EFFECT OF ANNEALING ON THE MOLECULAR STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF BREAD WHEAT STARCHES VARYING IN AMYLOSE CONTENT

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Effect of annealing on the molecular structure and physicochemical properties of bread wheat starches varying in amylose content

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List of Abbreviations

AACC	American Association of Cereal Chemists
ADP	Adenosine diphosphate
AFM	Atomic force microscopy
AGPase	ADP-glucose pyrophosphorylase
AM	Amylose
AML	Amylose leaching
AMP	Amylopectin
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
^{13}C-CP MAS/NMR	^{13}C cross polarization magic angle spinning/ nuclear magnetic resonance
$\overline{CL}$	Average chain length
Con A	Concanavalin A
Da	Dalton
DBE	Starch debranching enzymes
DMSO	Dimethyl sulphoxide
DNS	Dark northern spring wheat
DP	Degree of polymerization
DP_n	Degree of polymerization by number
DSC	Differential scanning calorimetry
EU	European Union
FFA	Free fatty acid
FSU	The Former Soviet Union
FTIR	Fourier transformed infrared spectroscopy
GBSS	Granule-bound starch synthase
ΔH	Enthalpy of gelatinization
ΔH_R	Enthalpy of retrogradation
HAMS	High amylose maize starch
HPAEC-PAD	High pressure anion exchange chromatography with pulsed amperometric detection
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
I_5^-	Pentaiodide
IAOM	Image analysis of optical microscopy
ICL	Internal chain length
LPL	Lysophospholipids
M	Molarity (Molar)
M_W	Average molecular weight

PV	Peak viscosity
Retro-DSC	Retrogradation DSC
RS	Resistant starch
RVA	Rapid visco analyzer
RVU	Rapid visco analyzer units
SAXS	Small angle X-ray scattering
SBE	Starch branching enzyme
SD	Standard deviation
SEM	Scanning electron microscopy
SF	Swelling factor
SP	Swelling power
SS	Starch synthases
TCA	Trichloroacetic acid
TDF	Total dietary fiber
T_c	Conclusion temperature
T_c-T_o	Gelatinization temperature range
TEM	Transmission electron microscopy
T_g	Glass transition temperature
T_m	Melting temperature
T_o	Onset temperature
T_p	Peak temperature
USA	United States of America
USDA	United States Department of Agriculture
WAXS	Wide angle X-ray scattering
WSN	White salted noodles
Wx	Waxy
XRD	X-ray diffraction
%T	The percentage light transmission
°C	Centigrade
λmax	Maximum wave length
v/v	Volume/volume
w/v	Weight/volume
w/w	Weight/weight
db	Dry basis

Abstract

Starch from normal (CDC teal), high amylose (line 11132) and waxy (99WAX27) bread wheat cultivars was isolated and its morphology, composition, structure and properties were studied before and after annealing. Granule diameters, total phosphorus, total amylose, lipid complexed amylose chains, crystallinity, gelatinization temperature range, gelatinization enthalpy, swelling factor (at 90°C), and amylose leaching (at 90°C) ranged from 2-38μm, 0.007-0.058%, 26.9-32.3%, 13.4-18.7%, 28.6-42.8%, 12.7-14.3°C, 11.3-13.3 J/g, 27.6-72.1 and 22.2-26.2%, respectively. Peak viscosity, thermal stability, set-back and susceptibility towards acid hydrolysis followed the order: 99WAX27 >CDC teal >11132, 11132 >CDC teal >99WAX27, CDC teal > 99WAX27 >11132, and 99WAX27 >11132 >CDC teal, respectively. Susceptibility towards α-amylase hydrolysis followed the order: 99WAX 27 > 11132 > CDC teal (<24h) and 11132 >CDC teal >99WAX27 (>24h). The extent of retrogradation measured by spectroscopy and differential scanning calorimetry followed the order: 11132 > CDC teal >99WAX27 and 99WAX27 >CDC teal >11132, respectively. In all starches, concentration of amylose (apparent & total), lipid complexed amylose chains, gelatinization temperature range, swelling factor, amylose leaching, peak viscosity, final viscosity, set-back, light transmission, susceptibility towards α-amylase and acid hydrolysis and the proportion of small (2 to 8 μm) B-type granules decreased on annealing. However,

thermal stability and crystallinity increased on annealing, whereas enthalpies of gelatinization and retrogradation and the amylopectin chain length distribution remained unchanged in all starches. Pores and indentations were formed on the granule surfaces of CDC teal and 99WAX27 starches on annealing.

Chapter 1. Introduction

1.1 Introduction

Wheat (*Triticum spp.*) is the most widely grown cereal grain, occupying 17 percent of the total cultivated land in the world. A large proportion of man's essential nutrients are contained in the wheat grain. Furthermore, the low water content, ease of transport and processing, and good storage qualities have made this crop the most important staple food of more than one billion people (35% of the world's population) (Emes *et al.* 2003). Wheat is used mainly for food, but substantial quantities are also used as feed for livestock. In addition to food and feed uses, wheat has industrial applications in textiles, distilling, loose-fill packaging materials and cosmetics.

Canada is one of the leading producers of wheat in the world. Three different varieties of wheat are predominantly grown in Canada: winter wheat, dark northern spring wheat, and durum wheat. Different food products are made from these different classes of wheat. Winter wheat is used to make French type hearth breads, pancake flour, noodles, pan and steamed breads. Durum wheat is used in the manufacture of macaroni, spaghetti, and other pasta products. Flour from hard red spring wheats contains a high percentage of gluten and is used to make bread and fine cakes, while soft white spring wheat varieties are paler and have starchy kernels and their flour is preferred for piecrust, biscuits, cookies, cakes, doughnuts,

and other fine pastries. Canadian wheat has been widely used for leavened bread. However, the emphasis now is to extend the use of Canadian wheat for the Asian market in flat bread and noodle production, especially Japanese white salted noodles (WSN). WSN are typically produced from flour exhibiting high starch swelling volume and peak viscosity (Crosbie 1991), which confer a soft, elastic texture to the noodles. Reduced amylose content, increased flour swelling ability, decreased paste gel rigidity, and enhanced deformation of gelatinized starch granules contribute to soft texture in WSN (Miura & Tanii 1994, Morita *et al.* 2002). Developing partial waxy wheats with varying amylose contents will provide a means for producing diverse noodle types with desired eating textures (Chibbar & Chakraborty 2005).

Currently, plant-breeding techniques have resulted in the production of waxy (amylose free) and high-amylose (30-37%) starches with improved functional properties. However, native starches from various plant sources have their own unique properties, and these inherent characteristics are not sufficient to meet the requirements of the modern food industry. Therefore, to satisfy these demands, the current research is focused on starch modification techniques such as chemical (cross-linking, substitution, conversion) and physical (pre-gelatinization, heat-moisture treatment and annealing) modifications (Thomas & Atwell 1999).

Annealing is a process whereby starch granules in excess (>60% w/w) or at

intermediate water content (40% w/w) are held at a temperature above the glass transition temperature (T_g) but below the onset (T_o) temperature of gelatinization for a set period of time (Tester & Debon 2000, Jacobs & Delcour 1998, Hoover & Vasanthan 1994a,b). Annealing has been described as a crystal growth/perfection, diffusion controlled non-equilibrium process (Tester & Debon 2000, Jacobs & Delcour 1998, Hoover & Vasanthan 1994a). Annealing of starches has been studied at various starch: water ratios (1: 1, 1 :3, 1 :5) and at temperatures ranging from 40 to 75°C (Kozlov *et al.* 2007, Kohyama & Sasaki 2006, Waduge *et al.* 2006, Vermeylen *et al.* 2006, Kiseleva *et al.* 2004, 2005, Genkina *et al.* 2004a,b,c, Qi *et al.* 2004, Nakazawa & Wang 2003, Tester *et al.* 2000, Jacobs et al. 1998a,b,c, Tester et al. 1998, Wang *et al.* 1997, Hoover & Manuel 1996, Jacobs *et al.* 1995, Hoover & Vasanthan 1994a,b, Cameron & Donald 1992, Larsson & Eliasson 1991, Knutson 1990, Krueger *et al.* 1987a,b). The above studies have shown that annealing results in structural changes within the amorphous and crystalline domains of starch granules. These changes in turn influence granular swelling, amylose leaching, pasting properties, gelatinization parameters and susceptibility towards enzymes and acid. Cereal starches (barley, wheat, rice and maize) are ideal for studying the part played by amylose during annealing, since they can be obtained with amylose contents ranging from 0 to 70%. Of the above cereal starches, only barley (Waduge *et al.* 2006, Kiseleva *et al.* 2003) and maize (Qi *et*

X-ray intensities, polymorphic form, granule crystallinity, gelatinization parameters, extent of amylose leaching, granular swelling, rheological characteristices, susceptibility to α-amylase and acid hydrolysis, and extent of retrogradation.

