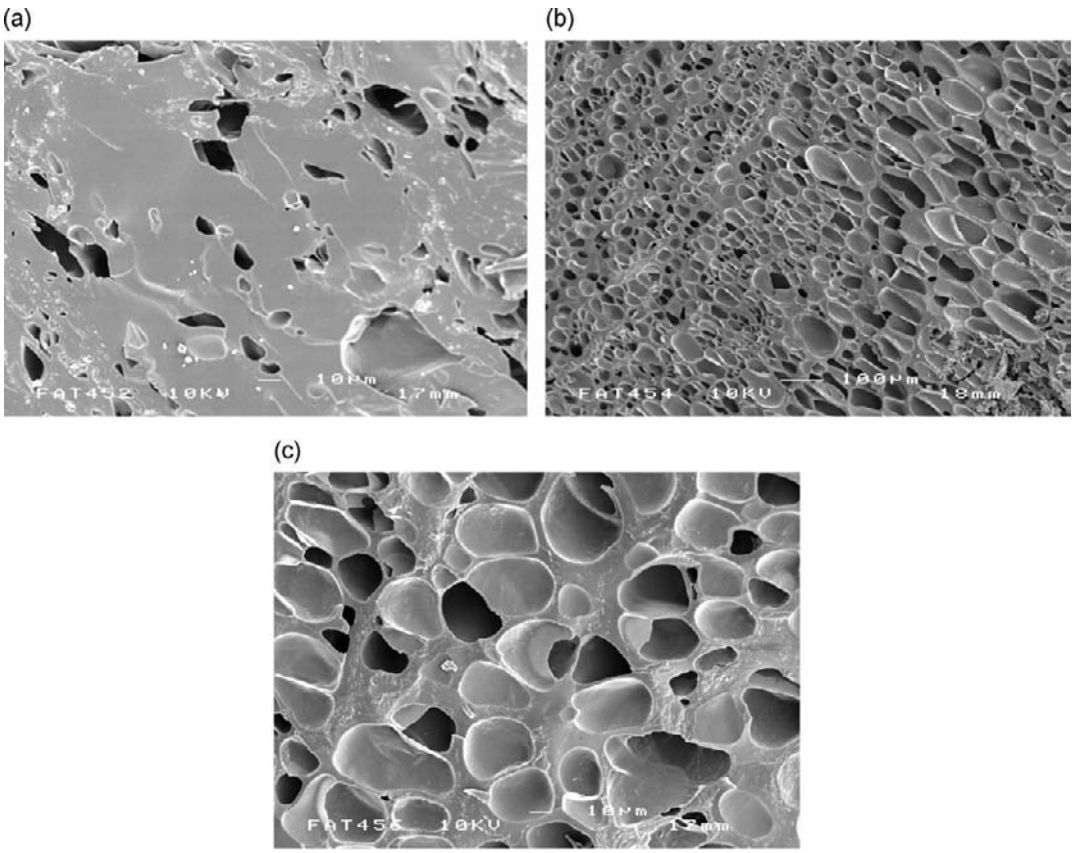

Figure 15.6A: Scanning electron micrographs (SEM) of fractured surfaces of (a) TPS-natural MMT nanocomposite containing 9.8 wt% clay, and (b) TPS-NH_4MMT nanocomposite containing 10.7 wt% clay, (c) is the enlarged image for (b) showing spontaneously formed regular foam structures with 84% porosity in TPS-ammonium-treated clay.
(Reprinted from: Chen et al., 2005, with permission from IOP Publishing Ltd.).

products for use in cushioning and insulation applications. Altieri and Tessler (1996) prepared water-resistant foams from blends of starch with starch esters. Polymers such as poly (caprolactone), cellulose acetate, poly (ethylene vinyl alcohol) and poly (ethylene-coacrylic acid) are some of the other polymers used to increase the hydrophobicity of starch-based foam packaging (Nabar et al., 2006). Addition of fiber (>30%) has been reported to increase the mechanical strength of starch-based foam trays (Yu et al., 2006). Recently, an application of potato starch as filler for a composite material with high-density polyethylene has been reported (Szymanowski et al., 2005). Starch surface properties were altered using methane radiofrequency glow discharge and then composite materials were prepared using modified potato starch with polyethylene. Polyethylene samples filled with modified starch presented an improvement of mechanical properties compared to those filled with unmodified starch.

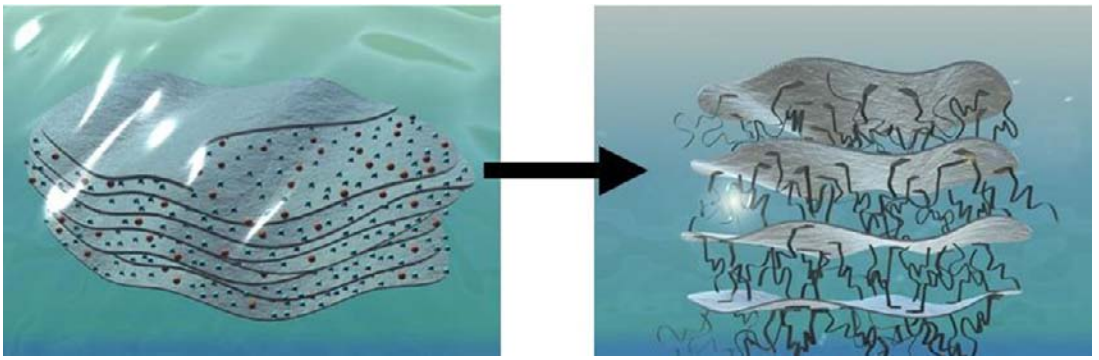

Figure 15.6B: Schematic picture of an ion-exchange reaction. The inorganic, relatively small (sodium) ions are exchanged against more voluminous organic onium cations. This ion-exchange reaction has two consequences: firstly, the gap between the single sheets is widened, enabling polymer chains to move in between them and secondly, the surface properties of each single sheet are changed from being hydrophilic to hydrophobic.
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Problems of applications of bioplastics arise not only from the (price) competition with the highly developed synthetic polymers but also from their insufficient property levels. Possibilities to decrease the hydrophility and to increase the values of mechanical properties so far are: (1) application of coating(s) of the processed bioplastic material with hydrophobic materials; (2) blending with different, hydrophobic, biodegradable synthetic polymers (polyesters) and (3) new ways of reactive extrusion of natural polymers (graft- and co-polymerization, esterification during extrusion process). A new possibility in this direction is seen in the creation of composite materials of thermoplastic organic biopolymer and nanoscopic inorganic particles incorporated on a molecular scale (Figure 15.6B, Fischer, 2003; Fischer et al., 2001).

Starch-based biodegradable plastics are the future of the global packaging industry, however, not enough attention has been provided to study the durability and degradability of these plastics. Degradation is a process in which deterioration occurs in polymer properties due to different factors such as light, thermal, mechanical, etc. And as a consequence of that, the shelf life of a material becomes limited, thus, the study of degradation and stabilization of these polymers should be carried out to better understand this phenomenon, which will ensure long life of the product (Pandey et al., 2005). Synthetic polymeric components used with starch remain in an undegraded state even after the starch is fully biodegraded. Also, when starch is blended with some polymeric materials, there is a problem of phase separation that has an undesirable effect on mechanical properties of the product (Kim et al., 2003). Re-inforcement of starch-based plastic films with short pulp fiber has been reported to improve mechanical properties (Kim et al., 2003; Tsiapouris and Dresden, 2000). One major advantage of using natural fibers to reinforce natural polymers is their compatibility (Yu et al., 2006).

15.3 Fiber-Reinforced Biodegradable Composites for Constructive Parts in Aerospace, Automobiles, and Other Areas

Fiber-reinforced biodegradable plastics are becoming increasingly popular for use in various applications because of their excellent mechanical properties, e.g. high strength and stiffness, light weight, and low cost. Different plant fibers studied for this purpose are jute, flax, ramie, oil palm fibers, and fibers made from regenerated cellulose. Natural fibers have excellent mechanical properties of breaking length, and elastic moduli characteristics such as E-glass and they enhance the mechanical strength of biodegradable polymers, such as potato starch (Riedel, 1999). These biocomposites are produced by embedding these fibers into a biopolymeric matrix made of derivatives of starch, lactic acid, cellulose, etc. (Yu et al., 2006). These have been formulated to meet the processing requirements for commonly used manufacturing techniques, such as hot press molding, extrusion cooking,and injection molding, etc. An essential requirement for a good fiber matrix adhesion system is optimized for impregnation of the reinforcing system (Riedel, 1999). Potato starch (usually TPS) reinforced by cellulose is a typical example of natural polymer composites. Starch needs to be modified physically or chemically to be suitable for the processing as thermoplastic resins. Another commonly used option is to add copolymers, which can even be of petrochemical origin (such as MATER-BI, Bastioli, 1998). However, esterification of potato starch by adding plasticizers or by acetylation can give the same effect.

Apart from anisotropic and specially tailored structural parts with continuous fiber reinforcements, biocomposites have been reported to be well-suited for paneling elements in cars, railways, and aeroplanes, using different kinds of non-wovens from single fibers (Riedel, 1999). Recently, a fiber-reinforced molded plastic part manufacturing process using cellulose propionate, thermoplastic potato starch, and flax fibers has been reported (Foelster et al., 2001). These molded plastic parts have excellent stability and strength and can be used as molded parts for trucks or passenger cars or rail vehicles or aircraft, particularly their body and/or their paneling. It has been suggested that biocomposites might be suitable for interior applications, such as furniture and packaging for electronics (Riedel and Nickel, 2003). However further research should be conducted to develop biocomposites with flame-retardant properties for use in some applications, such as interior cladding of railway carriages and aircraft bodies. Further applications of the starch-based biocomposites needs to be explored in automobile and railway design, in furniture industry, and in the field of leisure industry (Riedel, 1999).

15.4 Edible Films

Edible film and coating is defined as a thin, continuous layer of edible material used as a coating or as a film placed between food components to provide a barrier to mass transfer (Balasubramaniam et al., 1997; Guilbert et al., 1997). These films/coatings have the potential to replace conventional packaging in some applications. Starches such as potato starch, cellulose

derivatives, and plant gums are some of the materials used as edible coatings and films in food packaging and preservation (Bertuzzi et al., 2007). Use of these edible films could extend shelf life and improve quality and handling properties of food and pharmaceutical products (Talja et al., 2008). Edible films can be a possible means to incorporate substances, such as antimicrobial agents, flavors, and coloring agents to foods (Han, 2002). These can also be used for the controlled release of drugs (Tuovinen et al., 2003) or active components from foods and packaging materials (Talja et al., 2008).

An edible film should have good water vapor barrier properties (low or no water permeation and diffusion through film), which should not increase or increase very little with increasing relative vapor pressure (Lawton, 1996). Films should withstand mechanical stress and strain to such an extent that they do not break easily under a decent mechanical force (Talja et al., 2008). Thus, composition of starch-based films is an important factor influencing its barrier and mechanical properties. Also, starch-based edible films may have an impact on the sensory and textural characteristics of the food.

Edible coatings can be applied and formed directly on the food products, by the addition of a liquid film forming solution or by molten compounds, using a brush or by spraying, dipping, or fluidizing and are an integral part of the food product (Sorrentino et al., 2007). On the other hand, edible films are first formed using casting or extrusion processing and then applied to foods. Potato-starch-based films can provide an effective barrier to oxygen and carbon dioxide transport but they are a poor barrier to water vapor. The hydrophilic nature of starch is the major limitation for its use in edible films, which can be improved by the addition of lipids. The functional, organoleptic, nutritional, and mechanical properties of an edible film have been reported to be altered by the addition of plasticizers such as glycerol or surfactants or both in small amounts (Rodríguez et al., 2006).

Glycerol is a plasticizer compatible with starch, which is often used to modify mechanical characteristics of the film, such as film flexibility, facilitating its handling and preventing cracks. The presence of glycerol in the film slows down starch digestion, which is a feature of potential dietetic use (Hernández et al., 2008). Surfactants, such as Tween 20 improve wettability properties of film solutions by decreasing surface tension (Hiemenz and Rajagopalan, 1997; Rodríguez et al., 2006). The presence of a surfactant has been reported to enhance plasticity and increase water vapor permeability of the films with glycerol, which could be due to some interaction between glycerol and surfactants allowing more molecular mobility (Rodríguez et al., 2006).

15.5 Textiles and Paper

Potato starch is used in the textile industry for sizing cotton, worsted, and spun rayon warps (Stearns et al., 1994). In the sizing process, a film of the sizing agent is applied to the textile yarn

in order to bind the loose fibers tightly to the surface of the thread. This strengthens and protects the warp from abrasion during weaving. Potato starch films have been reported to have a higher degree of toughness and flexibility compared with other starches, which allows potato-starch-sized warps to be woven at lower humidity than those sized with corn starch (Stearns et al., 1994). Chemically modified (carboxymethylated) amylopectin potato starch has been reported to be used as a sizing agent for natural and/or synthetic textile yarns. This modified starch has very favorable sizing properties and imparts excellent weaving properties to the sized yarn and washing-out properties to the woven cloth (Huizenga et al., 1998). The amylose component of potato starch can be used to prepare zinc-oxide-soluble starch nanocomposites (Nano-ZnO) to impart antibacterial and UV-protection functions to cotton fabrics (Vigneshwaran et al., 2006). Cotton fabrics with Nano-ZnO impregnation show better protection against UV radiation in comparison with untreated cotton fabrics.

Native and chemically modified potato starch is the second most consumed starch in the paper industry, primarily for use as a furnish additive, only surpassed by corn starch. Starch is used in the paper industry during furnish preparation (as a flocculent, retention aid, drainage aid, and carrier for alkaline size); surface sizing (as an adhesive to enhance paper strength and stiffness); coating (as a binder for fine pigments to impart smoothness and gloss); effluent treatment (as a cationic polymer to control discharge of cellulose fiber and pigments) and during conversion of paperboard to packaging grades (as an adhesive in the manufacture of multiply board and as a glue for corrugation and laminating applications) (Maurer and Kearney, 1998).

Chemical modification of potato starch via grafting of vinyl monomers can be used to incorporate desirable properties into starch without sacrificing its biodegradable nature (Athawale and Lele, 2000). Potato starch-graft-polyacrylonitrile has been used as a super absorbent in diapers, e.g. sanitary napkins (Athawale and Lele, 1998; Fang et al., 2005). Fu et al. (2002) synthesized *p*-acryloazoanilide derivatives grafted onto potato starch copolymers, which may have potential utilization in heavy metal ion removal and paper or textile manufacturing industries. Graft copolymerization of potato starch with zwitterionic monomers may also hold great promise for the future as the resulting copolymers may be useful in many commercial applications concerned with textiles, dispersion agents, coatings, personal care formulation, and water remediation. Of particular interest is the possibility of viscosity maintenance or increase of such copolymers in the presence of electrolytes and surfactants due to shielding of coloumbic attractions (Zhang and Hu, 2002).

Wastewater and dying effluent generated by textile and other industries are generally discharged to the surrounding environment without any further treatment. These pollutants apart from adding color to water also cause toxicity to aquatic and other forms of life (Khan and Husain, 2007). Immobilized potato enzyme polyphenol oxidase (celite bound) has been reported to be

a cheaper option for decolorization of a number of reactive textile dyes, such as Reactive Blue 4 and Reactive Orange 86, present in polluted water.