Chapter 2. Literature Review

2.1 Wheat grain

2.1.1 Introduction

Wheat (*Triticum spp.*) belongs to the tribe *Triticeae* and the genus *Triticum* of the grass family Gramineae *(Poaceae)* (Ünlü and Sümer 2005). It has been grown throughout temperate regions of the world since prehistoric times. Species of wheat are classified according to the number of chromosomes found in the vegetative cell. They are divided into three species: the diploid containing 14 chromosomes (e.g. *Triticum speltoides, T. bicorne* and *T. tauschii*), the tetraploid containing 28 chromosomes (e.g. *T. timopheevii* and *T. turgidum*) and the hexaploid containing 42 chromosomes (e.g. *T. aestivum*) (Encyclopedia, Wheat Varieties and Their Uses, 2007).

A large proportion of man's essential nutrients are contained in the wheat grain (60–80% carbohydrate, 8–15% protein, 1.5–2% fats, 1.5–2% minerals and vitamins). The majority of the carbohydrate portion is attributed to starch which constitutes approximately 70% of mature grain dry weight (Dale and Housley 1986). In addition to its high nutritive value, the low water content, ease of transport and processing, and good storage qualities have made this crop the most important staple food of more than one billion people (35% of the world's population) (Emes *et al.* 2003).

2.1.2 Production and Utilization

Wheat is the most widely grown cereal grain, occupying 17 percent of the total cultivated land in the world. Global wheat production dropped by 28.6 million tons from 621.7 million tons in 2005/06 to 593.1 million tons in 2006/07, mostly the result of adverse weather [United States Department of Agriculture (USDA) -World Agricultural Production, 2007]. By far the leading producers are the European Union (EU), China, the United States of America (USA), the Former Soviet Union (FSU), Canada, Australia, India, and Argentina. These eight countries produce about 77% of the wheat in the world. Because of the concentration of wheat production in a few countries, a large volume of wheat is traded in the world market. The United States is the largest exporter, followed by Canada and Australia (USDA-World Agricultural Production 2007).

Canada, on average, produces 25.7 million tons and exports an average of 18.4 million tons of wheat, mainly to Iran, Indonesia, China and Japan. Canadian wheat is well known for its high protein quality, which has satisfied the domestic and foreign wheat markets that demand a wheat flour with superior leavened bread-making properties (Båga *et al.* 1999). However, the uses of Canadian wheat over the last decade have gradually expanded towards other products, mainly due to changes in the export markets for the grain (Annon 1998). The expansion of wheat markets in Asia has shifted the consumer demand from flour with optimal baking qualities to flour suitable for noodle and flat bread production. There has

also been an increased interest in creating and expanding new niche markets for wheat. For example, the wheat kernel components may be used as raw materials for production of cosmetics, plastics, ethanol, adhesives and coating materials (Stroh 1997). This diversification of wheat grain uses has inspired wheat breeders and geneticists to develop wheat cultivars with grain characteristics suited for these new applications.

Three different varieties of wheat are predominantly grown in Canada: winter wheat, dark northern spring wheat (DNS), and durum wheat. Canadian winter wheat is almost exclusively grown in the province of Ontario, which produces 82 percent of the total winter wheat in Canada, with the remaining being grown on the prairies of Alberta, Saskatchewan, and Manitoba. Winter wheat planting in Canada begins around first of September and should be completed by the end of October. Winter wheat heads around mid-May, with harvest beginning around the first of July and running through the first part of September (USDA Economic and Statistics System 2007).

DNS is grown mostly in the southern areas of Canada. Saskatchewan accounts for 55 percent of all the DNS grown in Canada, with Alberta contributing 26 percent to the total DNS wheat grown. The remainder of the spring wheat grown in Canada is raised in Manitoba, which contributes 17 percent to the total spring wheat production. Durum is raised, generally, in the same area that the DNS is

raised. The provinces are ranked the same for durum production as they are for spring wheat. Saskatchewan accounts for 76 percent of the durum produced in Canada, Alberta produces 18 percent, and Manitoba produces 6 percent. Both spring wheat and durum follow, generally, the same life cycle. Both are planted around the first of May, with planting running through the first of June. Both can be expected to begin heading around the first of July, with harvest beginning in mid-August and running through the middle of October (USDA Economic and Statistics System 2007).

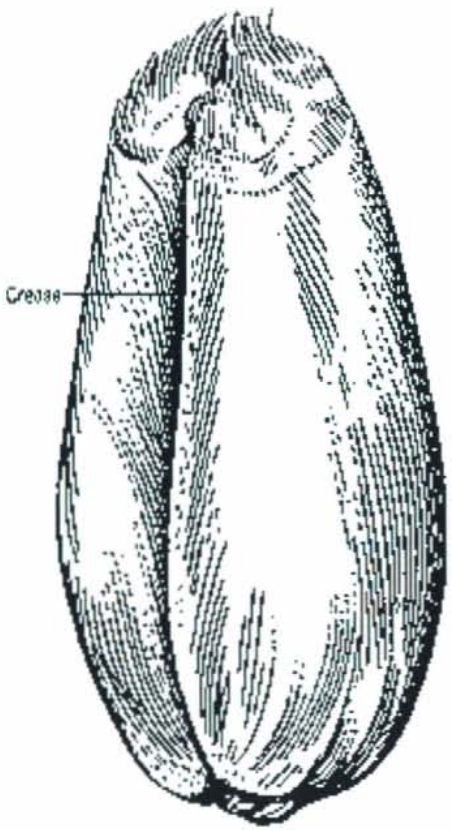
Wheat is used mainly for food, but substantial quantities are also used as feed for livestock. In addition to food and feed uses, wheat has industrial applications in textiles, distilling, loose-fill packaging materials and cosmetics (The Center for New Crops & Plant Products, at Purdue University, 2007).

For food, different food products are made from these wheats. Winter wheat is used to make French type hearth breads, pancake flour, noodles, pan and steamed breads. Flour from hard red spring wheats contains a high percentage of gluten and is used to make bread and fine cakes. The hardest-kerneled wheat is durum and its flour is used in the manufacture of macaroni, spaghetti, and other pasta products. Soft white spring wheat varieties are paler and have starchy kernels and their flour is preferred for piecrust, biscuits, cookies, cakes, doughnuts, and other fine pastries. Wheat is also used in the manufacture of whisky and beer.

Figure 2. 1A wheat grain and wheat grain cut lengthwise through crease

Source: Adopted from Song *et al.* (1998) with permission from Elsevier Science.

Whole grain of wheat



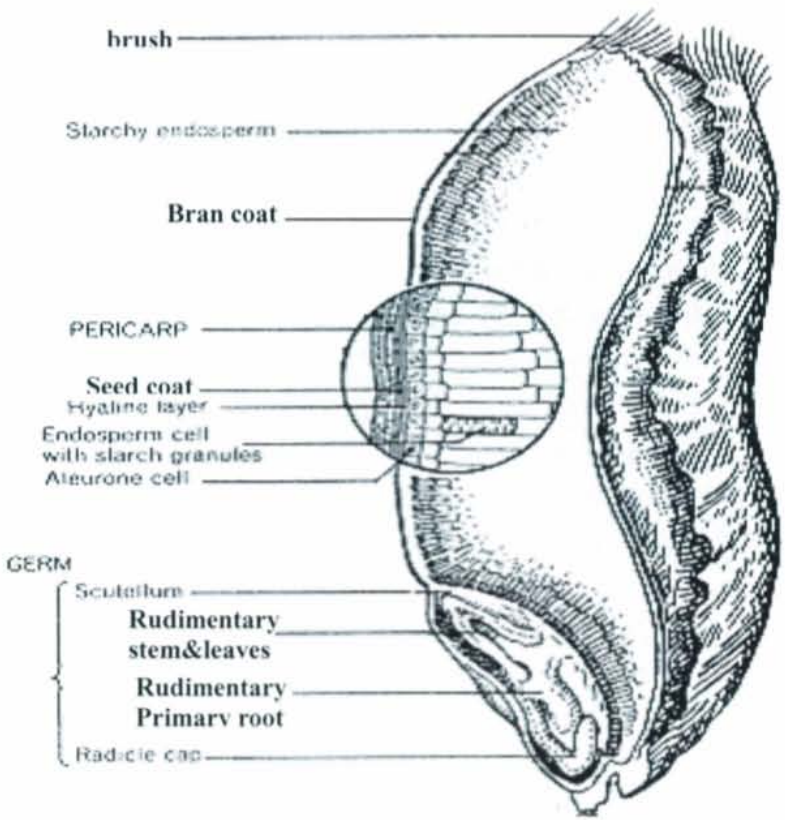


Table 2.1 Chemical composition of the whole wheat grain and its various parts (converted to percentages on a dry matter basis)