15.6 Starch Spherulites and Nanocrystals

Spherulites are semi-crystalline entities with some degree of radial symmetry displaying a 'Maltese cross' extinction pattern when viewed between crossed polarizers (Ziegler et al., 2003). The 'Maltese cross' is oriented with its arms parallel to the polarizer and analyzer vibration directions. These small particles or spherulites are birefringent, which is generally defined as the difference between the radial and tangential refractive indices. In synthetic polymer melts, they form at a high degree of under-cooling (rapid quenches) in media of high viscosity and in the presence of some impurity that can be rejected at the growing crystal front (Ziegler et al., 2003). Starch's ability to bind volatile flavor compounds can be exploited in the food industry by using nano-size starch spherulites for this purpose, which can bind more flavors because of their very exceptionally small size (Conde-Petit et al., 2006). Potato starch is the cheapest available biopolymer, and therefore can be used as a raw material to produce spherulites (Ziegler et al., 2003). Starch spherulites may also find applications as encapsulants for controlled release of drugs. Starch spherulites can be produced using differential scanning calorimetry (DSC) or steam jet cooking (Figures 15.7 and 15.8). Steam jet cooking has been used commercially for decades to prepare aqueous starch solutions for industrial application. Dispersions for steam jet cooking are prepared by dispersing starch (and fatty acid) in water at temperatures sufficient to cause starch granule swelling. DSC is the most common technique used for detecting both first-order

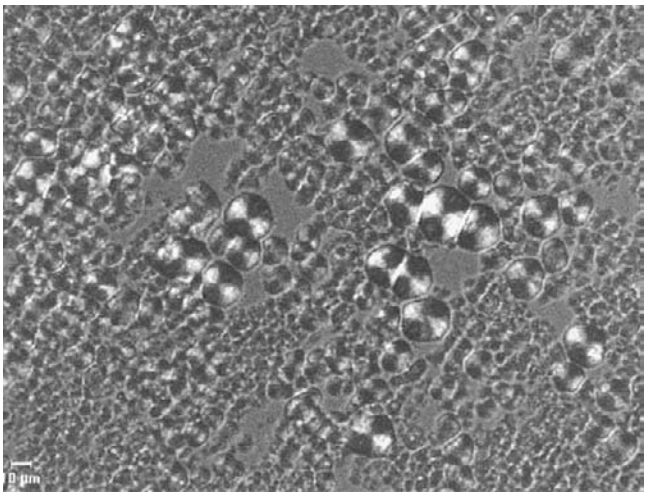

Figure 15.7: Morphology of aqueous potato starch dispersion (10%, w/w) heated to 180°C and quenched at 150°C/min to 10°C viewed between crossed polarizers. (Reprinted from: Ziegler et al., 2003, with permission from Elsevier).

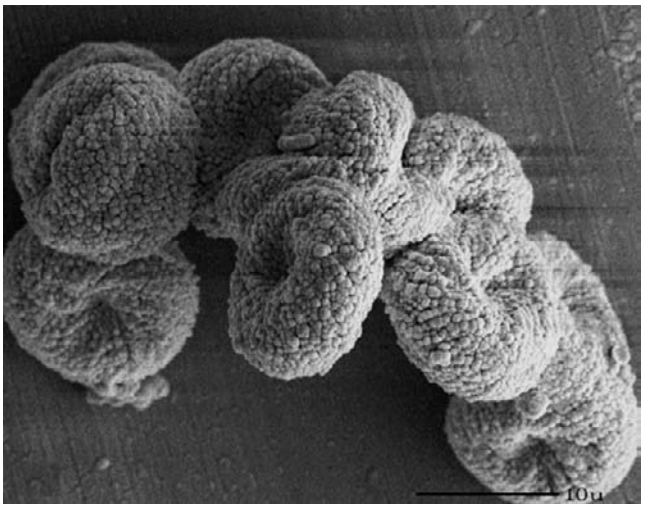

Figure 15.8: SEM image of spherulite aggregates of jet cooked high amylose starch dispersion slowly cooled without stirring. (Reprinted from: Fanta et al., 2008, with permission from Elsevier).

(melting) and second-order (glass) thermal transitions. Differential scanning calorimetry is an efficient tool for the study of starches and the production of spherulites due to its high sensitivity and ease of use. Furthermore, analyses are carried out using sealed pan, which prevents the loss of water from the suspension during heating. Native starch granules generally exhibit positive birefringence, since the refractive index is largest along the chain axis, which is oriented radially (Nordmark and Ziegler, 2002a,b). But for starch spherulites, studies (Nordmark and Ziegler, 2002a,b) revealed the presence of predominantly negative birefringence. Spherulites appear to consist of radially oriented 'fibers' that, if composed of chain-folded lamellae, double helices, or single helices with inclusion complexes with their main chain axis perpendicular to the lamella surface, would result in the negative birefringence.

The addition of different fatty acids in the starch may enhance the process of spherulite formation. The formation of spherulites from helical inclusion complexes of amylose with different lipids, fatty acids have been reported in the literature. Many studies on amylose–guest complexes carried out by electron microscopy and X-ray diffraction have demonstrated that these complexes form lamellar single crystals approximately 100 A° thick with the amylose chains perpendicular to the crystal surface in a chain-folded configuration (Shogren et al., 2006). Fanta et al. (2006) reported the formation of large spherical/lobed and the small torus-shaped spherulites using starch and different fatty acids. These spherulites consisted of layers of chain-folded amylose–lipid helices (i.e. crystalline lamellae) about 80 A° in thickness with the helices oriented radially. The morphology of spherulites has been reported to depend on the branching of lamellae and also to imperfect stacking of individual lamellae during the growth process

due to repulsive forces caused by portions of un-crystallized polymer chains that are not chain-folded and thus extend from lamella surfaces (Shogren et al., 2006). It was also suggested that spherulite morphology is influenced by the rate at which the spherulites are formed, which, in turn, would depend upon factors such as water solubility of the fatty acid, rate of cooling, and the amount of fatty acid used relative to starch (Fanta et al., 2008).

The formation of starch nanocrystals by acid hydrolysis of native starch has also been reported. By subjecting native starch to acid hydrolysis below its gelatinization temperature, the amorphous regions are hydrolyzed allowing the separation of crystalline residues (Angellier et al., 2004; Song et al., 2008). During the hydrolysis process the amorphous region of starch was hydrolyzed and removed, the nanocrystals around 5–7 nm thick with a length of 20–50 nm and a width of 15–30 nm was obtained. Nanocrystals have the shape of parallepiped nanoplatelets. Even if the parallepipedic shape is the general shape observed, many varying organizations can be distinguished. Few stacks of nanoplatelets oriented edge-on may be observed in a very little proportion. Such nanocrystals can be obtained from potato starch granules by acid hydrolysis, using H_2SO_4. It is used as a reinforcing phase in a polymeric matrix and displayed substantially improved mechanical properties. Starch disruption by acid hydrolysis depends on many factors such as the botanic origin, namely the crystalline type, the relative proportion of amylose and amylopectin, and the granule morphology. The conditions during acid hydrolysis such as acid type, acid concentration, temperature, and hydrolysis duration also have a significant effect (Angellier et al., 2004).

15.7 Miscellaneous Uses

Genetically modified (GM) potatoes have been introduced, which produce several times more fructose than the normal ones (Somasekhar, 2001). Fructose is generally produced by enzymatic conversion of corn starch through industrial processes. However scientists have applied gene fusion technology to convert starch (40–60%) stored in potato to fructose in the plant itself. Fructose is released upon heating and mashing of the potatoes. Potato was modified by inserting two genes coded enzymes, α-amylase and glucose isomerase. Apart from use in the food industry, fructose from these GM potatoes can also be used for low-cost ethanol production to fuel automobiles. Recently, Thanavala et al. (2005) conducted a double-blind placebo-controlled trial to evaluate the immunogenicity of hepatitis B surface antigen (HBsAg) expressed in transgenic potatoes and delivered orally to previously vaccinated individuals. They concluded that potato-tuber-derived orally delivered vaccine for prevention of hepatitis B virus should be considered as a viable component of a global immunization program.

Various research projects in Europe are trying to find a less-expensive manufacturing process for polyhydroxy alkanoates (PHA), which can be used for the production of biodegradable packaging materials, by identifying the individual genes, and gene sets, in the bacteria that are responsible for the production of PHA (e.g. 3HB, 3HV, and 4HV-PHA) and then expressing

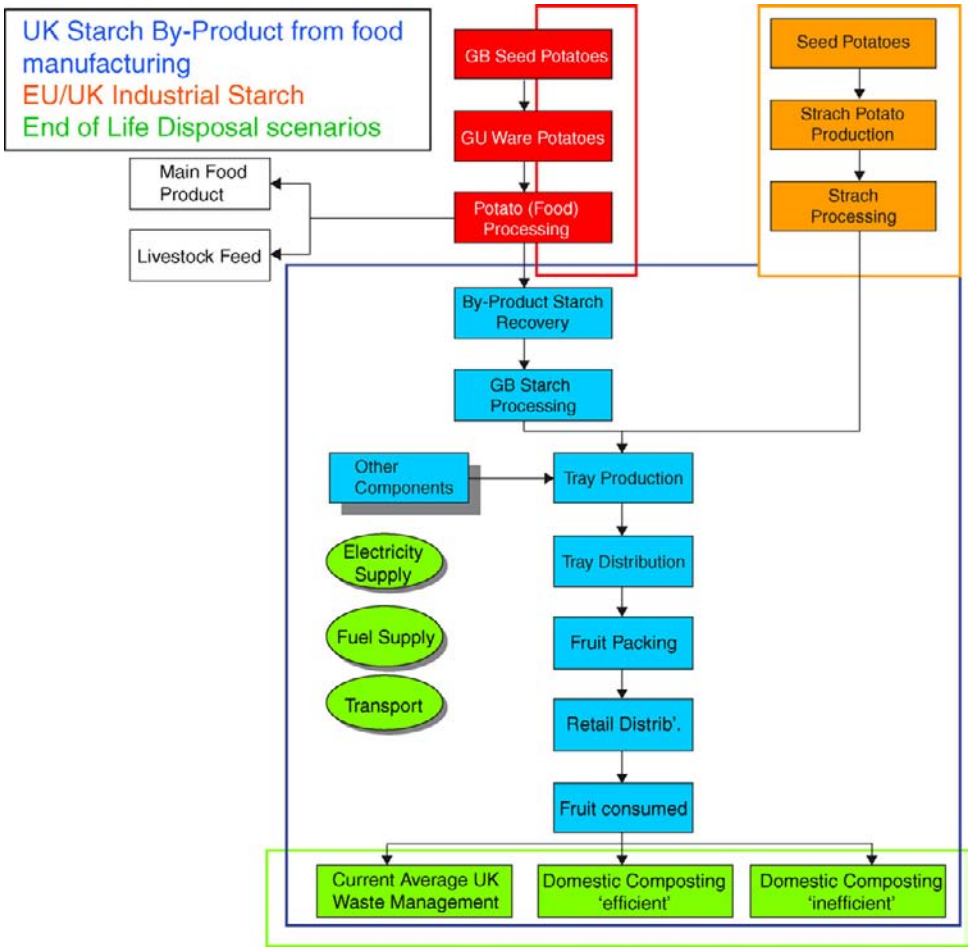

Figure 15.9: System boundaries of the Life Cycle Assessment of potato starch based packaging. (Reprinted from: Murphy et al., 2004, with permission from British Potato Council, UK).

them in plants like tobacco, rape, pea, and potato. This new manufacturing process has come to be known as molecular farming (Canadian Agri-Food Research Council, 2003).

Waste from the potato-processing industry has been reported to be turned into biodegradable packaging trays that offer major environmental advantages and give the same performance as those made from conventional plastics (Figure 15.9; Storey, 2003; Murphy et al., 2004). Around 17 000 tonnes a year of the waste has been estimated to be generated by the British potato product producers, which can be converted into biodegradable plastics to meet foreseeable packaging needs. The starch from processed waste material has been reported to perform even better than the pure potato starch and the packaging has been shown to be well-suited to single-use applications with fruit and vegetables that have a relatively short shelf life (Figure 15.10). Some researchers

Figure 15.10: Different potato starch based packaging products. (Reprinted with permission from: Storey, 2003; Murphy et al., 2004; Potatopak NZ Ltd.).

are converting potato waste to some high-value products, such as xanthan and polylactic acid, which is otherwise an expensive waste management challenge (Robertson, 2006). Potato starch is converted to glucose through enzymatic hydrolysis, which is a raw material for the production of lactic acid. Then the lactic acid is polymerized to produce polylactic acid (PLA). PLA is becoming increasingly popular in the production of a wide range of biodegradable materials (board, sheet, films, fiber, paint, etc.) because of low energy requirements during its production compared to other plastics of petroleum origin. Fiber-reinforced PLA composite materials have been used to interior components for automobiles (Kawamoto, 2007). However, the cost of production of these plastics is significantly high as compared to those of petroleum origin and it is essential to develop a technology that uses energy more efficiently and is cost-effective.

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CHAPTER 16

Novel Applications and Non-Food Uses of Potato: Potatoes in Biomedical/Pharmaceutical and Fermentation Applications

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16.1 Introduction

The increasing human population over recent decades has greatly influenced the demand for food products. Potato (*Solanum tuberosum* of the *Solanaceae* family) is a major world crop of which more than 300 million tons are produced worldwide annually. It is the world's most widely grown tuber crop and the fourth largest food crop in terms of fresh produce after rice, wheat, and maize (corn). It is the most important vegetable in European countries today. Potatoes are used for several purposes, including human consumption, industrial processing (potato starch, alcohol, etc.), and recultivation. Nutritionally, potatoes are best known for their carbohydrate content with starch being the predominant form of carbohydrate. A small but significant portion of the starch in potatoes called 'resistant starch' is considered to have similar physiological effects and health benefits of fiber (e.g., provide bulk, offer protection against colon cancer, improve glucose tolerance and insulin sensitivity, lower plasma cholesterol and triglyceride concentrations, increase satiety, and possibly even reduce fat storage) (Cummings et al., 1996; Hylla et al., 1998; Raban et al., 1994). Potatoes contain toxic glycoalkaloids, of which the most prevalent are solanine and chaconine (Feustel, 1987; Talburt, 1987).

Potato starch was first produced in Germany at the end of the 17th century. Industrial starch production includes steps such as cleaning and soil removal on rotating bar screens; opening cells by the high-speed raspers; separation of the starch granules and juice from cell walls (the pulp) on rotating conical screens; concentrating the crude starch milk (starch and juice) on hydrocyclones which is washed on multi-stage hydrocyclones in countercurrent with water; drying of dewatered

Table 16.1: Components of conventional wet potato pulp (Mayer, 1998)

Components	%(w/w) of wet pulp	% (w/w) of dry matter
Dry matter	13.0	–
Ashes	0.5	4.0
Starch	4.9	37.0
Cellulose	2.2	17.0
Hemicellulose	1.8	14.0
Pectin	2.2	17.0
Fiber (unidentified)	0.9	7.0
Protein/amino acids	0.5	4.0

cake. The starch yield in a modern process is close to 96% of the granular starch in the raw potatoes. Potato starch has been reported to have applications in biomedical/pharmaceutical industry as well as in fermentation for the production of different biomolecules.