	Whole grain	Mealy endosperm	Bran	Germ
Carbohydrates	68	82	16	40
Dietary fibers	11	1.5	53	25
Proteins	16	13	16	22
Fats	2	1.5	5	7
Minerals (ash)	1.8	0.5	7.2	4.5
Other components	1.2	1.5	2.8	1.5
Total	100	100	100	100

Source: Belderok B. (2000a)

2.1.3 Composition of wheat grain

Wheat grains have either a dark, orange-brown appearance (red wheats or red-seeded wheats) or a light, yellowish colour (white wheats or whiteseeded wheats). It is composed of three distinct parts: the bran, the germ (embryo) and the mealy endosperm (Figure 2.1). The bran coat, or testa, surrounds the endosperm and embryo. The endosperm, the largest organ in the seed is surrounded by a single layer of cells, the aleurone layer. The embryo is comprised of the embryonic axis (incorporating the embryonic root and the hypocotyl), a single cotyledon, containing the first true leaves, and the scutellum (Emes *et al.* 2003). Wheat grains contain 2–3% germ, 13–17% bran and 80–85% mealy endosperm (all constituents converted to a dry matter basis) (Belderok 2000a).

The chemical composition of wheat grain is presented in Table 2.1. Most of the mealy endosperm consists of food reserves: 82% carbohydrates (mainly starch), 13% proteins and 1.5% fats. The contents of minerals (ash) and of dietary fibres are low, 0.5% and 1.5%, respectively (Belderok 2000a).

More than half the bran consists of fibre components (53%). Proteins and carbohydrates each represent 16% of total dry matter. The mineral content is rather high (7.2%). The two external layers of the grain (pericarp and seed coat) are made up of empty cells and are dead. The cells of the inner bran layer, i.e. the aleurone layer, are filled with living protoplasts. This explains the rather high

levels of protein and carbohydrate in the bran. Finally, the germ is rich in proteins, fats, carbohydrates and dietary fibres. The mineral level is also rather high (4.5%) (Belderok 2000a). The chemical composition of wheat grain is influenced by both environmental conditions and genetic (varietal) factors.

2.2 Starch

2.2.1 Introduction

Starch is an insoluble polymer of glucose residues produced by the majority of higher plant species, and is a major storage product of many of the seeds and storage organs produced agriculturally and used for human consumption. All higher plant starches are synthesized inside plastids, but their function therein will depend upon the particular type of plastid, and the plant tissue from which they are derived. Transient starches synthesized in leaves during the day are degraded at night to provide carbon for non-photosynthetic metabolism. Starch produced in tuberous tissues also acts as a carbon store, and may need to be accessed as environmental conditions dictate, whilst storage starches in developing seeds are a long-term carbon store for the next generation (Tetlow *et al.* 2004).

Most of the starch utilized world-wide comes from a relatively small number of crops, the most important being maize, potato, wheat, and tapioca with smaller amounts from rice, sorghum, sweet potato, arrowroot, sago, and mung beans. In general, starches from tapioca and sorghum are used only for food, whereas those

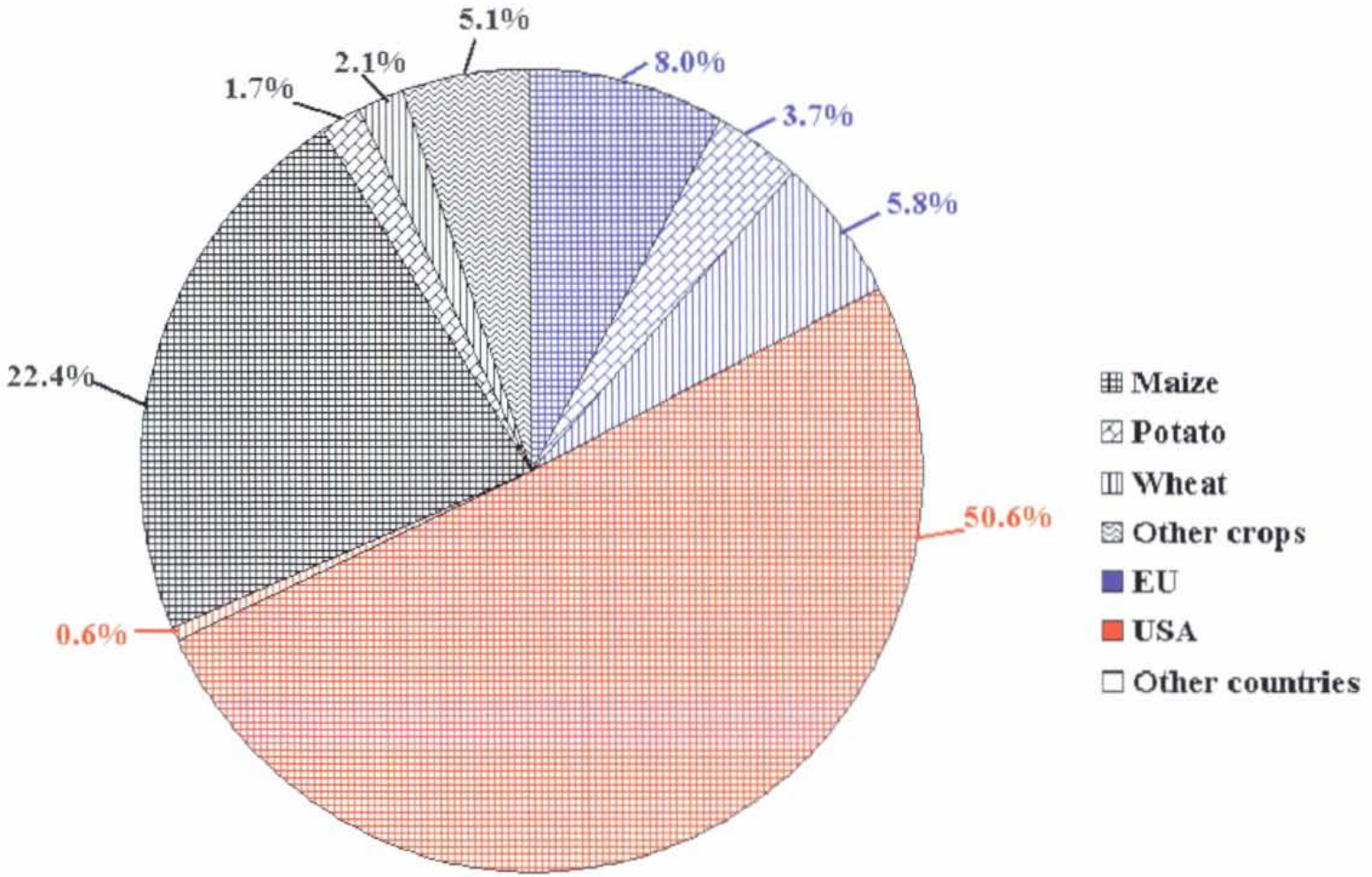
from maize, potato and wheat are used for food and other non-food purposes (Wang *et al.* 1998). Cereal crops accumulate starch in the seed endosperm as an energy reserve. This starch serves as the primary carbohydrate component in the diets of humans and livestock, and also has numerous important industrial applications (James *et al.* 2003). The amount of starch contained in a wheat grain may vary between 60% and 75% of the total dry weight of the grain. Starch occurs in seeds in the form of granules. Wheat has two types of starch granules: large lenticular and small spherical ones. The lenticular granules are formed during the first 15 days after pollination. The small granules, representing about 88% of the total number of granules, appear 10–30 days after pollination(Belderok 2000b).