During starch production from potatoes, a huge amount of a mass is also produced, which consists of fruit liquid (up to 90% of the mass), cell debris, intact starch cells, and cells or cell aggregates of the potato skin. The potato fruit liquid can be separated from the particulate fraction. It is characterized by a high content of proteins, free amino acids, and salts. This juice is rich in protein, which might be exploited for food, biotechnological, and pharmaceutical applications. It is primarily used for the enrichment of proteins and amino acids, and as a fertilizer because of its high nitrogen content.

The particulate fraction, called potato pulp, besides the fruit liquid and water in intact cells, contains starch, cellulose, hemicelluloses, pectin, and proteins. On a dry matter basis, the pulp contains 74% of protein in comparison to the content in potato tuber (Kempf, 1980). Components of conventional wet potato pulp are listed in Table 16.1.

In countries where a strong environmental regulation for industrial wastewater exists, purification of waste streams from potato factories regarding both the fruit water and the pulp is required. Several attempts have been made to dehydrate the by-products and to utilize them for different purposes. Its high moisture content (80%) requires an expensive drying due to the problem of spoilage, if left untreated. The starch industry tries to sell as much pulp as possible as wet or partially dried cattle feed. However, the need for potato pulp by farmers is limited. Potato pulp is being used as cattle feed as well as a solid-state fermentation media for the production of different biomolecules. Conventional applications of potato pulp are listed in Table 16.2.

Potato peels are waste by-products of the potato-processing industry. They are a good source of vitamin C, vitamin B_6, copper, potassium, manganese, and dietary fiber. They also contain a

Table 16.2: Conventional applications of potato pulp (Mayer, 1998)

Treatment/product	Application
Pulp supplemented by potato proteins or other nitrogen-containing components (wet or partially dried and pelleted)	Cattle feed
Preparation of pectin or pectin-starch mixtures	Nutritional and technical applications
Conversion into sugars and extraction of a syrup	Treatment of potato chips and pommes frites
Hydrolysis; for substrates used in fermentations	Alcohol production
Extraction of nitrogen-containing components from the liquid phase	Fertilizer
Dilution with water	Stabilizing factor in deep-drilling (lubricant)
Untreated, substrate for growth of yeast	Vitamin B_{12} production
Untreated; component of growth substrate	Biogas production

variety of phytonutrients which are a natural source of antioxidants that help to prevent cellular deterioration of the body. The phytonutrients found in potato skins as well as the flesh include polyphenols, carotenoids, flavonoids, and caffeic acid.

16.2 Biomedical Applications

Cells are often implanted into an artificial structure, typically called 'scaffolds,' that are capable of supporting three-dimensional tissue formation. These structures are often critical, both *ex vivo* as well as *in vivo*, to recapitulating the *in vivo* milieu and allowing cells to influence their own microenvironments. Scaffolds usually serve at least one of the following purposes: allow cell attachment and migration; deliver and retain cells and biochemical factors; enable diffusion of vital cell nutrients and expressed products; exert certain mechanical and biological influences to modify the behavior of the cell phase. Biodegradability is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal. The rate at which degradation occurs has to coincide as much as possible with the rate of tissue formation. Injectability is also important for clinical uses. Many different materials (natural and synthetic, biodegradable and permanent) have been investigated. New biomaterials have been engineered to have ideal properties and functional customization such as injectability, synthetic manufacture, biocompatibility, non-immunogenicity, transparency, nano-scale fibers, low concentration and resorption rates. A commonly used synthetic material is polylactic acid (PLA). This is a polyester which degrades within the human body to form lactic acid, a naturally occurring chemical which is easily removed from the body. Similar materials are polyglycolic acid (PGA) and polycaprolactone (PCL). Repair or regeneration of bone is one of the most challenging areas in the tissue-engineering field, due to the specific characteristics of the skeleton. A wide array of properties in materials is desirable for bone-tissue-engineering applications. One approach regarded as being very promising combines the use of adequate

scaffold materials and site-specific cells, in order to create a hybrid material that can enhance repair.

The use of starch-based materials can be justified by the well-known acidification phenomenon when using systems constituted by PLA. Using materials of natural origin has an advantage because starch can be degraded within the body by several enzymes (Gomes et al., 2001) resulting in degradation products that can be readily metabolized and excreted. Its natural origin, together with its mechanical properties (Gomes et al., 2002; Reis et al., 1996; Sousa et al., 2002) and biocompatibility (Marques et al., 2001; Mendes et al., 2001) support the potential in the biomedical field. Starch-based polymers are being studied for a wide range of bone-related therapy applications, including tissue engineering scaffolds (Reis and Cunha, 2001) and bone cements (Boesel et al., 2003; Elvira et al., 2002; Pereira et al., 1998).

Silva et al. (2004) studied synthesis and evaluation of novel bioactive composite starch/bioactive glass microparticles. The biodegradable character, good controlled-release properties, and natural origin of starch-based biomaterials were combined with the bioactive and bone-bonding properties of bioactive glass (BG). Novel, bioactive composite starch–BG microparticles were synthesized starting from a blend of starch and PLA (50/50 w/w) with BG 45S5 powder using a simple emulsion method. Their bioactive nature was confirmed by immersing them in a solution simulating body fluids, for periods up to 3 weeks. The short-term cytotoxicity of these materials was also tested by placing 24 h leachables of the materials extracted in culture medium in contact with a fibroblastic cell line (L929) up to 72 h. The results showed that these materials are not cytotoxic. Silva et al. (2005a) reported on the synthesis and the bioactivity of newly developed polymer-soluble potato starch and composite (with BG 45S5) micron-size particles. They found that both polymer and composite particles were able to form a calcium phosphate layer at their surface. They also found that both types of trials allow rat bone marrow cells to attach and to proliferate on their surface and to express osteogenic markers, such as alkaline phosphatase and osteopontin. The results obtained indicated that these carriers might be used as substrates for cell culture *in vitro*, in order to form constructs that might be used as a part of a tissue-engineering strategy.

Silva et al. (2006) studied starch-based microparticles as a novel strategy for tissue engineering applications. They developed starch-based microparticles, and evaluated them for bioactivity, cytotoxicity, ability to serve as substrates for cell adhesion, as well as their potential to be used as delivery systems either for anti-inflammatory agents or growth factors. Two starch-based materials were used for the development of starch-based particulate systems: (1) a blend of starch and polylactic acid (SPLA) (50:50 w/w) and (2) a chemically modified potato starch, Paselli II (Pa). Both materials enabled the synthesis of particulate systems, both polymer and composite (with BG 45S5). A simple solvent extraction method was employed for the synthesis of SPLA and SPLA/BG microparticles, while for Pa and Pa/BG

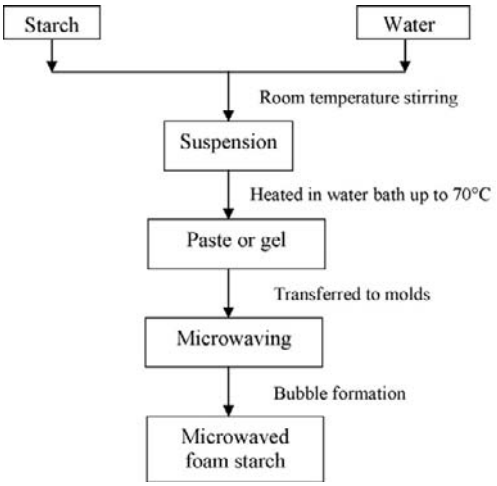

Figure 16.1: Manufacturing of microwaved starch scaffolds (Torres et al., 2006).

microparticles an emulsion cross-linking method using trisodium trimetaphosphate as a cross-linker was developed.

Torres et al. (2006) reported a novel microwave processing technique to produce biodegradable scaffolds for tissue engineering from different types of starch-based polymers. Potato, sweet potato, corn starch, and non-isolated amaranth and quinoa starch were used along with water and glycerol as plasticizers to produce porous structures. Figure 16.1 shows the manufacturing procedure of microwaved starch scaffolds.

Vigneshwaran et al. (2006) synthesized stable silver nanoparticles by using soluble starch as both the reducing and stabilizing agents. The use of environmentally benign and renewable materials like soluble starch offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications.

16.3 Pharmaceutical Applications

Synthetic and natural biodegradable polymers have been a major focus of interest in pharmaceutical research. They are used to control the drug release rate from parenteral controlled delivery systems (Asano et al., 1991). Furthermore, drugs encapsulated within injectable biodegradable micro- or nanospheres can be targeted directly to the site of action (Laakso et al., 1987). In addition, they have enormous potential in the delivery of peptides and proteins by protecting them from premature inactivation (Woo et al., 2001). Because of the considerable advantage of their clearance from the body after the release of therapeutic agents, biodegradable polymers are among the most widely used materials for controlled drug delivery applications. Various starches and derivative films have been widely studied due to their good molding and

film-forming properties, high oxygen barrier and good mechanical strength (Forssell et al., 2002; Gilleland et al., 2001; Lawton, 1996; Lee and Rhim 2000; Mali et al., 2002).

Native starches are used as disintegrants, diluents, and wet binders. However, their poor flow and high lubricant sensitivity make them less favorable in direct compression. Different chemical, mechanical, and physical modifications of native starches have been used to improve both their direct compression and controlled-release properties (Sanghvi, 1993; van Aerde and Remon, 1988). Schinzinger and Schmidt (2005) used potato starch as an excipient and compared its granulating behavior with α-lactose-monohydrate and di-calcium phosphate anhydrous in a laboratory fluidized bed granulator using statistical methods.

Yoshizawa and Koishi (1990) reported on the coating of an interactive powder mixture for sustained-release preparations. The interactive powder mixture consisted of drug particles coated on the surface of potato starch which were then encapsulated in magnesium stearate to have sustained-release preparation. The nanoparticles of indomethacin were deposited directly on the excipient powder, i.e. potato starch by fluidizing the powder by vibration (Nagare et al., 2004) and by pulsed laser deposition using rotation method (Nagare et al., 2007).

Korhonen et al. (2000) evaluated starch acetates and the effects of substitution on the starch acetates on the physical and tablet properties. The resulting starch acetates were compared to commercially available direct compression excipients. Tuovinen et al. (2003) compared the drug release rates from the native and acetylated starches. Their results showed that the acetylation of potato starch could substantially retard drug release, and that the release profiles could be controlled by the degree of substitution. Tuovinen et al. (2004) studied drug release from starch-acetate microparticles and films with and without incorporation of α-amylase. The study concluded that the release of model drugs of different molecular weight from the starch acetate microparticles and films was slow as compared to that from the native starch preparations. Figure 16.2 shows the preparation of starch acetate microparticles by water-in-oil-in-water double emulsion technique. Szepes et al. (2008) studied characterization and drug delivery behavior of starch-based (potato and maize starch) hydrogels prepared via isostatic ultra-high pressure. They found that the sample containing potato starch as a gel-forming polymer exhibited faster drug dissolution compared to an aqueous theophylline suspension, used as a reference, while the pressurization of maize starch resulted in a gel exhibiting sustained drug release.

Tarvainen et al. (2002) studied the film-forming ability of starch acetate (DS 2.8) and the effect of commonly used plasticizers on the physical properties of starch acetate films. The properties were compared with ethylcellulose films. Mechanical studies, water vapor and drug permeability tests, and thermal analysis by differential scanning calorimetry (DSC) were used to characterize the film-forming ability of starch acetate and efficiency of tested plasticizers. Starch acetate films were found to be tougher and stronger than ethylcellulose films at the same plasticizer concentration. Also, in most cases, the water vapor permeability of starch acetate

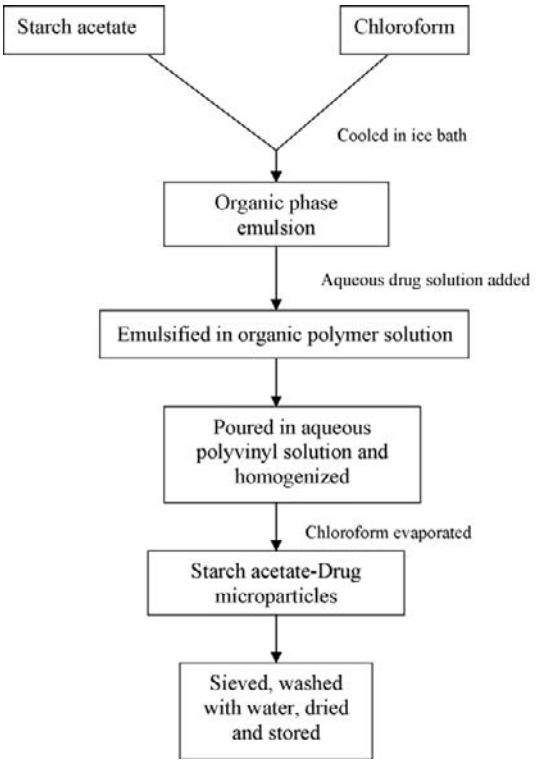

Figure 16.2: Preparation of starch acetate microparticles by water-in-oil-in-water double emulsion technique.

films was lower than that of ethylcellulose films. DSC thermograms supported the findings of the tensile test: plasticizers with several small ester groups (e.g., triacetin and triethyl citrate) were the most compatible with starch acetate. Due to good mechanical properties, low water vapor, and drug permeabilities of the films, starch acetate seems to be a promising film-former for pharmaceutical coatings. Tarvainen et al. (2004) evaluated film-formation properties of a novel, organic solvent-free aqueous dispersion of potato starch acetate (degree of substitution 2.8) and its ability to control drug release from a coated tablet. The starch acetate dispersion was found to be suitable for the fluid-bed coating process, forming strong films with complete coalescent polymeric spheres. These results clearly demonstrated starch acetate dispersion to have high utility as a novel aqueous coating material for controlled-release products.

Oral controlled drug-release systems are increasingly used for short half-life drugs to reduce peak blood levels and side-effects, to maintain optimum drug concentration and to stimulate patient compliance. In order to maintain a constant blood-level of the drug during an extended period, a constant *in vitro* drug release rate is desired. The most popular controlled-release system is the matrix tablet (Desai et al., 1965). Te Wierik et al. (1996) reported on

preparation and binding properties of high surface area potato starch products. Te Wierik et al. (1997a, 1997b) reported that retrograded modified starch is a suitable candidate as an excipient in controlled-release matrix tablets. Besides the ease of tablet manufacturing, it is able to sustain the release of many active ingredients with different physico-chemical properties. Acceleration or retardation to a desired profile can be achieved easily by changing tablet geometries, drug load, and incorporation of water-soluble excipients or weak acid or basic compounds or by a combination of two or more of these factors.

Grafting of polyacrylic acid into starch has been considered as an alternative procedure to produce non-irritant delivery systems in tablet form with good bioadhesion and controlled-release properties for buccal application. Geresh et al. (2004) studied grafted starch copolymers as platforms for peroral drug delivery. Grafting onto starch was performed with acrylic acid salts of mono- and divalent cations. The release kinetics of theophylline from graft copolymer tablets was studied as a function of variables such as radiation time, ratio of starch to acrylic acid, type of cation, the type of starch (corn, rice, or potato), and the pH of the dissolution medium. The suitability of the tablets for buccal application was assessed by determination of the extent of tablet erosion and swelling.

Stepto (1997) focused on the injection molding of potato starch including the basis of the process. In addition, the rheological behavior of starch/water melts during the refill part of the injection molding cycle was analyzed quantitatively to give apparent melt viscosities. Finally, the mechanical properties of molded starch materials and the drug-delivery behavior of starch capsules were also discussed.

Starch microparticles have been shown to be excellent for the controlled release of meclofenamic acid, an anti-inflammatory agent (Malafaya et al., 2001), and for the release of glucocorticoid agents such as dexamethasone (Silva et al., 2005b). When combining these properties with the advantageous bone-bonding properties of BG 45S5, there is a distinct potential for these particles to be used as controlled-release systems of either bone-acting drugs or growth factors. In principle, these systems would be able to bond to the bone and at the same time act as drug-release systems. In theory, the presence of a bone-bonding material (BG) would enhance small-defect bone repair whereas, simultaneously, the biodegradable material would act as a scaffold for cell growth, by releasing incorporated growth factors. This release would stimulate cell proliferation and differentiation, thus achieving a faster repair.

16.4 Fermentation Applications

Bioethanol production by yeasts is widely used for biodegradation of potato. However, yeasts cannot ferment starch directly, and a two-step enzymatic reaction to glucose is necessary. Different potato wastes such as industrial residues, low-grade potatoes, and spoiled potatoes can be used for acetone/ethanol production (Nimcevic et al., 1998). They used whole potato media

to study the solvent production by *Clostridium beijerinckii* NRRL B592 and showed that potato can serve as an excellent substrate for acetone/butanol fermentation. Their investigations were also directed towards the development of a continuous culture with online product removal, whereby productivity and yield could be significantly improved, effluent problems could be reduced, and complete utilization of the substrate could be done.

Global market for lactic acid and lactate (polymers excluded) production ranges at about 100 000 tons per year and shows an annual 15% growth rate (Akerberg and Zacchi, 2000). Lactic acid production is currently attracting a great deal of R&D interest since it is one of the most important organic acids, which has the potential of becoming a very large volume of commodity-chemical intermediate produced from renewable carbohydrates for use as feedstock for biodegradable polymers, oxygenated chemicals, plant growth regulators, environmentally friendly 'green' solvents and special chemical intermediates (Datta et al., 1995; Huang et al., 2005a). One of its most promising applications is its use for biodegradable and biocompatible PLA polymers, an environmentally friendly alternative to non-biodegradable plastics derived from petrochemicals. Jin et al. (2003) reported a new strain of *Rhizopus arrhizus* DAR 36017, for simultaneous saccharification and fermentation of starch waste effluents for lactic acid production. They found supplementation of nitrogen source to be unnecessary if potato or corn starch waste effluent was used as the production medium. Similar reports were obtained by Jin et al. (2005) quoting a single-stage simultaneous saccharification and fermentation process using potato, corn, wheat, and pineapple waste streams as production media. Direct fermentation of lactic acid production from starch waste water with respect to growth pH, temperature, and substrate was also reported by Huang et al. (2005b). The performance of direct fermentation was characterized by starch hydrolysis, accumulation of reducing sugar, production of lactic acid, and fungal biomass.

A practical technique for lactic acid fermentation of potato pulp has been developed (Oda et al., 2002). They screened 38 strains of the fungus *Rhizopus oryzae*; either lactic acid or fumaric acid and ethanol were formed, and the ratio differed among the strains tested. Saito et al. (2003) studied the effect of pectinolytic enzymes on lactic acid fermentation of potato pulp by different *Rhizopus oryzae* NRRL 395 and NBRC 4707 strains. When a commercial preparation of pectinase was added to potato pulp inoculated with fungal spores and incubated for 7 days, both strains effectively produced larger amounts of lactic acid and ethanol. These data indicated that the fermentation of potato pulp depends on the degradation of pectic substances in NRRL 395 and NBRC 4707. Saito et al. (2006) evaluated the potato pulp obtained in different seasons and found pectin content to be dependent on the dates of extraction.

Potato pulp and potato pulp residue (after acidic treatment) and nutrient salt solution and potato protein liquor, respectively, were used for the production of cellulases and hemicellulases by *Trichoderma reesei* Rut C30 in a continuously operated bioreactor, (Klingspohn and Schügerl

1993). Trojanowski et al. (1995) reported the utilization of potato pulp and potato liquor for the production of laccase by basidiomycetes.

Shukla and Kar (2006) studied potato peel as a solid-state substrate for thermostable α-amylase production by thermophilic *Bacillus* isolates. Under optimal conditions, *B. licheniformis* produced 270 units/ml and 175 units/ml of α-amylase on potato peel and wheat bran, respectively, while the corresponding values for *B. subtilis* were 600 units/ml and 265 units/ml. Mukherjee et al. (2008) used potato peel and *I. cylindrica* grass mixed in a ratio of 1:1 (w/w) for the maximum production of alkaline protease. Arotupin (2007) studied growth and production of polygalacturonase of *Aspergillus flavus* isolated from cropped soils on different raw and commercial carbon substrates. The ripe banana peel supported the maximum growth followed by orange bagasse, unripe plantain peel, and potato peel, whereas potato peel supported the highest polygalacturonase production followed by ripe banana peel, then orange bagasse, ripe plantain peel, unripe plantain peel, soluble starch, unripe banana, and cassava peel. Mabrouk and El Ahwany (2008) studied the production of β-mannanase by *Bacillus amylolequifaciens* 10A1 cultured on potato peels. They reported that potato peels at 14 g/l as carbon source and ammonium nitrate as a nitrogen source produced maximum enzyme activity (61.5 U/mg protein). The optimum incubation temperature and pH for enzyme production were 35°C and 7, respectively.

Biosurfactants (surface-active molecules produced by microorganisms) are commonly used in specialty markets such as food and textiles and have potential for improved oil recovery. Currently, biosurfactants are not widely utilized in the petroleum industry due to high production costs associated with the use of expensive substrates and inefficient product recovery methods. The economics of biosurfactant production could be significantly impacted through use of media optimization and application of inexpensive carbon substrates such as agricultural process residuals. Utilization of biosurfactants produced from agricultural residuals may result in an economic advantage for surfactant production and technology application, and convert a substantial agricultural waste stream to a value-added product. Bala et al. (2002) discussed on the production of surfactants from microbiological growth media based on simple sugars, chemically pure starch medium, simulated liquid and solid potato-process effluent media, a commercially prepared potato starch in mineral salts, and process effluent from a potato processor. Thompson et al. (2001) studied the effect of pretreatments such as heat, removal of starch particulates, and acid hydrolysis on surfactin production from potato process effluent by *Bacillus subtilis*. Thompson et al. (2000) tested high-solids and low-solids potato process effluents as substrates for surfactin production. They concluded that low-solids could potentially be used without sterilization for surfactin production for low-value applications such as environmental remediation or oil recovery. In continuation, Noah et al. (2002) described continuous surfactin production from low-solids potato process effluent by *Bacillus subtilis* in an airlift reactor.

Table 16.3: Fermentation products obtained by using potato starch as carbon source

Product	Microorganism	Reference
Thermostable pullulanase	*Clostridium thermosulfurogenes* SV2	(Rama Mohan Reddy et al., 1999)
Glucoamylase	*S. cerevisiae* strain C468	(Pavezzi et al., 2008)
Lactic acid	*Rhizopus arrhizus* WEBL 0501	(Zhang et al., 2007)
	Lactobacillus amylophilus GV6	(Vishnu et al., 2002)
Citric acid	*A. niger* GCB-47 (parental strain) and GCMC-7 (mutant strain	(Haq et al., 2003)
Cholesterol oxidase	*Streptomyces lavendulae* NCIM 2421	(Varma and Nene, 2003)
Thermostable β-amylase	*Clostridium thermosulfurogenes* SV2	(Rama Mohan Reddy et al., 2003)
Amylase inhibitor	*Streptomyces nigrifaciens* NTU-3314	(Su et al., 1984)
Ethanol	*Aspergillus niger* and *Saccharomyces cerevisiae*	(Jeon et al., 2007)

Table 16.3 compiles the use of potato starch as a carbon source for the production of different biomolecules such as enzymes and organic acids.

16.5 Other Applications

16.5.1 Animal feed

Silage, which is produced to preserve forage with high moisture content by controlled fermentation, is an important winter feed for cattle. Recent efforts towards an increased use of potato pulp were primarily directed to a broader application as animal feed (Lisinska and Leszczynski, 1989). Okine et al. (2005) studied the effect of addition of two bacterial inoculants as *Lactobacillus rhamnosus* and *Rhizopus oryzae* at ensiling on the fermentation quality, change in nutrient composition, and the nutritive value of potato pulp silage. They concluded that the potato pulp can ensile well with or without bacterial inoculants.

16.5.2 Technical applications

Only small fractions of pulp are used for technical applications such as for production of glue (Mayer, 1998). Mayer and Hillebrandt (1997) reported microbiological characterization, physical modification, and application of potato pulp. They identified autochthonic microbial flora (bacterial, fungi) and studied them with a view towards the degradative potential of the microorganisms, and ways of conserving the pulp for subsequent technical applications such as animal feed and production of glue.

16.5.3 Functional food

Tapé is a popular Indonesian delicacy with a sweet-acid taste and mild alcoholic flavor. It is prepared by fermenting glutinous rice or cassava tuber using *ragi tapé* and consumed as a sweet dessert or snack. Among the microorganisms present in *ragi tapé*, the fungus *Amylomyces*

rouxii plays a crucial role in *tapé* fermentation. *A. rouxii* degrades starch to glucose, which sustains its growth and that of some yeasts and bacteria, and simultaneously synthesizes both lactic acid and ethanol. Abe et al. (2004) reported that potato pulp fermented by *ragi tapé* may be consumed as other *tape* products. They converted potato pulp to palatable foodstuffs by microbial fermentation and compared microflora of *tapé* made with potato pulp to *tapé ketan*, the conventional *tape* made with glutinous rice.

In recent years, there has been an increasing interest in finding natural antioxidants, since they can protect the human body from free radicals and retard the progress of many chronic diseases. Potato peel, a waste by-product from potato processing, could be considered as a new source of natural antioxidant. Potato peel is found to contain phenolic acids (Lisinska and Leszczynski, 1987). Recently the antioxidant activity of potato peel extract has been studied in food systems (Rodriguez de Sotillo et al., 1994a, 1994b). Singh et al. (2008) reported potato peel extract to have the potential to offer protection against acute liver injury in rats because of its antioxidant propensity. Singh and Rajini (2008) investigated the ability of potato peel extract to protect erythrocytes against oxidative damage, *in vitro*. The protection rendered by extract in erythrocytes was studied in terms of resistance to oxidative damage, morphological alterations as well as membrane structural alterations.

Fats and oils undergo pronounced oxidative changes at elevated temperature during storage, thereby decreasing the nutritional value and the consumer acceptability. However, addition of some suitable antioxidant in fats and oils retards the oxidation process. Synthetic antioxidants, especially butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used to prevent the oxidation process (Sobedio et al., 1991). These synthetic antioxidants are known to have toxic and carcinogenic effects on humans (Ito et al., 1986). Zia-ur-Rehman et al. (2004) evaluated potato peel extract as a natural antioxidant during 60 days storage of refined soybean oil at 25 and 45°C. Free fatty acids, peroxide values, and iodine values were used as criteria to assess the antioxidant activity. They concluded potato peel extract to exhibit an antioxidant activity which was almost equal to BHA and BHT. Therefore, potato peel extract in oils, fats, and other food products can safely be used as natural antioxidant to suppress lipid oxidation.

Dietary fiber comes from the portion of plants that is not digested by enzymes in the intestinal tract. Different types of plants have varying amounts and kinds of fiber, including pectin, gum, mucilage, cellulose, hemicelluloses, and lignin. Insoluble fiber is helpful in the treatment and prevention of constipation, hemorrhoids, and diverticulosis. Water-soluble fiber binds bile acids, suggesting that a high-fiber diet may result in an increased excretion of cholesterol. Dietary fiber is known to reduce the risk of some cancers, especially colon cancer. High-fiber diets are also useful in a weight-loss regimen. Kaack et al. (2006) compared the functional properties of five fiber fractions by substitution of wheat flour using dry potato pulp, a commercial potato fiber,

two fibers prepared from potato pulp by enzymatic hydrolysis, and one solubilized fiber in bread making. The effect of chemical composition of fiber on texture, color, specific weight, and volume of wheat bread was studied. They concluded that the enzymatically solubilized 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, and delicious texture and flavor.

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CHAPTER 17

Potatoes for Human Life Support in Space

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17.1 Plants for Life Support in Space

Can this be serious – plants, no less the versatile potato, in space? When one thinks about the future of human kind, eventually we will travel on to other planets and establish long duration outposts. The 'when' and 'why' are largely political, but the 'how' is technological, and achievable. To do this will require innovative approaches for even the most fundamental requirements, such as meeting human life support requirements for breathable air, clean water, and food. Space missions to date have stowed life support provisions because the supply line has been relatively short. But as missions venture farther and stay longer, stowage and resupply become more costly and methods for regenerating life support consumables will be needed. There have been modest attempts to use regenerative physico-chemical systems on the Russian Mir and the International Space Stations, where, for example, oxygen (O_2) was produced using electrolysis and condensed humidity was collected and purified for reuse. These approaches can be expanded for future space missions, but such physico-chemical technologies become more massive and costly for long-duration missions and provide no means for producing food. An alternative would be to use biological technologies, such as plant photosynthesis to scrub the CO_2 from the air and generate O_2 (Galston, 1992; Myers, 1954). Photosynthesis produces the O_2 on Earth that humans now breathe and removes the CO_2 that we exhale, and it could be used in a similar fashion in space. In addition, if crops are used, the photosynthetic process could also produce food. Moreover, plant production systems in space could be used to assist in water purification, where, for example, gray water or pre-treated wastewater could be recycled to the plants; the plants would take up the water and generate humidity through transpiration, which could be condensed to provide clean water (Wolverton et al., 1983). Microbial communities associated with the plant root systems could breakdown the organic compounds in the wastewater and the plants could recycle the nutrients (Loader et al., 1999). Thus plants could provide four separate life support functions in one combined system: (1) removal of CO_2; (2) a source of O_2; (3) a source of food; and (4) a means for purifying and recycling wastewater (Figure 17.1). A key factor for driving this entire process is light, which could

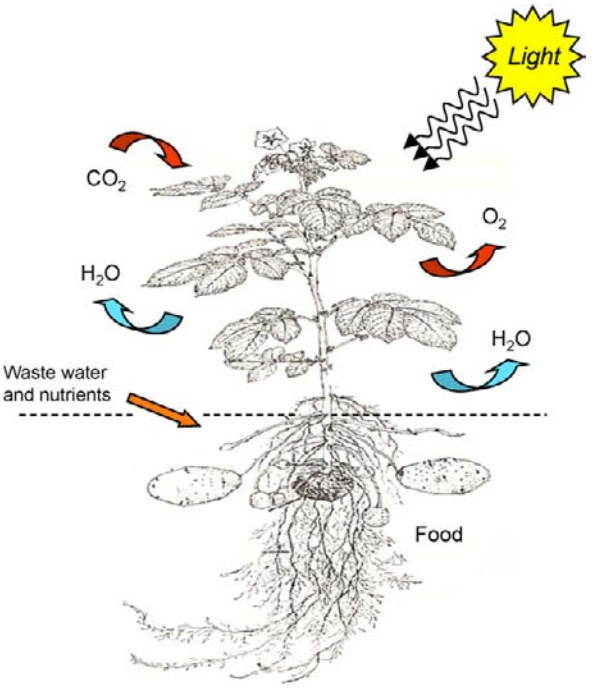

Figure 17.1: Plants as life support machines for space travel. Plant photosynthesis could provide oxygen and food, while removing carbon dioxide. Plant growing systems could also help waste water processing where the plant transpiration serves as a final distillation step after which the humidity is condensed as clean water.

be provided either from the Sun or with the use of electric lamps, and I will return to this later.