2.2.2 Starch production and utilization

Starch comprises over 30% of the average diet on a dry weight basis and more than 25% on an available energy basis (Galliard & Bowler 1987).World wheat starch production was 4.1 million tons in 2000, originating from 8 million tons of wheat and occuping second position in the global starch market (>8%) after corn starch, which yields 39.4 million tons and supplies over 80% of the global starch market (Van Der Borght 2005). Starches from potato, cassava rice, barley, oats, sweet potatoes, sago, etc. share the rest of the production. The EU (42%) and USA (11%) are the two largest wheat starch producers in the world (Fig 2.2) (LMC International Ltd, 2002).

Figure 2.2 World starch production by raw material in 2000
(total world starch production—48.6 million tons)

Information from: LMC International Ltd (2002)


5.1%
2.1%
1.7%
8.0%
3.7%
5.8%
22.4%
50.6%
0.6%
Maize
Potato
Wheat
Other crops
EU
USA
Other countries

The ability of starch products to form a viscous paste when heated in water is their most important characteristic, and its hydrocolloidal properties make it suitable for a great variety of applications. Starch and its derivatives are widely used for industries as diverse as food, brewing, paper, textiles, adhesives, pharmaceuticals and building materials (Singh *et al.* 2003, Table 2.2). Starch may be converted to glucose syrup for use in foods, beverages and confectionery. Starches high in amylose content are used in the textile industry as a sizing and finishing agent; in the paper industry for adhesives, binding agents and surface sizing applications; and in the food industry as a thickening, stabilizing, gelling and encapsulating agent (Young 1984). The food industry utilizes the thickening and filling properties of starch in products such as soups, sauces, pie fillings, sausages and canned foods. Frequently, food starches are physically and chemically modified, since native starches lack certain desired functional properties such as consistent viscosity form one starch to another, region to region and year to year; color consistency; good paste clarity; cool water solubility; resistance of viscosity breakdown at low pH, or high processing temperatures and under shear, etc (Batey and Mueller 1991). Nowadays the world leading exporters of modified potato and corn starches are the EU and USA, respectively.

Wheat is unique because gluten, a storage protein of wheat, is the only cereal protein to form a strong, cohesive dough that will retain gas and produce light

Table 2.2 Application of starch and starch derivatives in food and non-food industries

Indusry type	Purpose	Starch type
Food	Thickener, stablilizer, binder, moisture retainer, fat replacer, adhesive, glaze	Native and modified starches, maltodextrins, high fructose syrups
Confectionary	Ice cream, candy, gums, marshmallows, canning, marmalade, jams	Starch, maltodextrins, maltose syrups
Beverage	Soft drinks, beer, alcohol, instant coffee	Sweeteners
Pharmaceuticals	Diluents, binders, drug delivery, encapsulation	Starch, glucose syrups, malto- and cyclodextrins, polyols
Cosmetic	Emulsifiers, humectants, face activators	Starch, sorbitan esters
Paper & cardboard	Wet end additives, spraying, surface sizing, coating	Native, cationic and hydroxyethyl starches
Textile	Sizing, finishing, printing, fire resistances	Native and modified starche
Plastics	Biodegradable filler	Starch
Detergent	Surfactants, builders, bleach activators	Sucrose derivatives
Adhesive	Case sealing, laminating, tube widing, corrugated board	Starch, dextrins
Biochemistry	Organic acids, amino acids, biopolymers, polyols, enzymes, alcohols, antibiotics	Starch hydrolysates
Other	Ceramics, coal, water treatment, gypsum, mineral fiber, oil drilling, concrete	Native and modified starches

Source: International Starch Institute (2008), Ellis *et al.* (1998), Huang (1995).

baked products. Starch gives structural and textural properties to baked products through its gelatinization and the formation of a three dimensional network between the wheat gluten. Therefore, starch, gluten, and lipid components of the flour are essential to the bread-making process. Canada, as a major wheat exporter, is trying to expand towards other products, especially facing a fast-growing Asian market for noodles and flat breads. Considerable research effort is now focused on isolating wheat genotypes with desirable starch pasting properties, as these properties have been identified as the major quality attributes for certain types of noodles and flat bread (Båga *et al.* 1999). This has resulted in the production of waxy (0-3%), normal (25-28%), and high-amylose (30-37% amylose) maize, wheat, rice, and barley cultivars (Van Hung *et al.* 2006). Furthermore, there is a growing interest in small granule starches because these starches show superior penetration power into the fabric and are less affected by humidity, which can be used to manufacuture biodegradable plastics. Starches utilized commercially have large granules (e.g. corn, potato), which are mainly used in foods such as soup mixes, fruit pie filling, gravy, salad dressings and pastes, since they show industrially desirable characteristics such as high viscosity, paste clarity, and other functional properties (Lindeboom *et al.* 2004). Small granules are also ideal for the manufacture of fine printing paper, formulations of toiletries, "talcum" powders, aerosol sprays, cold water laundry-stiffening agents, and fat replacers. Interest in

new value-added products to the industry has resulted in many studies being carried out on separation and biochemical characterization of A- and B-type starch granules in the wheat endosperm (Ao and Jane 2007, Geera *et al.* 2006, and Peng *et al.* 1999). However, commercial production and utilization of small or large granule starches is still hampered by practical limitations of starch isolation, purification, granule loss and the associated costs (Lindeboom *et al.* 2004).

2.2.3 Starch biosynthesis

During photosynthesis, transitory starch is synthesised in chloroplasts. During the night the transitory starch is degraded and transported as sucrose to amyloplasts of storage organs, where it is incorporated into storage starch. Four different type of enzymes [adenosine diphosphate (ADP) - glucose pyrophosphorylase, starch synthases, branching enzymes and debranching enzymes] have been shown to be involved in starch biosythesis (Davis *et al.* 2003, Denyer *et al.* 2001, Myers *et al.* 2000, Buléon *et al.* 1998).

2.2.3.1 ADP-glucose pyrophosphorylase

ADP-glucose pyrophosphorylase (AGPase) catalyzes the rate limiting step [production of ADP-glucose from glucose-1-phosphate and adenosine triphosphate (ATP)] in starch biosynthesis. Plastidal and cytosolic isoforms of AGPases have been identified with the cytosolic activity predominating in the endosperm (Stark *et al.* 1992).

2.2.3.2 Starch synthases

Starch synthases (SS) catalyze the elongation of glucan chains through the introduction of α(1→4) glucosidic linkages between the incoming glucose residues of ADP-glucose and the growing glucan chains. At the present time, four classes of SS are known. One class is exclusively granule bound [granule-bound starch synthase (GBSS)-type I and II], while the three other classes of starch synthases (I, II and III) may be located partially or entirely in the soluble phase (Myers *et al.* 2000). GBSS I has been shown to be responsible for amylose synthesis in plant storage organs (Nelson & Rines 1962). Amylose synthesis has been shown to occur by two different mechanisms (Ball *et al.* 1998). In the first mechanism, small-sized malto- oligosaccharides are used by GBSS1 as a primer for chain elongation. In the second mechanism, GBSS1 extends a long outer chain of amylopectin within the starch granule, which is eventually followed by a cleavage step to form a free amylose molecule. Recently, another isoform of GBSS, GBSS II, was found in the pericarp, aleurone layer and embryos of both wild type and waxy wheats (Nakamura *et al.* 1998). Therefore, GBSS II is thought to be involved in amylose synthesis in tissues other than the endosperm. SSI, SSII and SSIII have been shown to be involved in amylopectin synthesis, although the function of these enzymes is still not clear. SSI and SSII have been found in both the soluble phase and as starch granule proteins in a number of different species (Peng *et al.* 2001, Gao & Chibbar 2000). In contrast to GBSS1, which is

functional only when bound to a starch granule, it is not clear whether SSI, SSII and SII are functional enzymes when bound to the starch granule or simply become trapped inside the granule as grows (Davis *et al.* 2003).

2.2.3.3 Starch branching enzymes

Starch debranching enzymes (DBE) cleave α(1→6) glucosidic bonds of a branched α-glucan in one single step resulting in the release of the entire α(1→4) glucan chain. Based on their amino acid sequences and substrate specificities, DBE are classified into two groups: 1) limit dextrinase (also called pullulanase or renzyme), and 2) isoamylase (Nakamura *et al.* 1998). Both classes of DBE are soluble enzymes. DBE have also been shown to be involved in starch biosyntheses (Myers *et al.* 2000). However, the exact mechanism by which this occurs is still not known.