17.2 Why the Potato?

One of the first meetings to discuss crops for space travelers was the Biologistics Symposium held at Wright Patterson Air Force Base, US in 1962 (Boeing Comp., 1962). The recommendations focused largely on vegetables and perishable crops that could supplement the diet of stowed foods. Related conferences held about 15 years later generated more comprehensive lists to meet the broader needs of human diets (e.g., a more complete supply of carbohydrate, protein, and fat), and considered yield potential, harvest index (ratio of edible to total biomass), food processing, and horticultural requirements, such as planting, pollination, and harvesting (Hoff et al., 1982; Masuda et al., 2005; Mitchell et al., 1996; Salisbury and Clark, 1996; Tibbitts and Alford, 1982; Waters et al., 2002). Most of these lists contained a mix of staple crops that provide carbohydrate, protein, and fat, along with a balance of vegetables and small fruits. But it was recognized that meeting 100% of the dietary needs including all the micronutrients

Figure 17.2: Dr. Ted Tibbitts of the University of Wisconsin, Madison, WI, USA, working with potato plants in a growth chamber. Ted Tibbitts was the principal investigator for NASA-sponsored studies with potatoes from 1982 through 1994, and work from his laboratory has provided baseline information on controlled environment production techniques bioregenerative life support systems in space.

would require large plantings with numerous species, and that using some dietary supplements to supply micronutrients would be more cost effective for near-term missions.

A crop common to most of these lists was the potato, *Solanum tuberosum* L. Potatoes are highly productive, rich in digestible carbohydrate, a significant source of protein, and are easily propagated (Tibbitts and Alford, 1982). In addition, potatoes do not require extensive processing steps for consumption, as do crops like soybean and some grains, and when potatoes are strongly induced to tuberize, their harvest index can exceed 80%, which is nearly double that of grain crops (Wheeler and Tibbitts, 1987). This high harvest index increases the intrinsic yield potential per unit area and reduces the amount of inedible biomass for recycling. Reducing the amount of inedible biomass in turn minimizes the amount of O_2 required (and CO_2 produced) during waste processing in closed systems (Wheeler, 2003).

Despite these virtues, experience with potatoes was largely limited to field settings with little information available on controlled environment production. With this in mind, the US National Aeronautics and Space Administration (NASA) initiated a series of grants (1982–1994) to Ted Tibbitts (Figure 17.2) at the University Wisconsin, US to study potato growth and development in environmental chambers at the University of Wisconsin Biotron. Following this, additional growth chamber testing and larger-scale production studies with potatoes and other crops were initiated at NASA's Kennedy Space Center beginning ca. 1988 (Wheeler et al., 2001). The following reviews some findings from this NASA-sponsored research with potatoes and is largely excerpted from Wheeler (2006) and Wheeler et al. (2008a).

17.3 Cultivars for Space

Obviously plants cannot withstand the harsh vacuum, extreme temperatures, and high UV radiation of space; hence controlled environments will be required for plant production, perhaps not unlike growth chambers we use on Earth. Initial NASA testing focused on identifying lines that would perform well in controlled environments. A comparison of four North America cultivars, Norland (early season), Superior (early season), Norchip (mid season), and Kennebec (late season), showed the greatest tuber yields from Norland under 12, 16, and 20 h of light, while Kennebec showed the lowest yields under the longer photoperiods (Wheeler and Tibbitts, 1986a) (Figures 17.3 and 17.4). Subsequent testing with these cultivars along with Russet Burbank (late season) and Denali (late season) showed that cvs. Norland and Denali produced tubers consistently under many different environments (Wheeler and Tibbitts, 1986b; Yandell et al., 1988; Wheeler et al., 1991). Additional tests with 23 cultivars for tolerance to continuous light were conducted later and several cultivars from Alaska, Norway and Netherlands performed well (Tibbitts et al., 1994). Total glycoalkaloids (TGA) were measured in tubers from some of these studies, and TGA levels in Denali > Russet Burbank > Norland, but all were within acceptable limits for human consumption (Nitithamyon et al., 1999). To maintain consistency with prior studies, University of Wisconsin and Kennedy Space Center continued to use cvs. Norland and Denali, but further testing with different cultivars and/or targeted development of genetically engineered cultivars will be needed to maximize yields for space life support systems.

Figure 17.3: Potato tubers harvested from (left to right) cvs. Norchip, Kennebec, Norland, and Superior after 105 days growth at 20°C and a 12-h photoperiod with 400 μmol m^{-2} s^{-1} of PAR. A meter stick was place in the photo for reference.

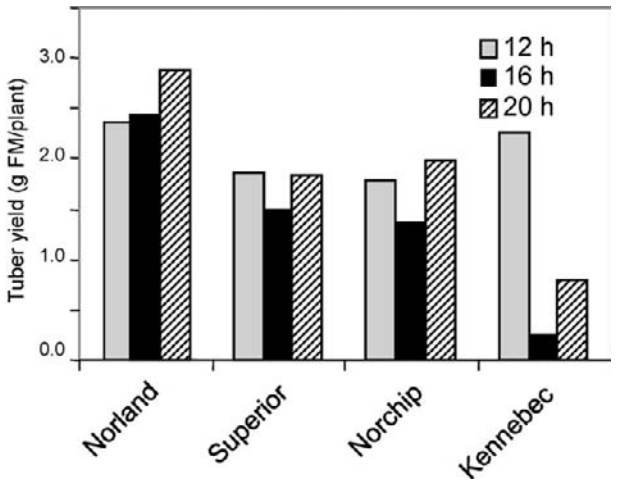

Figure 17.4: Tuber yields from four North American cultivars grown under 12, 16, and 20 h of 400 μmol m^{-2} s^{-1} PAR. Kennebec, a late-season cultivar did not produce well under the longer photoperiods, while Norland, and early season cultivar produced well under all the photoperiods (Wheeler and Tibbitts, 1986a).

17.4 Horticultural Considerations

17.4.1 Cultivation techniques

Horticultural strategies for growing crops in space must consider the effects of reduced gravity (Wheeler et al., 2001). In low-Earth orbit, such as on the Space Shuttle or the International Space Station, or during transit missions to the Moon and Mars, one must deal with weightlessness (μ-gravity). On the surface of the Moon and Mars, approximately 1/6 and 3/8 gravity exist (Salisbury, 1991). All of these reduced gravities will affect watering strategies for plants, but especially in weightlessness where water can only move by capillary forces (Wright et al., 1988; Porterfield, 2002). On the Moon and Mars, water should drain through solid media, although it will be slower than under 1 g, but recirculating hydroponics systems should be adequate for plant cultivation (Bugbee and Salisbury, 1989). It is interesting to note that a NASA-sponsored workshop in 1987 focused entirely on issues facing lunar base agriculture and the potential for using lunar regolith as a growing medium for plants (Ming and Henninger, 1989).

Most studies at the University of Wisconsin used pots containing peat–vermiculite (50:50 vol.) medium and drip irrigation with a complete nutrient solution with 7.5 mM nitrate (Figure 17.5; Wheeler and Tibbitts, 1986a; Tibbitts et al., 1994). Other studies used arcillite (turface), which consists of calcined, clay particles (McCown and Kass, 1977; Tibbitts et al., 1994). Growth in arcillite was not as good as on peat-vermiculite, but the arcillite had excellent drainage and could be washed cleanly from the roots allowing easy reuse, and it has since been used in several

Figure 17.5: A typical experiment with potatoes conducted at the University of Wisconsin Biotron in the 1980s and 1990s. Plants were propagated with in vitro grown nodal cuttings and planted into a peat–vermiculite (50:50) and watered to excess with a nutrient solution four times daily. The watering tubes for the pots are shown hanging from an overhead delivery line (Wheeler and Tibbitts, 1987).

space flight experiments with plants (Croxdale et al., 1997; Levinskikh et al., 2000; Morrow et al., 1995; Stutte et al., 2005). Some comparisons of pot sizes were also conducted and in general, for long-duration studies (ca. 100 days or more), larger pots sustained better growth (Tibbitts et al., 1994).

Exploratory tests were conducted at Wisconsin with hydroponic and aeroponic cultures, where roots and stolons were either submerged in a recirculating nutrient solution or suspended in dark chambers and continuously sprayed with nutrient solutions (Tibbitts et al., 1994). Hydroponic techniques had been used previously for potato research (Chapman, 1958; Fong and Ulrich, 1969; Krauss, 1978; Sattelmacher and Marschner, 1978) and seemed to hold a lot of potential. Unfortunately, the plants grown in the standing solution cultures, under continuous misting, or even in wet arcillite with continuous flowing nutrient solution showed good shoot growth but tuberization was inconsistent and stolons and tubers often developed callus and became pigmented (Figure 17.6) (Fong and Ulrich, 1969; Tibbitts et al., 1994). Later testing with nutrient film technique–NFT (Resh, 1989) at Kennedy Space Center proved more successful: the stolons and tubers were not submerged and the solution could be recirculated allowing control of pH and electrical conductivity (Wheeler et al., 1990, 1997) (Figure 17.7). The combined results with hydroponic testing suggested that stolons and tubers require good aeration (gas exchange), which is consistent with observations poor yields from wet or poorly drained fields (Smith, 1977) and recent advances in the use of hydroponic techniques with potato (Muro et al., 1997; Ritter et al., 2001).

Figure 17.6: Callus development around the lenticels of a tuber (cv. Norland) grown in media that was too wet. Similar responses were noted with submerged tubers grown in solution cultures or aeroponic cultures with continuous misting (source: Tibbitts et al., 1994).

Figure 17.7: Tubers from cv Norland plants grown in nutrient film technique (NFT) for 105 days at NASA's Kennedy Space Center, FL, USA (source: Wheeler et al., 1990). Nutrient solution was pumped continuously to the back end of the trays and flowed to a drain at the front for return to the reservoir. NFT proved more successful for producing good tuber yields and allowed easy harvesting.

For recirculating NFT studies, the electrical conductivity (EC) was maintained near 0.12 S m^{-1} with daily additions of a stock solution, and pH was maintained automatically near 5.8 with additions of dilute (0.4 M) nitric acid. The approach worked well but resulted in high nitrogen levels both in the shoot biomass and tubers (Wheeler et al., 1994a; McKeehen et al., 1996). In some cases, greater than 30% of the nitrogen came from the nitric acid in this hydroponic approach (Wheeler et al., 1990). Much of the nitrogen in the shoot was in the form of nitrate, but fortunately there was little nitrate in the tubers, which would raise food safety concerns (McKeehen et al., 1996). Nitrogen in the tubers was a combination of protein and other non-protein nitrogen, possibly including nucleic acids, amino acids, amides and peptides (McKeehen et al., 1996).

17.4.2 Mineral nutrition

Numerous tests were conducted at the Wisconsin to study different concentrations of essential nutrients on potato growth and development. Total plant growth in NFT was reduced at 0.1 mM and 9.8 mM K concentrations compared to 0.5, 1.6, 3.2, and 6.4 mM K (Cao and Tibbitts, 1991a). Similar studies with Mg at 0.05, 0.12, 0.25, 1.0, 2.0, and 4.0 mM showed total plant and tuber growth increased with increased Mg up to 1.0 mM and then decreased with further increases in Mg. A comparison of different combinations of NH_4/NO_3 showed better growth with mixed N forms compared to only NH_4 or only NO_3 (Cao and Tibbitts, 1993), and that NH_4 in solutions increased P and Cl, and decreased Ca and Mg in shoot tissue (Cao and Tibbitts, 1993). Subsequent studies lasting 84 days showed no advantage to using NO_3/NH_4 mixes vs. NO_3 only on final tuber yield, and that NO_3 levels could be reduced from 7.5 to 1.0 mM for the last half of growth with no significant loss in tuber yield (Goins et al., 2004). Moreover, reductions in nitrogen later in growth increased harvest index (Goins et al., 2004). When NO_3 was used alone, maximum growth occurred at 2, 4, and 8 mM levels but growth was reduced at 0.5 and 12 mM (Cao and Tibbitts, 1998). When ammonium was used alone, maximum growth occurred at 2 mM with decreased growth at 0.5 and at 4, 8, and 12 mM (Cao and Tibbitts, 1998).

17.4.3 Propagation

For all of the NASA sponsored testing, in vitro nodal cuttings were used to start the plants (Tibbitts et al., 1994). This assured uniform, disease-free planting stock for the experiments. Plantlets about 10 cm long were transplanted ex vitro into solid media by burying about 2/3 of the plantlet. Transplants were then covered with glass beakers for 3 days to allow acclimation. Most plantlets were grown on an MS type medium with 6% sucrose, but related testing showed that sucrose levels could be reduced if some air exchange and CO_2 could reach the plants (Kozai et al., 1988; Yorio et al., 1995a). Other tests showed that light spectral quality could be used to control stem and internodal elongation (Wilson et al., 1993). Although the in vitro propagation was useful for research, it might be cumbersome and costly for space and further testing is needed to study the use of micro and mini-tubers harvested from plants as propagules.

17.5 Physiological Responses in Controlled Environments

17.5.1 Light

Tuberization is known to be a short-day response (Garner and Allard, 1923; Gregory, 1965) and even shows the classic red / far-red reversal for phytochrome control (Batutis and Ewing, 1982). Yet studies showed that some potato cultivars tuberized even under continuous light (Arthur et al., 1930; Harvey, 1922). Because of this, early NASA testing focused on the photoperiod responses of potato cultivars, and in particular which cultivars might tolerate continuous light. The rationale for this was simple: If total growth and tuber yield could be increased per unit area with longer photoperiods, the crop area required to sustain humans in a life support system could be reduced (Salisbury, 1991). The good performance of the early cultivar Norland and the poor performance of the late cv. Kennebec under 20 h of light indicated genotypic differences in response to photoperiod (Figure 17.4). In addition, leaves of the Kennebec plants folded upward and became chlorotic under the 20 h photoperiod, suggesting a physiologically intolerance to the long photoperiod (Wheeler and Tibbitts, 1986a). To explore this further, cvs. Norland, Norchip, Russet Burbank, Superior, and Kennebec were grown under a 12/12 h light/dark cycle using 400 μmol m^{-2} s^{-1} PAR (controls), a 24 h photoperiod with 400 μmol m^{-2} s^{-1} PAR, and 12 h of 400 + 12 h of 5 μmol m^{-2} s^{-1} PAR. All cultivars tuberized well under 12 h light / 12 h dark treatment, while cvs. Norland and Russet Burbank also grew well and tuberized under continuous 400 μmol m^{-2} s^{-1}, which had twice the total light as the 12 h treatment. Cv. Norchip showed moderate tuberization under continuous 400 μmol m^{-2} s^{-1}, but cvs. Superior and Kennebec had poor tuber development and chlorotic leaves with rustic flecks (Wheeler and Tibbitts, 1986b; Cao and Tibbitts, 1991b). In contrast, plants grown with the dim day length extension showed little tuberization but healthy, dark green leaves and stems (Wheeler and Tibbitts, 1986b). This suggested that all the cvs. responded well and tuberized under short days but that requirements for a dark period could be overcome with greater amounts of total light in cvs. Norland and Russet Burbank. In contrast, cvs. Superior, Norchip, and Kennebec were physiologically intolerant to continuous light at 400 μmol m^{-2} s^{-1} PAR (Wheeler and Tibbitts, 1986b). Subsequent studies showed injuries to intolerant cultivars could be mitigated by providing a thermoperiod (Tibbitts et al., 1990; Cao and Tibbitts, 1991a, 1992b). Closer examinations showed that continuous light injury resulted in loss of chloroplast membrane integrity and photosynthetic competence in intolerant cultivars (Cushman et al., 1995), with ethylene also playing a role (Cushman and Tibbitts, 1998).

Despite the ability of some cultivars to grow and tuberize under long photoperiods, short-day tendencies were still apparent: Harvest index, which indicates the partitioning of growth to tubers, was nearly always greater under short photoperiods (Wheeler and Tibbitts, 1986a; Wheeler et al., 1988, 1991). This indicated that yields might be optimized if strong induction could be combined with high total light. This idea was tested by moving plants between 12 h light / 12 h dark and a 24 h light chambers at different stages of growth. The results with cv.

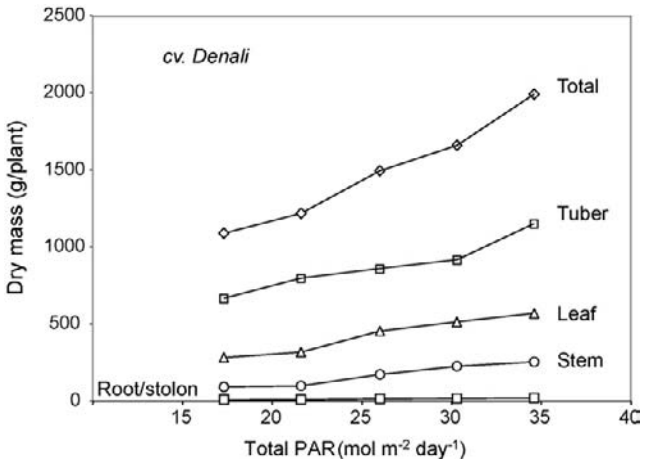

Figure 17.8: For most of the NASA-sponsored studies with potatoes, increased light (PAR) resulted in increased biomass. The relative partitioning of biomass among plant parts is shown for a range of PAR from studies by Wheeler and Tibbitts (1997).

Denali showed that plants given short days early in growth followed by long days later in growth produced greater tuber yields than plants given long days followed by short days (Wheeler and Tibbitts, 1997).

Depending on the system constraints in space, use of short photoperiods throughout growth still might be desirable. For example, if electrical power for lighting is limited but growing volume is not, larger areas might be planted and alternate halves illuminated at 12-h intervals. Lamps over each of the areas could be turned on for 12 h, or the lamps could be mounted on a track to move back and forth to provide alternate 12-h light cycles. Either of these approaches would be more efficient than continuous light for tuber yield per MJ of energy (Wheeler et al., 1992).

Except for when injury occurred with continuous light-intolerant cvs., total growth and tuber yield generally increased with total irradiance, regardless of photoperiods or other environmental treatments (Wheeler et al., 1991; Wheeler and Tibbitts, 1997; Yandell et al., 1988) (Figure 17.8). This emphasized the strong influence of light and its importance in designing bioregenerative life support systems for space. Similar findings have been reported for wheat, lettuce, and soybean in life support studies (Bugbee and Salisbury, 1988; Knight and Mitchell, 1988; Wheeler et al., 2001).

17.5.2 Temperature

The strong influence of temperature on potato growth and tuberization is well documented (Burton, 1972; Gregory, 1965; Cao and Tibbitts, 1995; Marinus and Bodlaender, 1975). Nonetheless several temperature studies were conducted for the NASA studies. Tests with

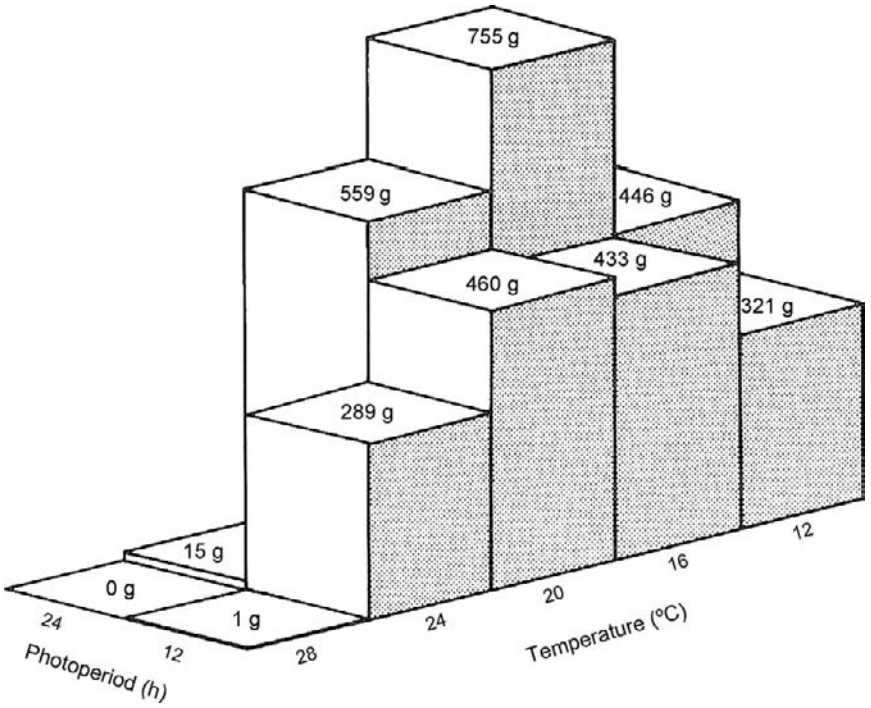

Figure 17.9: The effect of temperature on tuber yield from 56-day-old cv. Norland potato plants grown under 12 h or 24 h (continuous) 400 μmol m^{-2} s^{-1} PAR. Peak yields under 12 h occurred at 20°C, while peak yields under 24 h occurred at 16°C. Few or no tubers were produced at 28°C (Wheeler et al., 1986a).

Russet Burbank and Norland grown under continuous light showed that 17.5°C and 18.7°C were the optimum for tuber yield, respectively (Yandell et al., 1988). Prior studies with Norland showed that tuber yields were greatest at 20°C when a 12-h photoperiod was used, and at 16°C when continuous light was used (Figure 17.9). For all studies, stem lengths increased with temperature, regardless of the photoperiod. Thus cooler temperatures could be used to offset the less inductive influence of a long photoperiod, and warmer temperatures were tolerable if short photoperiods were used (Wheeler et al., 1986b). Other studies showed that a thermoperiod improved tuberization under both short and long photoperiod, and that thermoperiods reduced injury from continuous light (Bennett et al., 1991; Cao et al, 1992b; Tibbitts et al., 1990). Using warm temperatures early in growth followed by cool temperatures later in growth also promoted good tuberization, but the reverse did not (Cao and Tibbitts, 1994a).

17.5.3 Carbon dioxide

Closed environments in space will require continuous control of atmospheric composition and pressure, including the partial pressure of carbon dioxide (CO_2). At the beginning of NASA's

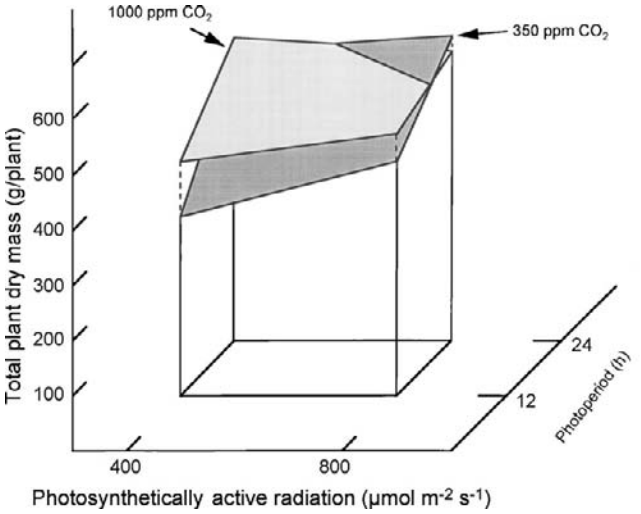

Figure 17.10: Total biomass of potato plants grown under two PARs (400 and 800 μmol m^{-2} s^{-1}), two CO_2 concentrations (350 and 1000 ppm), and two photoperiods (12 and 24 h). Data are averages for three cultivars, Norland, Russet Burbank, and Denali, grown for 90 days. Tuber yields showed a similar response pattern to total biomass. CO_2 enrichment showed the greatest proportionate benefit under the 12-h photoperiod and 400 μmol m^{-2} s^{-1} PAR, and no benefit or even had a negative effect under 800 μmol m^{-2} s^{-1} and 24 h lighting (Wheeler et al., 1991).

testing in 1982, little research had occurred on CO_2 effects on potato. Single leaf studies showed the classic C_3 responses with increased photosynthetic rates at elevated CO_2 levels (Ku et al., 1976), while whole-plant studies showed both positive (Collins, 1976) and negative effects (Goudriaan and de Ruiter, 1983). Initial studies at the Wisconsin showed that increasing the CO_2 from ~360 to 1000 ppm (0.036 to 0.10 kPa) increased single leaf photosynthetic rates for cv. Norland and increased tuber yields slightly for both cvs., but plants were grown under a 24-h photoperiod for those studies (Wheeler and Tibbitts, 1989). This led to a series of studies with cvs. Norland, Russet Burbank, and Denali where plants were grown for 90 days under 12- or 24-h photoperiods, 400 and 800 μmol m^{-2} s^{-1} PAR, and ambient (~360 ppm) and 1000 ppm CO_2. Total biomass and tuber yields increased with elevated CO_2 especially at the lower PAR and 12-h (short) photoperiod. In contrast, elevated CO_2 had had little or even a negative effect under continuous light (Wheeler et al., 1991) (Figure 17.10). These studies showed an average increase in yield of 39% when the CO_2 was enriched to 1000 ppm under the 12-h photoperiod with 400 μmol m^{-2} s^{-1} PAR and 27% increase under the 12-h photoperiod and 800 μmol m^{-2} s^{-1}. Free Air CO_2 Enrichment (FACE) studies in field plots in Europe found similar results – 40% increase in yield at 660 ppm (cv. Primura) and 32% increase in yield at 550 ppm (cv. Desirée) (Finnan et al., 2002; Miglietta et al., 1998). Subsequent studies investigating the effect of elevated CO_2 on cv. Denali plants showed a 29% increase in tuber yield from CO_2 enrichment (1000 ppm) under short days (Wheeler and Tibbitts, 1997). Collectively, the findings suggest

Figure 17.11: Neil Yorio at NASA's Kennedy Space Center taking leaf porometer measurements with potatoes to monitor stomatal conductance and transpiration rates. When measurements were taken at 400 ppm CO_2, a mask was worn to removed exhaled breath from the chamber to avoid elevating the CO_2 concentration.

the greatest benefits to potatoes from CO_2 enrichment occur at lower light levels and/or short photoperiods.

Although a CO_2 concentration of 1000 ppm is elevated in comparison to current Earth ambient, CO_2 concentrations in the Space Shuttle and International Space Station typically range from ~2000 to 6000 ppm, with episodes even exceeding 10 000 ppm with large crews. Consequently, we were curious about plant responses to 'super-elevated' CO_2 levels, i.e., $CO_2 > 2000$ ppm. When cvs. Norland and Denali plants were grown under 400, 1000, and 10 000 ppm CO_2, some bleaching was noted on leaves after 90 days growth at 10 000, but there was no difference in total biomass when compared to 1000 ppm-grown plants (Mackowiak and Wheeler, 1996). But transpiration and stomatal conductance measurements with a leaf porometer (Figure 17.11) showed significantly higher rates at 10 000 compared to 1000 ppm (Mackowiak and Wheeler, 1996), which was unexpected based on the research literature (Drake et al., 1997; Morison, 1987). Subsequent studies showed that stomatal conductance at 10 000 > 400 > 1000 ppm, and that the diurnal rhythms were damped at the super-elevated 10 000 ppm (Figure 17.12). Similar responses have been seen in soybean, sweetpotato, bean, and radish (Wheeler et al., 1999) and provide a good example of some unexpected and intriguing consequences of growing plants in space-like environments.

17.6 Further Testing for Space Environmental Physiology

An obvious concern for growing potatoes or any other plant in space is the different gravity environment. Watering plants in low gravity is a challenge but the typical problems associated

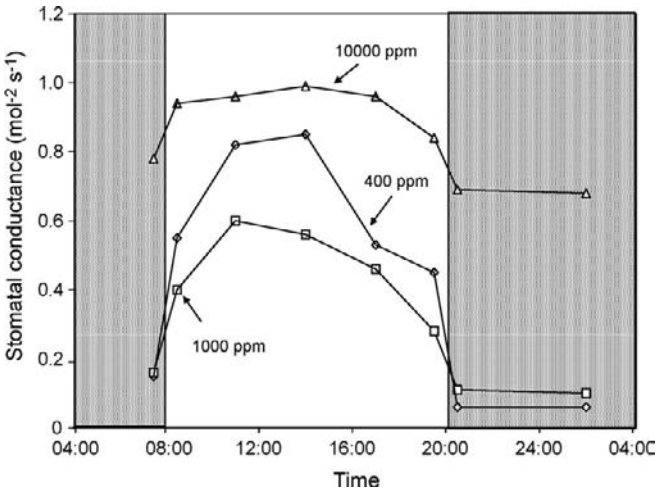

Figure 17.12: Stomatal conductance of potatoes grown at 400, 1000, and 10 000 ppm carbon dioxide. Conductance and transpiration were lowest at 1000 ppm and highest at 10 000 ppm. Super-elevated concentrations like 10 000 ppm might can occur in closed environments in space (source: Wheeler et al., 1999).

with this, such as poor aeration of roots zones, are secondary and not a direct effect of gravity on the plants (Hoehn et al., 2000; Morrow et al., 1995; Porterfield, 2002). Spaceflight conditions can cause some chromosomal aberrations (Krikorian and O'Connor, 1984) and affect some aspects of plant growth and development (Kiss et al., 2000; Musgrave et al., 1997), and initial tests to grow wheat plants in space resulted in heads with no seed (Levinskikh et al., 2000). But this was later determined to be a result of elevated ethylene on the Russian Mir space station and not weightlessness. Plant shoots can be oriented with light in the absence of gravity (Halstead and Dutcher, 1987), provided there is sufficient blue light (400–500 nm) for phototropism (Morrow et al., 1995). Thus if an adequate environment is provided for the plants, fractional or even microgravity do not appear to pose fundamental impediments (Monje et al., 2005; Stutte et al., 2005).