2.2.3.4 Enzymes in wheat starch biosynthesis

In wheat, the major GBSS1 synthase with a molecular weight of about 60 kDa, the so called waxy (*Wx*) protein is responsible for amylose production. The soluble starch synthases act together with the branching enzymes to synthesize amylopectin. These isoforms of the wheat *Wx* proteins, *Wx-A1*, *Wx-B1* and *Wx-D1*, have been identified (Nakamura *et al.* 1993). They are encoded by three homocologous *Wx* loci, *Wx-A1*, *Wx-B1* and *Wx-D1* located on 7AS, 4AL and 7DS, respectively (Chao *et al.* 1989). Yamamori *et al.* (1994) have found various

cultivars with null alleles for each of the three controlling loci. Miura & Tanii (1994) and Yamamori *et al.* (1992) have shown a correlation between the presence of *Wx* null alleles and a lower amylose content across cultivars. Miura & Sugawara (1996) and Miura & Tanii (1994) have shown that the three *Wx* genes have different effects on modifying amylose content, with the *Wx-B1b* allele providing the largest reduction in amylose through the lack of the *Wx-B1* protein in comparision with the other null alleles, *Wx-A1b* and *Wx-D1b*. This suggests that the wild type *Wx-B1a* predominates for amylose syntheis, followed by *Wx-D1a* and *Wx-A1a* (Miura *et al.* 1999).

2.2.3.5 Production and uses of waxy, partial waxy and high amylose wheat starches

Waxy (amylose free) wheat has been produced by combining three null GBSS1 alleles, whereas, partial waxy wheats (low amylose content) contain one or two null alleles. The reduction in amylose content seems to be the only difference between the starch of partial waxy and wild-type (three active *wx* alleles), hence, both partial waxy and wild type wheat starches are often termed 'normal' (Hayakawa *et al.* 1997). The extent of amylose reduction in partial waxy lines appears to be dependent both on the number of null alleles present, and the genetic background. Double null lines typically have lower amylose contents than single null lines, which in turn, may have lower amylose contents than wild type lines

(Yamamori *et al.* 1994). The reported ranges in amylose content of single null and wild-type wheats overlap. Even when two null alleles are present, amylose contents are reduced only by about 5%, relative to wild type (Graybosch 1998). Yamamori *et al.* (2000) have shown that elimination of SSII polypeptides in wheat increased apparent amylose concentration to 30-37%. Recently, Chibbar (2008) have developed high amylose starches (26-33% amylose) by producing wheat lines deficient in SSII A and B genome polypeptides. The wheat lines deficient in SSII from A and B genomes are currently being backcrossed into several wheat cultivars adapted to Saskatchewan growing conditions.

Commercial application of waxy wheat starch awaits the development of waxy wheat cultivars. The most likely application would be those in which waxy wheat is used as a blending wheat to develop flours with specific amylose contents. Regulating amylose content through blending of waxy and normal wheat flours could also enhance the quality of wheat-based extruded products such as pet foods, mixed-grain snack foods, or breakfast cereals. Partial waxy wheats may be of greatest value in the production of certain Asian noodles (Japanese white salted noodles [WSN]). WSN are typically produced from flour exhibiting high starch swelling volume and peak viscosity (Crosbie 1991), which confer a soft, elastic texture to the noodles. Reduced amylose content, increased flour swelling ability, decreased paste gel rigidity, and enhanced deformation of gelatinized starch

granules contribute to soft texture in WSN (Miura & Tanii 1994, Morita *et al.* 2002). Developing partial waxy wheats with varying amylose contents will provide a means for producing diverse noodle types with desired eating textures (Chibbar & Chakraborty 2005).

Starch with elevated amylose concentration (≥40%) is preferred in confectionary and pulp industries. Recently, an increased demand for high amylose wheat starch within the food industry has emerged as high-amylose starch can be converted to resistant starch (RS) upon heating and subsequent cooling. RS is not digested in the small intestine, but is broken down by the bacteria in the colon. As a result, RS acts as dietary fiber (DF), it reduces the calories from food, has low glycemic index and is considered beneficial for colon health (Topping & Clifton 2001).

2.2.4 Starch granule morphology

Visualization of starch granule is of great importance for a better understanding of the morphology and size (Jane *et al.* 1994), surface features (Jayakody & Hoover 2002), internal features (Lineback 1984), behavior of starch and starch pastes (Velde *et al.* 2002), and topography (Aguilera 2000). Morphological characteristics of starches from different plant sources vary with the genotype and cultural practices. The variation in the size and shape of starch granules is attributed to the biological origin. The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast, as well as physiology of the

Figure 2.3 Pictorial representation of the length scales within starch granules together with techniques used to characterise the structural features

Source: Tester and Debon (2000) with permission from: Elsevier Science.

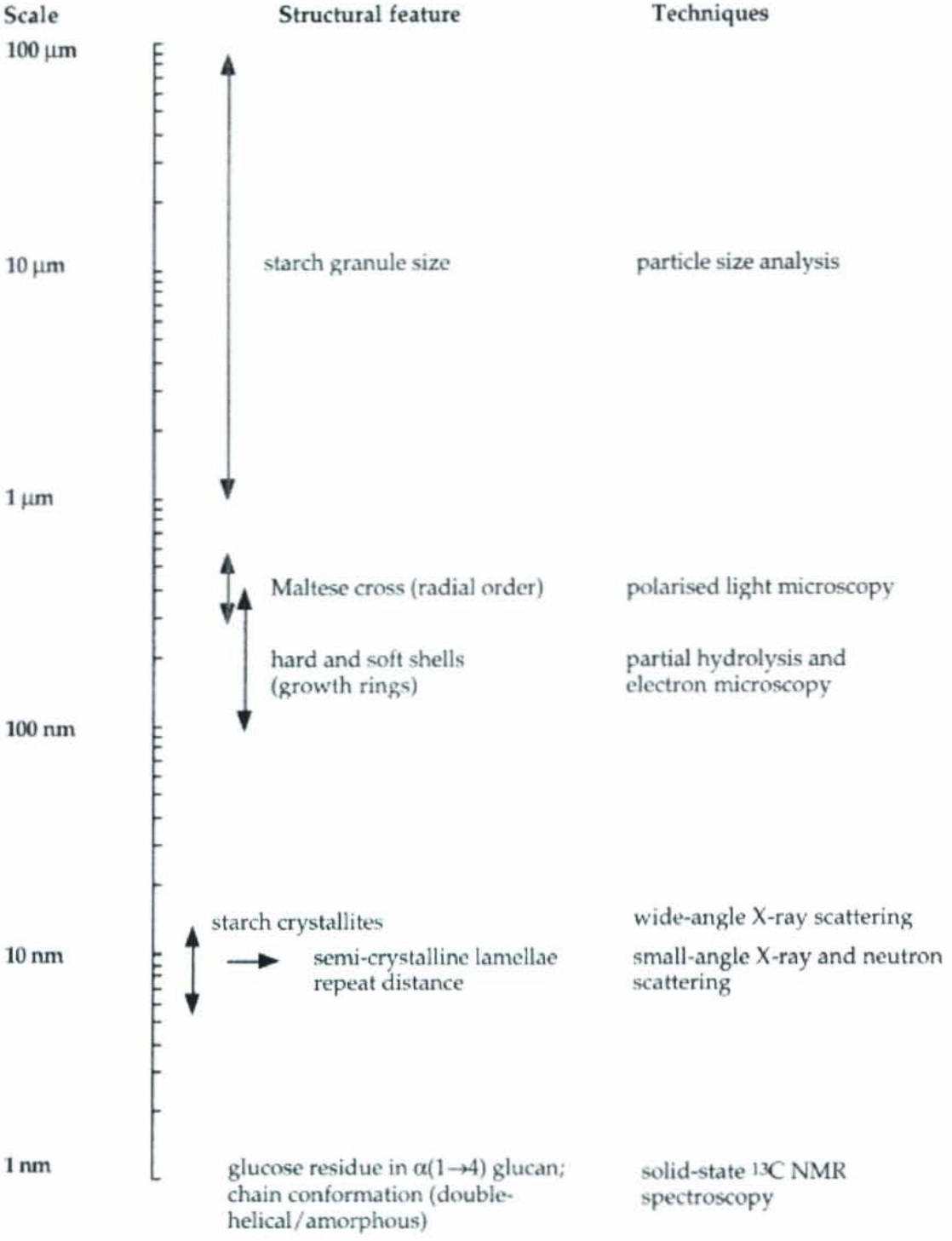

Scale
Structural feature
Techniques
100 μm
10 μm
1 μm
100 nm
10 nm
1 nm
starch granule size
particle size analysis
Maltese cross (radial order)
polarised light microscopy
hard and soft shells (growth rings)
partial hydrolysis and electron microscopy
starch crystallites
wide-angle X-ray scattering
semi-crystalline lamellae repeat distance
small-angle X-ray and neutron scattering
glucose residue in α(1→4) glucan; chain conformation (double-helical/amorphous)
solid-state 13C NMR spectroscopy