Another unique but controllable aspect about space environments for both humans and plants will be atmospheric pressure. Recent missions on NASA's Space Shuttle, the International Space Station, and the Russian Mir Space Station all operated at 1 atm (101 kPa) with about 21% (21 kPa) of oxygen. But early NASA missions with Mercury, Gemini, and Apollo operated at 1/3 atm (34 kPa) with 100% oxygen (34 kPa), while Skylab, NASA's first Space Station, operated at 1/3 (34 kPa) total pressure with 70% (24.3 kPa) oxygen (Lange et al., 2005). NASA's future missions to the Moon and Mars will likely operate at 54 kPa (0.54 atm). By using lower pressures both structural mass and gas leakage can be reduced. In addition, EVAs (space walks) can occur without any pre-breath time, allowing rapid responses to emergencies. If pressures are

sufficiently low, separate inflatable greenhouse structures might be possible for growing plants (Clawson et al., 2005). Pressure testing with plants to date has been sparse, but results suggest that plants tolerate pressures down to a 1/4 atm or less, provided sufficient oxygen is available for respiration and CO_2 for photosynthesis (Corey et al., 2002; He et al., 2007). Perhaps one of the more consistent responses of plants to reduced pressures is increased transpiration (Daunicht and Brinkjans, 1992; Massimino and Andre, 1999). This can be explained largely by increased gas diffusion rates at reduced pressures, which in turn can result in cooler temperatures of any surfaces associated with evaporating water, such as leaves (Rygalov et al., 2005).

One of the biggest risks for any living organisms in space will be the high energy radiation that can damage the molecular structure of cells. This includes galactic cosmic radiation (GCR), solar energetic particles (SEP), and so-called trapped radiation circulating planets with magnetic fields, such as Earth's Van Allen Belts (NRC, 2006). For spacecraft in low-Earth orbit, living organisms are inside the Van Allen Belts and somewhat shielded by the Earth's magnetic field, but in interplanetary travel or on the surfaces of the Moon or Mars, the effects of GCR and SEP can be serious. In addition, secondary radiation such as high energy neutrons can occur from collisions with surface regolith. Extensive interest and testing have focused on radiation effects on humans and astronaut safety (NRC, 2006), but less is known of plant responses to high energy radiation, and this remains an important area of research.

17.7 Atmospheric Regeneration Rates from Potato Photosynthesis

When potatoes were grown in the atmospherically closed Biomass Production Chamber at Kennedy Space Center, photosynthetic gas exchange rates could be tracked throughout growth for an entire 20 m^2 stand (Wheeler et al., 2003, 2008a). These measurements showed several distinctive features: First, stand (canopy) net photosynthetic rates increased rapidly as stand ground cover increased and approached 100% ground cover ca. 35 days-age (Figure 17.13). This has been observed with other crops as well and emphasizes the importance of light interception by the canopy (Monje and Bugbee, 1998; Wheeler et al., 1994). A closer look at Figure 17.13 shows that photosynthesis continued to rise after full canopy cover (ca. days 35–50), but this was a result of the canopy growing closer to the lamps and receiving greater light. A second observation was that stand photosynthetic rates were a strong function of incident light, with the study conducted at 865 $\mu mol\, m^{-2}\, s^{-1}$ PAR showing higher rates than the study at 655 $\mu mol\, m^{-2}\, s^{-1}$ (Figure 17.13). Short-duration tests where the light intensity was changed showed that photosynthetic rates increased linearly up to $\sim$1000 $\mu mol\, m^{-2}\, s^{-1}$ at 50 days after planting, with a light compensation point near 200 $\mu mol\, m^{-2}\, s^{-1}$ and stand respiration rates near 10 $\mu mol\, m^{-2}\, s^{-1}$ when grown at high light intensities (Wheeler et al., 2008a). Third, net photosynthetic rates decreased with age depending on the extent of leaf senescence (Figure 17.13). This was especially apparent in one test in which the photoperiod was changed from 12-h to continuous light (Figure 17.14). Immediately following this photosynthetic rates dropped but when the photoperiod

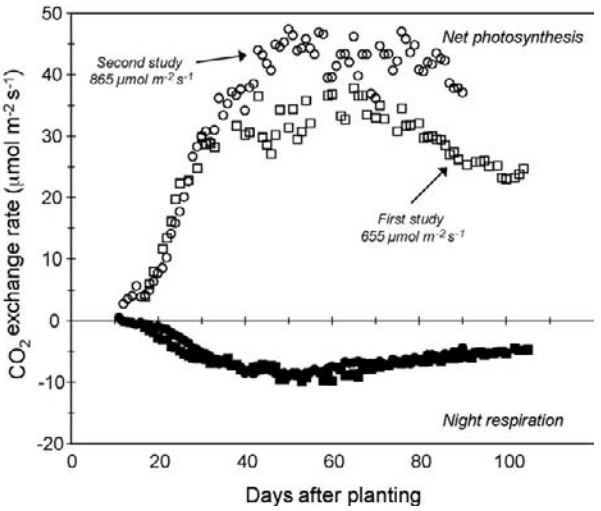

Figure 17.13: Carbon dioxide exchange rates of 20 m^2 potato stands grown at 865 or 655 µmol m^{-2} s^{-1} PAR. Positive values reflect net photosynthetic rates during the light period and negative values reflect respiration rates during the dark period (source: Wheeler et al., 2008a).

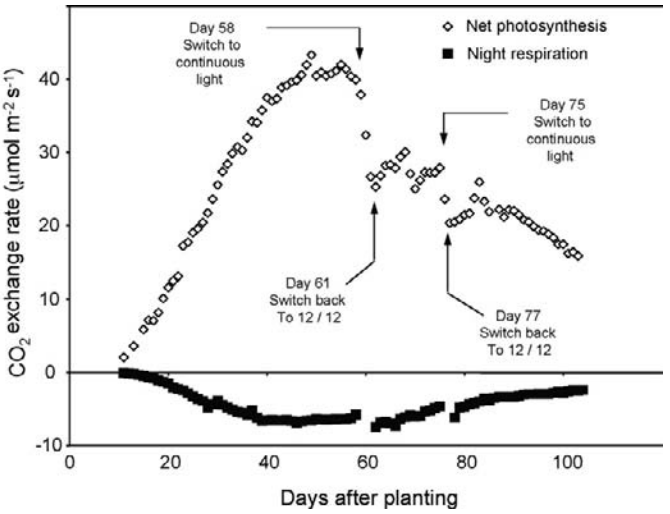

Figure 17.14: Effect of changes in photoperiod on the carbon dioxide exchange rate of a 20 m^2 potato stand. On day 58, the photoperiod was changed from 12-h to 24-h (i.e., continuous light), which resulted in drop in the instantaneous photosynthetic rates. Switching back to 12 h at day 61 allowed photosynthetic rates to slowly increase. A similar effect was observed between days 75 and 77 (source: Wheeler et al., 2008a).

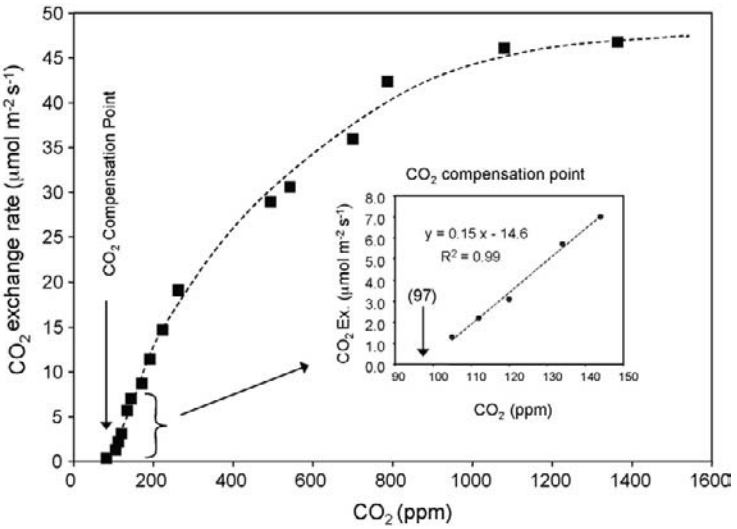

Figure 17.15: Carbon dioxide exchange rate (net photosynthetic rate) of a 20 m^2 potato stand at different CO_2 concentrations. The photosynthetic rates saturated above 1200 ppm while the CO_2 compensation point occurred at 97 ppm (source: Wheeler et al., 2008a).

was returned to 12 h, photosynthetic rates increased again. This suggested some feedback inhibition on instantaneous photosynthetic rates under the long photoperiod (Wheeler et al., 2008a). A fourth observation was that photosynthetic rates of potato stands showed a classic C_3 response to CO_2, where rates increased rapidly as CO_2 was increased up to ~400–500 ppm and saturated near ~1200 ppm (Drake et al., 1997; Wheeler et al., 2008a) (Figure 17.15).

During these same closed chamber studies, concentrations of ethylene gas could be monitored throughout growth and development. Ethylene is a plant hormone that is produced during normal metabolism (Abeles et al., 1992) but it can accumulate in tightly closed atmospheres. Ethylene production by potato stands was generally low in comparison to wheat, soybean, and lettuce but even a relatively low concentration of 40 ppb caused epinasty in young expanding leaves (Wheeler et al., 2004) (Figure 17.16). When the photoperiod was changed from 12 to 24-h, ethylene levels rose rapidly following this change from a basal rate of 0.4 nmol m^{-2} stand area day^{-1} to 6.2 nmol m^{-2} day^{-1}, presumably from stress to the plants (Wheeler et al., 2004). When the photoperiod was reduced from 24 to 12 h, ethylene levels decreased.

Stand transpiration rates for 20 m^2 stands using the NFT approach ranged from 3.4 to 5.2 L m^{-2} day^{-1} (3.4–5.2 mm day^{-1}) throughout growth, while maximum rates for canopies exceeded 9 L m^{-2} day^{-1}, or 9 mm day^{-1} (Figure 17.17) (Wheeler, 2005; Wheeler et al., 2008a). Higher transpiration rates occurred at higher PAR but it is not clear whether this was due to increased leaf

Figure 17.16: Epinastic potato leaves on secondary branches that developed in an atmosphere containing about 40 ppb ethylene (source: Wheeler et al., 2004).

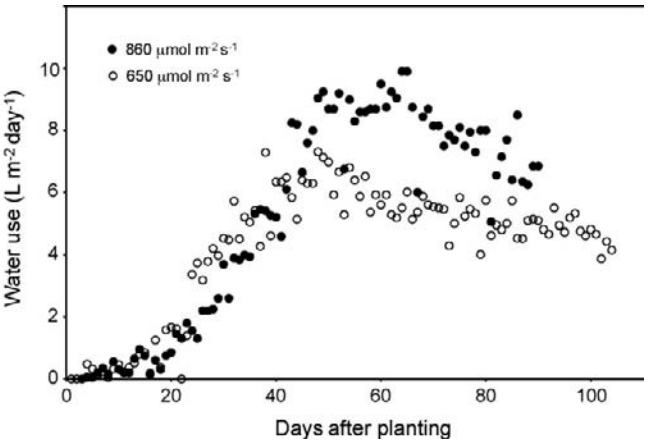

Figure 17.17: Water use (transpiration) of 20 m^2 potato stands grown at different PAR (light) intensities. Peak rates exceeded 9 L m^{-2} day^{-1} (9 mm day^{-1}) under high PAR.

temperatures, increased stomatal opening, or both. As with stand CO_2 exchange, time course measurements of transpiration typically showed a rapid rise early in growth as the canopy cover filled in, followed by a relatively constant rate during mature growth and tuber bulking (Figure 17.17).

Figure 17.18: Tuber yields from cv Norland plants grown under 12 h (3.6 kg) and 24 h (4.9 kg) photoperiods of 400 μmol m^{-2} s^{-1} PAR. Plants were harvested at 147 days.

17.8 Potatoes for Food in Space

There will be no 'growing seasons' in space and crop cultivation can be continuous. Thus estimating yield rates or productivities (g m^{-2} day^{-1}) is more meaningful than traditional yield per unit area (g m^{-2}, t ha^{-1}, etc.). These productivities can then be used to estimate the crop area needed to meet the food and oxygen requirements for a given number of people. An additional consideration is when to harvest. Sequential harvests with cv. Norland plants showed that yields continued to increase up to 148 days but that maximum productivity (i.e., g m^{-2} day^{-1}) occurred as early as 105 days with continuous light (Wheeler and Tibbitts, 1987). At that point, it was more efficient to harvest and replant rather than going on until 148 days. Plants in these studies were grown in large pots and confined to a cross-sectional area of 0.2 m^2, and their tuber yields reached 3.4 kg $plant^{-1}$ and 4.3 kg $plant^{-1}$ fresh mass under 12-h photoperiod and continuous light, respectively (Figure 17.18). This equaled 0.57 kg $plant^{-1}$ and 0.79 kg $plant^{-1}$ dry mass (DM) with a harvest index of 81% (Wheeler and Tibbitts, 1987). Dividing these yields by the cross-sectional area and number of growing days showed tuber productivity of 29.4 g DM m^{-2} day^{-1} at 126 days for continuous light and 19.5 g DM m^{-2} day^{-1} at 148 days for a 12-h photoperiod. Although these plants were confined to and area of 0.2 m^2 using screen cages, plants received side lighting through the cages, which means that the total light reaching the plants was underestimated. As a consequence, yields per unit area were inflated, although in theory the extra light might be provided within the canopy using imbedded lamps or light pipes (Tibbitts et al., 1994.). A less equivocal approach would be to grow the plants in a contiguous stand where side lighting is eliminated (Figure 17.19). When this was done with cv. Norland, productivities were 21.9 g DM m^{-2} day^{-1} at 110 days (Wheeler and Tibbitts, 1989), which is 23% less than the 28.5 g DM m^{-2} day^{-1} observed with

Figure 17.19: Ann Fitzpatrick of the University of Wisconsin taking leaf photosynthetic measurements from a closed stands of potatoes in a large growth room at the Biotron.

individually caged plants harvested at a similar age (Wheeler and Tibbitts, 1987). This points out the importance of getting accurate measurements of harvested areas and light provided to the plants.

Follow-up studies were conducted with cv. Denali using stands where only the center plants were harvested. Plants in the first study were given short days for the first 40 days to initiate strong tuber sinks followed by continuous light for 92 days to promote tuber bulking (Wheeler, 2006). Plants were spaced closely (0.02 m^2 $plant^{-1}$) and grown at lower light for the first 18 days to reduce overall area use, after which they were transplanted to larger pots spaced at 0.25 m^2 $plant^{-1}$ for the final growout. Final tuber yields from this test reached 19.7 kg FM m^{-2} (197 t ha^{-1}) or 4.35 kg DM m^{-2} (Table 17.1). These yields are roughly twice that for record field yields (Knowles and Thornton, 2000). Adjusting for the reduced area requirement prior to transplanting, the tuber productivity in this study was 37.5 g DM m^{-2} day^{-1} (Table 17.1). Radiation use efficiencies from this study were approximately 0.71 g DM mol^{-1} PAR for tuber biomass and 0.97 g DM mol^{-1} PAR for total biomass (Table 17.1) (Wheeler, 2006). When a second study was conducted but with a 12-h photoperiod for entire 132 days, tuber productivity was slightly less (31.1 g m^{-2} day^{-1}) but radiation use efficiencies increased to 0.82 g DM mol^{-1}PAR for tuber biomass and 1.15 g DM mol^{-1} PAR for total biomass. In all cases, these radiation use efficiencies included the reduced area required prior to transplanting.