Table 2.3 Advantages and disadvantages of different granule size determination techniques

Technique	Positive	Negative
Microscopy		
Light microscopy	in situ analysis morphology data	laborious
Image analysis of optical microscopy (IAOM)	Morphology data	undamaged starch prerequisite; homogeneous sample needed
SEM	detailed morphology data	limited differentiation with non-starch
Microsieving		affected by aggregation; affected by shape low efficiency
Electrical resistance	unaffected by shape; unaffected by density	inaccurate at size<3 μm
Laser light scattering	evaluation till 0.1μm	undamaged starch prerequisite; affected by shape
Field flow fractionation	Fractionation high resolution	undamaged starch prerequisite; affected by shape; affected by density

Source: Lindeboom *et al.* (2004)

plant (Singh *et al.* 2003). With the introduction of improved analytical and microscopic techniques, the different levels of starch granule organization have begun to be visualized. The choice of microscope for high resolution visualisation of starch granule structure largely depends on the type of information required, i.e. surface or internal. Surface information may be acquired using either scanning electron microscopy (SEM) or by atomic force microscopy (AFM). Internal information regarding architectural organization at high resolution requires the use of transmission electron microscopy (TEM) (Gallant *et al.* 1997). A pictorial representation of the length scales within starch granules together with techniques used for their quantification are presented in Figure 2.3 (Tester and Debon 2000). Besides microscopic methods, nonmicroscopic techniques are also used in granule determination morphology and size. For example, micro-sieving is a nonmicroscopic technique whereby granules of different sizes are separated in a specially-designed sieving apparatus. The relative weights of starch granules above and below one or more aperture sizes are thereby determined. The electrical resistance method using the Coulter counter discriminates among particles by how they affect the electrical resistance of an electrically conductive liquid. With lower angle laser light scattering, the light of a parallel-laser beam is deflected at an angle dependent on the diameter and optical properties of the granules. Field flow fractionation techniques are a group of analytical elution methods that suspended

particles are separated in a thin flow channel by the action of a gravitational or centrifugal field directed at right angles to the channel. These are suitable for both size determination and fractionation of macromolecules and particles (Lindeboom *et al.* 2004). Table 2.3 summarizes the advantages and disadvantages of the various granule morphology size determination methods (Lindeboom *et al.* 2004).

2.2.4.1 Granule shape, size and size distribution

Generally, granule size refers to the average diameter of the starch granules. For this, spherical granules are assumed, which is seldom correct. Granule size may also be expressed as the average length of the major and minor axes, mean maximum diameter, mean granule volume or mean surface area. Granule size distributions are usually classified as monomodal (similar size) or bimodal. A bimodal size distribution of large and small granules is characteristic of wheat starches as well as those from rye and barley (Lindeboom *et al.* 2004). Variations in starch granule size (~1–100 μm in diameter), shape (round, lenticular, polygonal), size distribution (uni- or bi-modal), association as individual (simple) or granule clusters (compound) and composition (α-glucan, lipid, moisture, protein and mineral content) reflect the botanical origin (Tester *et al.* 2004a, Table 2.4). In rice, several polyhedral small (2–7μm) granules are produced in one amyloplast. They form parts of compound granules. In contrast, only one granule is produced in one amyloplast of, e.g. maize (polygonal or round, 10–15μm), wheat and potato

Table 2.4 Characteristics of starch granules from different botanical sources

Starch	Type	Shape	Distribution	Size (μm)
Barley	Cereal	Lenticular (A-type)	Bimodal	15–25
		spherical (B-type)		2–5
Maize (waxy and normal)	Cereal	Spherical/polyhedral	Unimodal	2–30
Amylomaize	Cereal	Irregular	Unimodal	2–30
Oat	Cereal	Polyhedral	Unimodal	3–10 (single)
				80 (compound)
Pea	Legume	Rentiform(single)	Unimodal	5–10
Potato	Tuber	Lenticular	Unimodal	5–100
Rice	Cereal	Polyhedral	Unimodal	3–8 (single)
				150 (compound)
Rye	Cereal	Lenticular (A-type)	Bimodal	10–40
		Spherical (B-type)		5–10
Sorghum	Cereal	Spherical	Unimodal	5–20
Tapioca	Root	Spherical/lenticular	Unimodal	5–45
Triticale	Cereal	Spherical	Unimodal	1–30
Sago	Cereal	Oval	Unimodal	20–40
Wheat	Cereal	Lenticular (A-type)	Bimodal	15–35
		Spherical (B-type)		2–10

Source: Tester *et al* (2004a).

tubers (oval, 15–100μm). Many starches have a unimodal distribution of granules, whereas those from *Triticicae* have a bimodal distribution composed of large A-type (lenticular, 10–35μm) and small B-type (spherical, 1–8μm) granule populations (Vandeputte and Delcour 2004). Mutations in the genes affecting amylose and amylopectin content show some effects on the granule size distribution. Starch from waxy barley was reported to have a greater number of starch granules per endosperm, but the average granule size was smaller than that of wild-type starches. In contrast, the gene that causes a relatively high amylose content in barley starch was associated with a reduction in the mean volume of A-granules and an increase in the mean volume of B-granules The relative quantities (numbers) of A- and B-granules were unaffected by either mutation (Lindeboom *et al.* 2004). In rice, the starch granules of high amylose mutants are irregular and rounded, while those of the wild-type are polyhedral (Ellis *et al.* 1988).

Mature wheat endosperm contains at least two distinct starch granule populations, commonly referred to as A- and B-type granules. A third population of starch granules, the C-type, was reported by Bechtel *et al.* (1990). For practical purposes, C-type granules are most often considered a subpopulation of the B-type granule fraction, as they represent only a minor portion (~3%) of the total endosperm starch by weight. For the two predominant wheat starch granule populations,

A-type granules are larger in size (>10 μm), lenticular in shape, and initiated early in the grain filling period as compared with B-type starch granules, which are smaller in size (<10μm), more spherical in shape, and initiated during the later stages of grain filling (Geera *et al.* 2006). By number, B-type (including C-type) granules comprise up to 99% of granules within the wheat endosperm, wheareas A-type granules constitute the majority of the starch (50-87%) by weight. Aside from morphological differences, significant effort has been expended to characterize and define wheat starch A- and B- type granule charateristics and properties(Geera *et al.* 2006). The two starch granule fractions differ with regard to granule composition (Ao and Jane 2007), molecular structure(Ao and Jane 2007, Geera *et al.* 2006), granule swelling (Van Hung and Morita 2005), gelatinization properties(Ao and Jane 2007, Peng *et al.* 1999), pasting/rheological behavior(Ao and Jane 2007), and reactivity to modifying agents (Van Hung and Morita 2005). These differences result in the two starch granule types being utilized differently, both in food and nonfood applications. For example, starch with predominantly B-type starch granules can be used as a fat substitute, while starch with a high percentage of A-type starch granules has applications in the manufacture of biodegradable plastic film and carbonless copy paper. Thus, wheat cultivars with predominantly B- or A-type starch granules would be very useful to the food and nonfood industries, respectively (Peng *et al.* 1999).

Granule size has an appreciable influence on its properties, because granule composition changes as the granule grows and ages (Baldwin 2001). Several factors such as starch composition (Meredith 1981), amylose-lipid complex (Chiotelli and Meste 2002), chain length distribution (Naka *et al.* 1985), gelatinization (Yusuph *et al.* 2003, Stevens and Elton 1971), crystallinity (Wong and Lelivére 1982), pasting properties (Singh *et al.* 2006, Jayakody *et al.* 2005), granule swelling (Liu *et al.* 2003), solubility (Lindeboom *et al.* 2004), susceptibility to enzyme and acid (Kulp 1973), and backing quality (Kulp 1973) have been shown to reflect granule size (Jayakody *et al.* 2007).

Starch granule size is an important factor for mouth-feel and overall texture of food products, smoothness of cosmetic products, coating of films, paper and carbon-less copy paper and extractability during malting of barley and gluten extraction from wheat. However, little is known about the factors determining starch granule size. Genetic mapping studies in wheat and barley have indicated that several loci regulate starch granule size and its distribution in these cereals (Batey *et al.* 2001, Borém *et al.* 1999). The involvement of several factors is further strengthened by studies of barley mutants with altered starch granule size distribution in which the activity of several starch biosynthetic enzymes is affected (Stahl *et al.* 2004). In wheat, a novel starch branching enzyme I (SBEI) isoform, SBEIc, may be involved in determining bimodal starch granule size distribution

pigeon pea (Hoover *et al.* 1993), and wheat (Thomas & Atwell 1999) starches. The presence of channels has been reported for corn and sorghum starches. It has been shown that channels are more abundant in normal maize than in waxy corn starch (Huber & BeMiller 1997). Central cavities, which are voids at the hilum of mature starch granules, have been reported in granules of B-type wheat (Baldwin *et al.* 1994), potato (Baldwin *et al.* 1994), canna (Hall and Sayre 1970), normal corn (Huber & BeMiller 1997, Hall & Sayre 1973), waxy corn (Chabot *et al.* 1978), sorghum (Huber & BeMiller 1997, Hall & Sayre 1973), barley (Hall and Sayre 1973), lentils (Revilla & Tárrago 1986), and rice (Baldwin *et al.* 1994) starches.

Pores at the surfaces of corn, sorghum and millet starch granules and along the equatorial grooves of large wheat, barley and rye starch granules were postulated by Fannon *et al.* (1992 & 1993) to be openings to the granule interior. Baldwin *et al.* (1994) observed void spaces on cavities in the interior regins of potato, rice and wheat starch granules, and demonstrated the partial role of dehydration in the development of these cavities. Huber & Bemiller (2000 & 1997) demonstrated the existence of cavities within corn, sorghum and millet starch granules, and provided evidence that cavities within these granules were connected to the granule exterior by a network of channels extending to the granule surface. These microstructural features were further shown to impact gramular patterns of

reaction by facilitationg the flow of reagent into the granule matrix during starch modification (Huber & Bemiller 2001, 2000).

While pores, channels and cavities of corn starch have been elucidated in more detail, these features within wheat starch granules are relatively less understood. Several studies have suggested the existence of channels and cavities in wheat starch granules. Patterns of enzymatic attack of wheat starch have been observed to produce large pin holes at granule surfaces (along the equatorial groove). This pattern of enzyme digestion is similar to that observed for corn starch (Aggarwal & Dollimore 1998), which possesses both pores and channels. Han *et al.* (2005) observed a network of channel-like protein structures within commercial wheat starch granules using a protein specific dye, suggesting that channels in wheat starch granules possess a protein component. More recently, Glaring *et al.* (2006) observed channels traversing the growth rings within larger A-type granules of wheat starch. However, most investigations to date have been confined primarily to A-type granules and have not fully assessed the nature of channels specific to wheat starch granules. Therefore, there is a need to characterize and elucidate the pore and channel structures of A- and B-type granules of normal, waxy and high amylose wheat starches.

2.2.5 Starch composition and structure

The structure of starch can be described in terms of physicochemical properties of the constituent molecules, compositional variation, interactions at the molecular level associations of molecular interactions (architecture) and the macro level of the whole granule itself. These elements are discussed in detail below.

2.2.5.1 Composition

2.2.5.1.1 Major components

Starch granules are composed of two α-glucan biopolymers, amylose and amylopectin, which represent approximately 98–99% of the dry weight. The ratio of the two polysaccharides varies according to the botanical origin of the starch (Tester *et al.* 2004a). In most common types of cereal endosperm starches, the relative weight percentages of amylose and amylopectin range between 72 and 82% amylopectin, and 18 and 33% amylose (Buléon *et al.* 1998). Generally, the 'waxy' starches contain less than 15% amylose, 'normal' 20–35% and 'high' (amylo-) amylose starches greater than about 40% (Tester *et al.* 2004a). The structure of the α-glucans is discussed below in more detail.

2.2.5.1.2 Minor components

In addition to amylose and amylopectin, starch granules contain small quantities of other 'minor' components, such as proteins, lipids, pentosans, and minerals (e.g. phosphorus and silica). The exact quantities of protein and lipid associated with

the starch depends on both the botanical origin of the starch and its degree of purification during extraction, however, a typical well-washed cereal starch sample contains ~0.25% protein and up to ~1.0% lipid, whilst a typical root or tuber starch (e.g. potato) contains 0.05% protein and 0.05 – 0.1% lipid (Baldwin 2001). Although on a quantitative scale these constituents are deemed minor, there is increasing awareness that their presence significantly affects both physicochemical properties of the granule as a whole (granular swelling, gelatinization, amylose leaching, acid and enzyme digestibility, paste clarity, pasting properties, and retrogradation) and the properties of starch-derived products(Han & Hamaker 2002, Baldwin 2001).

2.2.5.1.2.1 Lipids

Based on solubility under specific extraction conditions, lipids are classified into three categories: (1) surface or free lipids (lipids present on the surface of the granule), (2) internal, bound or starch lipids (lipids present complexed with starch chains), and (3) non-starch lipids (present in the aleurone layers and germ of the grain) (Morrison 1988). Surface lipids are mainly triacyglycerols, followed by free fatty acids, glycolipids and phospholipids (Vasanthan & Hoover 1992a) and can be extracted by mixture of chloroform and methanol in the ratio of 2:1. Wheat starch lipids constitute 1% of the granular weight, having surface lipids to the extent of 0.05% (Singh *et al.* 2003). The location of the lipids at the surface of the

starch granule is still unknown (Buléon *et al.* 1998) and very little is known about the deposition of lipid or its regulation during starch biosynthesis. The lipids bound to starch are generally polar and thus more polar solvents (e.g. n-propanol-water or water saturated butanol) and a long refluxing time (~72h) are needed to completely extract bound lipids from native starches (Vasanthan & Hoover 1992b). The major constituents of bound lipids are lysophospholipids, in particular lysophosphatidylcholine (lysolecithin) (Van Der Borght 2005).

Normal cereal starches contain approximately up to 1.5% of lipid by weight (Tester & Karkalas 2001) and trace quantities of lipids are present in legume and tuber starches (0.1-0.2%) (Hoover 2001). Most waxy starches have negligible lipids (Buléon *et al.* 1998). Cereal starches contain monoacyl lipids (free fatty acids (FFA) and lysophospholipids (LPL). Wheat, barley, rye, and other triticale starches contain almost exclusively LPL (-choline, -ethanolamine and -glycerol) with variable amounts of free fatty acids (FFAs), whereas other cereals such as maize contain mainly free fatty acids together with trace quantities of lysophospholipids (Buléon *et al.* 1998, Table 2.5).

2.2.5.1.2.2 Protein

Nitrogen present in the starch is generally considered to be present as protein. However, Morrison (1988 & 1981) reported that the nitrogen content of isolated starches represents contamination from storage proteins and lipids that contain

Table 2.5 Free fatty acids and lysophospholipids present in purified cereal starches

Source	Free fatty acid content range	Lysophospholipid content range
Barley		
Waxy	0.03–0.04	0.12–0.75
Normal	0.03–0.05	0.47–1.14
High amylose	0.05–0.09	0.86–1.36
Maize		
Waxy	0.01–0.05	0.01–0.03
Normal	0.30–0.53	0.16–0.35
High amylose	0.38–0.67	0.26–0.61
Rice		
Normal	0.22–0.50	0.41–0.86
Wheat		
Normal	0	0.78–1.19
Waxy	0	0.07–0.17

Source: Buléon *et al.* (1998); Values represent % total dry weight.

choline, ethanolamine and serine. Thus, the amount of protein content in a purified starch is a good indicator of starch purity. In general, purified starches contain less than 0.6% protein (Tester *et al.* 2004a). Average nitrogen content of well purified starches is 0.05-0.06%, and 0.25-0.5% in potato and cereal starches, respectively (Baldwin 2001, Martin & Smith 1995).

In common with starch lipids, proteins occur on the surface (and include non-starch derived proteins) and, regardless of origin, are embedded within the matrix of granules. Collectively, the proteins are referred to as starch granule associated proteins and may be associated with lipids on granule surfaces (Baldwin 2001). In wheat, the starch surface protein, friabilin, has received much attention because of its proposed association with grain hardness (Tester *et al.* 2004a, Baldwin 2001). Integral proteins have a higher molecular weight than surface proteins (~50–150 and ~15–30 kDa, respectively) and include residues of enzymes involved in starch synthesis, especially starch synthase (Baldwin 2001). Starch surface protein can be readily extracted with diluted NaCl, aqueous alkali solution or 1-2% sodium dodecyl sulfate solution at room temperature (Seguchi & Yamada 1989). However, internal protein can be extracted only after starch gelatinization. This indicates that internal proteins are interspersed with the starch matrix, whereas surface proteins are deposited on the granule surface as aggregates (Mu-Forster & Wasserman 1998).