Assuming one human requires about 2500 kcal day^{-1} and there are ~3.7 kcal g^{-1} DM for potato (Wheeler et al., 1994), then 38 g m^{-2} day^{-1} × 3.7 kcal g^{-1} = 141 kcal m^{-2} day^{-1}. Then dividing 2500 kcal $person^{-1}$ day^{-1} by 141 kcal m^{-2} day^{-1} ≈ 17.8 m^2 of potatoes would be required to continuously provide the food (dietary energy) for one person (Wheeler, 2006). The total area required to remove the CO_2 (~1000 g day^{-1}) and supply the O_2 (~800 g day^{-1}) for one person

Table 17.1: Some high yields from potatoes grown in controlled environments (source: Wheeler, 2006)

	Study Duration	Tuber FM	Tuber DM	Total DM Productivity	Tuber DM Productivity	PAR	Radiation Use Efficiency (Total)	Radiation Use Efficiency (Tuber)
	(days)	($kg\,m^{-2}$)	($kg\,m^{-2}$)	($g\,m^{-2}\,day^{-1}$)	($g\,m^{-2}\,day^{-1}$)	($mol\,m^{-2}\,day^{-1}$)	($g\,mol^{-1}$)	($g\,mol^{-1}$)
Univ. Wisconsin (12 h photperiod)	132	18.01	3.61	38.3	27.3	37.8	1.01	0.72
Values adjusted for transplanting				43.6	31.1	37.8	1.15	0.82
Univ. Wisconsin (12 then 24 h photoperiod)	132	19.7	4.35	44.8	33.0	52.4	0.85	0.63
Values adjusted for transplanting				51.0	37.5	52.4	0.97	0.71
Ken. Space Cen. (12 h then 16 h photoperiod)	105	10.5	1.88	27.2	18.4	42.2	0.64	0.44
Estimated Values with transplanting				32.1	21.7	42.2	0.76	0.51

FM = fresh mass; DM = dry mass; PAR = photosynthetically active radiation.

would be somewhat less, since the gas exchange is a function of the total biomass produced and not just tuber yields. The total biomass productivity from this test was 43.6 g m^{-2} day^{-1} (Table 17.1); assuming this biomass was mostly carbohydrate (CH_2O) and that all of the C came from CO_2 fixed during photosynthesis, an equivalent amount of CO_2 needed to produce this could be estimated by dividing 0.68, which is the ratio of 30 (g mol^{-1} of CH_2O) / 44 (g mol^{-1} of CO_2) (Wheeler, 1996). Then (43.6 g m^{-2} day^{-1}) / 0.68 = 63.9 g CO_2 m^{-2} day^{-1}. Then (1000 g CO_2 $person^{-1}$ day^{-1}) / 63.9 g CO_2 m^{-2} day^{-1} ≈ 15.6 m^2 of potatoes to remove the CO_2 and supply the O_2 for one person in this study. This assumes a 1:1 molar ratio of CO_2 produced to O_2 consumed by the human, which is reasonably close for a high carbohydrate diet (Wheeler, 1996).

17.9 Spaceflight Testing

With the relatively short supply line to spacecraft in low-Earth orbit, food can be replenished easily from Earth. Hence no space studies have occurred to date where plants were grown specifically for life support. But exploratory studies have been conducted with a range of crops, including potatoes (Levinskikh et al. 2000; Musgrave 2002; Nechitailo and Mashinsky, 1993; Stutte et al., 2005). The first potato study involved flying tubers cv. Priekulsky packed in moist moss for 18 days on a Russian Soyuz flight in 1970 (Nechitailo and Mashinsky, 1993). All three tubers flown in space sprouted and formed small roots but development was slightly delayed when compared to ground controls. When these tubers from space and ground were grown out as whole plants, little difference was noted (Nechitailo and Mashinsky, 1993). A second study used in vitro potato plantlets that were grown in the laboratory for 4 weeks on Earth and then launched to the Mir Space Station in May of 1991 (Kordyum et al., 1997). Plantlets were maintained in dark containers for 8 days on board Mir, during which they formed small spherical tubers containing starch, similar to plantlets kept on Earth. The starch grain size in the space tubers was smaller than those on Earth, and the lamellae within the amyloplasts were enlarged (Kordyum et al., 1997). The third experiment used excised leaves with axillary buds to determine whether photosynthetically driven tuber formation could occur under space flight conditions (see Ewing, 1985; Wheeler et al., 1988a). Five leaves from induced cv. Norland plants were placed in the University of Wisconsin's Astroculture plant chamber (Morrow et al., 1995) and flown on the Space Shuttle mission STS-73 in November of 1995. The cut ends were buried in arcillite particles kept moist by a porous tube watering system (Morrow et al., 1995). Light was provided to the leaves using a combination of red and blue light emitting diodes (LEDs) to provide a 12-h photoperiod of 150 μmol m^{-2} s^{-1} PAR (Croxdale et al., 1997). In-flight data showed that CO_2 concentrations in the chamber rose in the dark, and then drew down to a set-point of 500 μmol mol^{-1} during the light, indicating the leaves were respiring and photosynthetically active (Brown et al., 1997). After 16 days in space the leaves had senesced significantly but tubers developed at all five leaf axils (Figure 17.20) (Cook et al., 1998; Croxdale et al., 1997). The size and shape of tubers from space were similar to those grown in a ground

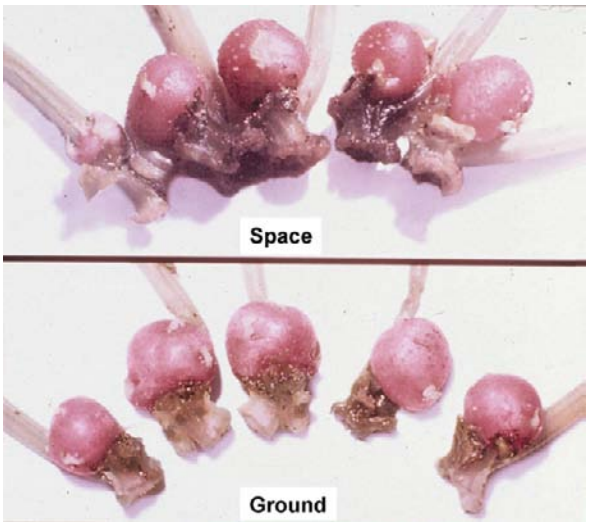

Figure 17.20: Potato tubers (1.5 cm in diameter) formed at the axils of leaf cuttings sent into space for 16 days on NASA's Space Shuttle (source: Croxdale et al., 1997; Tibbitts et al., 1999). Tubers formed in the μ-gravity environment of space with no negative effects.

control chamber, as were the distributions of starch grains and proteinaceous crystals (Cook and Croxdale, 2003). There were more small starch grains in the space-grown tubers (Cook et al., 1998; Croxdale et al., 1997), similar to results reported in experiments on the Russian Mir Space Station by Kordyum et al. (1997). Collectively the results indicate that gravity is not required for tuber formation.

17.10 Bioregenerative Systems for the Future in Space

It is still unclear whether electric lamps, direct solar lighting, or some combination of the two will be used for crops in space. The use of solar lighting will depend on the setting: For example, on the moon there are roughly 14 days of dark and 14 days of light (Salisbury, 1991). On Mars, the diurnal cycle is 24.6 h, similar the Earth's, but the solar intensity at Mars' orbit is only 43% that of Earth's, and extensive dust storms can occur at some latitudes on Mars (Salisbury, 1991; Wheeler, 2004). In addition, techniques for capturing solar light and delivering it to a protected environment will be needed (Wheeler and Martin-Brennan, 2000). On the other hand, electric lighting can be used in any setting, provided sufficient electrical power is available. But the effects of lamp spectra must be considered. For example, high-pressure sodium lamps are electrically efficient, but are relatively deficient in blue wavelengths, which can cause elongated stems (Yorio et al., 1995b). Novel approaches such as light-emitting diodes (LEDs) have the advantage of a long operating life and low thermal radiation, but selecting optimal color combinations of LEDs for growing plants needs further study (Bula et al., 1991; Goins et al., 1997).

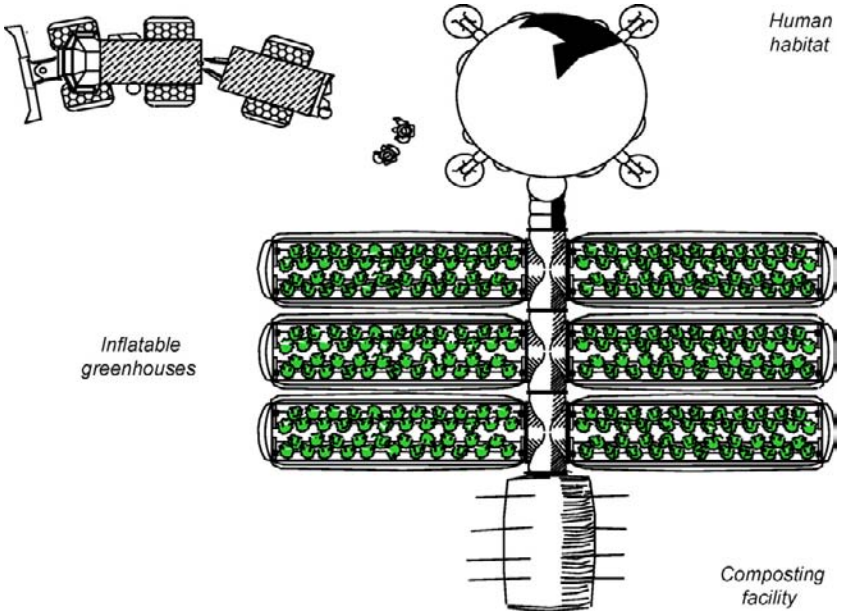

Figure 17.21: A concept for inflatable greenhouses (overhead view) that might be deployed on the surface of Mars (source: Sadler and Giacomelli, 2002). Inflatable structure are light weight and could be stowed in a small volume, but will require materials that are air tight, transparent, and tolerant of the UV radiation on the surface of Mars.

The costs associated with lighting tend to be the major economic factor in trade studies of plant-based life support approaches (Drysdale et al., 2003). For example, highly productive plant systems for life support might require about 200 W m^{-2} ($\sim$1000 μmol m^{-2} s^{-1}) of photosynthetically active radiation (PAR) (Wheeler et al., 2001). Assuming a lighting system is 20% efficient for converting electric power to PAR reaching the plants (Cathey and Campbell, 1977), then1 kW of electric power would be required per m^2 of plant growing area. Studies done to date with planting of multiple species indicate that about 50 m^2 of plant growing area would be required to meet the food needs (daily calories) for one human (Gitelson et al., 1989; Tako et al., 2005; Wheeler et al., 2001), thus 50 m^2 $person^{-1}$ × 1 kW m^{-2} = 50 kW $person^{-1}$ just for the electric lighting. This does not include the power for cooling, air circulation, water pumps, sensors, etc., which might double the number to 100 kW $person^{-1}$. Thus hundreds of kilowatts or perhaps even megawatts of power might be required for large, plant based life support systems with electric lighting. Alternatively, solar light might be used. This would cut the electric power requirements significantly but would require a means for delivering the light into the protected growing environment. This might be possible with transparent structures, such as inflatable 'greenhouses' (Figure 17.21) or perhaps solar collectors connected to fiber optic or light conduits (Figure 17.22) (Cuello et al., 2000; Sadler and Giacomelli, 2002; Wheeler, 2004). The former approach would require durable transparent materials that are gas tight, resistant

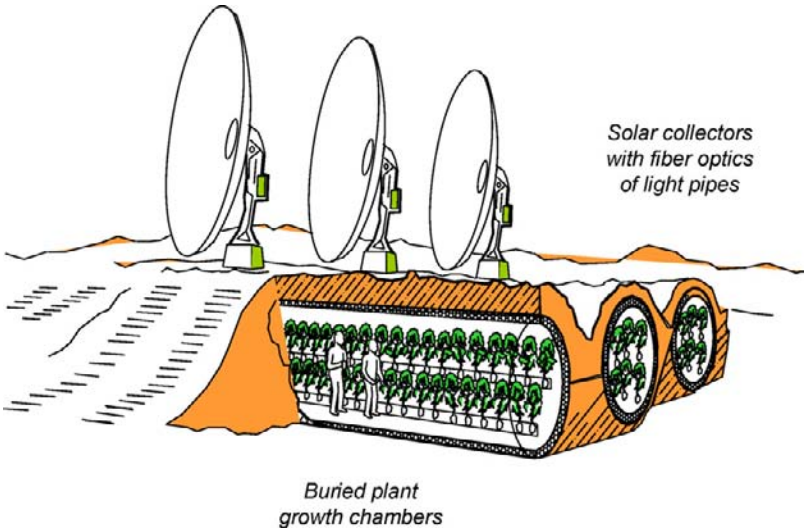

Figure 17.22: A concept of plant growth chamber covered with surface regolith for radiation shielding. Light could be collected with solar concentrators and piped into the plant chamber (source: Sadler and Giacomelli, 2002).

to the high energy radiation of space, and provide at least some thermal insulation (Clawson et al., 2005). These are substantial challenges but might be assisted by dropping the internal atmospheric pressure to reduce the force on the structure and/or by using external covers at night to provide insulation (Boston, 1981). Regardless of the configuration, solar lighting approaches would be possible only in settings that receive sufficient light. Use of solar lighting only at low latitudes on the Moon would not be practical because of the long (14-day) dark period (Salisbury, 1991). But at higher latitudes, such as the rim of Shackleton Crater on the South Pole of the Moon, sunlight is available for all but a few days each month and use of solar light for plant cultivation should be possible. Mars receives only 43% of the sunlight that reaches Earth and has a diurnal rotation cycle similar to Earth's (Salisbury, 1991). In addition, certain areas of Mars are prone to dust storms, which would interfere with transparent structure and solar collector approaches. Nonetheless, analyses of dust events and light transmittance data from Mars suggest that some Martian settings receive up to 25 to 30 mol m^{-2} day^{-1} of PAR (Clawson, 2007), which is comparable to many settings on Earth (Albright et al., 2005). Dealing with these various lighting constraints will pose an interesting challenge for agriculture engineers of the future.

17.11 Concluding Comments

Findings from controlled environment studies for NASA were consistent with many previous physiological studies with potatoes, but also revealed some interesting phenomena, including

physiological intolerance of some cvs. to continuous light, increased stomatal conductance at super-elevated CO_2 concentrations, ethylene production by whole canopies, successful growth in NFT culture, and yields approaching 200 t ha^{-1} under high light and CO_2 enrichment. Small-scale space flight experiments showed that tubers can form and sprout in weightlessness. Clearly these are just modest steps toward the ultimate use of plants for human life support in space, but I am convinced that potatoes will one day supply food and oxygen to humans living on other planets, just as they have for hundreds of years on Earth.

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