Several physicochemical proterties, such as swelling, solubilization, gelatinization, pasting, retrogradation, and enzyme resistance characteristics, could be influenced by the presence, orientation and nature of starch granule-associated proteins (Baldwin 2001, Appelqvist & Debet 1997). Both the starch lipids and proteins have the potential to moderate starch functionality including the flavor, foam formation and color (Appelqvist & Debet 1997, Martin & Smith 1995).

2.2.5.1.2.3 Minerals

Starches also contain relatively small quantities (<0.4%) of minerals (calcium, magnesium, phosphorus, potassium and sodium) which are, with the exception of phosphorus, of little functional significance (Tester *et al.* 2004a). The phosphorus is found in three major forms: phosphate monoesters, phospholipids and inorganic phosphates and can easily quantified using nuclear magnetic resonance (^{31}P-NMR) and colorimetry and the results calculated by these two methods is in good agreement with each other (Muhrbeck & Tellier 1991). Root and tuber starches contain primarily phosphate monoesters, with an exceptionally high level in potato (0.089%). Phosphate monoesters are selectively bound to specific regions within the amylopectin molecule mainly on the primary C-6 alcohol function (61%) and less on the secondary C-3 function of the glucosyl unit (38%) (Blennow *et al.* 2002, , Bay-Smidt *et al.* 1994). Mineral fractions are negligible in cereal starches in contrast to tuber starches (Buléon *et al.* 1998). Normal cereal (wheat, maize,

rice, oat and millet) and waxy (du-waxy maize, rice) starches contain phosphorus that is mainly in the form of phospholipids such as lysophospholipids (LPL) and there is a relatively small amount of alpha-glucan phosphate monoesters (Tester *et al.* 2004a, Kasemsuwan and Jane, 1996, Hizukuri 1996).

Many desirable starch properties such as low rates of retrogradation (Jane *et al.* 1996), increased paste clarity and light transmittance (Singh *et al.* 2006, Galliard & Bowler 1987), increased peak viscosity (Wang *et al.* 2006, Jane *et al.* 1996), decreased gelatinization temperature (Palasinsky 1980), resistance to freezing and thawing (Wang *et al.* 2006, Jane *et al.* 1996, Hoover *et al.* 1988), and improved textural properties (Vasanthan *et al.* 1999) have been attributed to starch phosphate content. The high phosphate monoester content of potato starch has been shown to increase paste clarity, peak consistency, shear thinning and to decrease the rate of retrogradation (Jane *et al.* 1996, Galliard & Bowler 1987). Phosphate esters bound to C-6 carbons have been shown reduce the gelatinization enthalpy, whereas phosphate esters at C-3 have very little influence on starch gelatinization (Muhrbeck & Eliasson 1991). Phosphate esters have been shown influence starch crystallinity (Muhrbeck *et al.* 1991).

The degree of phosphorylation depends on the cultivar, granule size, growth conditions, growth temperature, type and level of fertilizer, and storage (Blennow *et al.* 2002, Cottrell *et al.* 1995, Nielson *et al.* 1994, Hizukuri *et al.* 1970).

Vasanthan *et al.* (1999) showed that in potato starch, smaller granules contain more phosphorous than the larger granules.

2.2.5.1.3 Amylose

Amylose is a relatively long, linear α-glucan containing around 99% α-(1→4)-linkage and 1% α-(1→6) linkages and differs in size and structure depending on botanical origin. Generally, the distribution of molecular size of amylose by gel permeation chromatography or high performance liquid chromatography (HPLC) is monomodal (Takeda *et al.* 1986). Amylose has a molecular weight of approximately $1x10^5$–$1x10^6$, a degree of polymerisation (DP) by number (DP_n) of 324–4920 with around 9–20 branch points equivalent to 3–11 chains per molecule (Tester *et al.* 2004a). The branched amylose molecule has been suggested to have an intermediate structure between that of linear amylose and amylopectin and is consequently often referred to as intermediate material (Takeda *et al.* 1993). Each chain contains approximately 200–700 glucose residues equivalent to a molecular weight of 32,400– 113,400. Rice starch amyloses have DP_n values of 920–1110 and they are slightly branched with 2–5 chains on average (Takeda *et al.* 1986). Wheat (DP_n 1300) and maize (DP_n 930) amyloses have a similar DP_n, whereas potato (DP_n 4920) and tapioca (DP_n 2600) amyloses have higher DP_n values (Vandeputte and Delcour 2004). A summary of the general characteristics of amylose is presented in Table 2.6. Typical levels of

amyloses in starches are 15-25%. However, waxy (*wx*) rice and *wx* maize and *wx* wheat starches can be virtually amylose free. On the contrary, mutants with high levels of amylose are also known. Amylose extender (*ae*) mutants of maize have amylose contents in a range of 50–85% (Vandeputte and Delcour 2004). Amylose content has been shown to be influenced by the date of planting (Hizukuri 1969), environmental temperature (Debon *et al.* 1998, Cottrell *et al.* 1995, Hizukuri 1969), seasonal variation (Madamba & San Pedro 1976), age of tuber (Sugimoto *et al.* 1987, Geddes *et al.* 1965), age of crop (Moorthy 2002), harvesting period (Liu *et al.* 2003), granule age and size (Jane 2006, Geddes *et al.* 1965) and length of storage (Sriroth *et al.* 1999).

The amylose molecule is presently believed to be arranged in a left-handed helix due to the natural twist present in the chair conformation of glucose (Kowblansky 1985). The helix consists of six glucose units per turn, with exterior, and central cavity diameters of 12.97Å, and 5Å, respectively (Fonslick & Khan 1989). The amylose helix is stabilized by hydrogen bonds between the hydroxyl groups of adjacent glucosyl residues and interturn hydrogen bonds located on the outer surface of the helix (Banks & Greewood 1975). Studies of light scattering, viscosity analysis and molecular weight have shown that the conformation of the amylose helix appears to be either a random helical coil (6 glucose units per turn), interrupted helix (i. e. segregated helical with 10-15 turns and linear parts in the

Table 2.6 Properties of amylose from various cereal starches

Property	Maize		Wheat (normal)	Barley		
	Normal	Amylomaize		Waxy	Normal	High-amylose
Amylose content	-	48-68	25.5-26.5	4 -11.9	25.7-28.2	37.4-40.5
Iodine affinity	20.0	19.4-19.6	19.3-20.2	19.5-19.8	20.0-20.1	1.36-1.43
Blue value	-	1.32-1.39	1.36-1.48	1.35-1.42	1.33-1.63	18.8-20.0
β-amylolysis limit	84	75-78	76-82	77-82	76-87	70-73
λ_{max}	-	645-650	645-657	643-655	643-664	643-646
DP_w (range)	390-13100	210-8940	-	-	180-17200	130-19000
DP_w (mean)	2550	1810-1990	-	-	3440-5580	4080-4920
DP_n (mean)	960	690-740	1020-1380	1560-1680	940-1570	950-1080
DP_w/DP_n	2.66	2.45-2.88	-	-	3.56-4.1	4.3-4.6
Chain length	305	215-255	270-380	460-510	210-530	350-450
Chain number	3.1	2.9-3.2	4.8	3.3-3.4	1.8-5.3	2.4-2.7
Unbranched amylose (mol%)	52	53-58	63-77	55-74	65-83	80-85
Reference	Morrison & Karkalas1990	Takeda *et al.* 1988	Morrison & Karkalas1990, Yoshimoto *et al.* 2004	Yoshimoto *et al.* 2002	Takeda *et al.* 1999, Yoshimoto *et al.* 2004	Yoshimoto *et al.* 2000

Figure 2.4 Models proposed for amylose helix conformations and amylose-lipid complex

(A) amylose conformation in aqueous solutions: (a) a random helical coil, (b) interrupted helix, (c) deformed helix/worm-like coil (B) amylose-lipid complex (C) linear-chain structure of amylose. Adapted from Banks & Greenwood (1975), and Carlson *et al.* (1979) with permission from: Edinburgh University Press, Wiley InterScience.

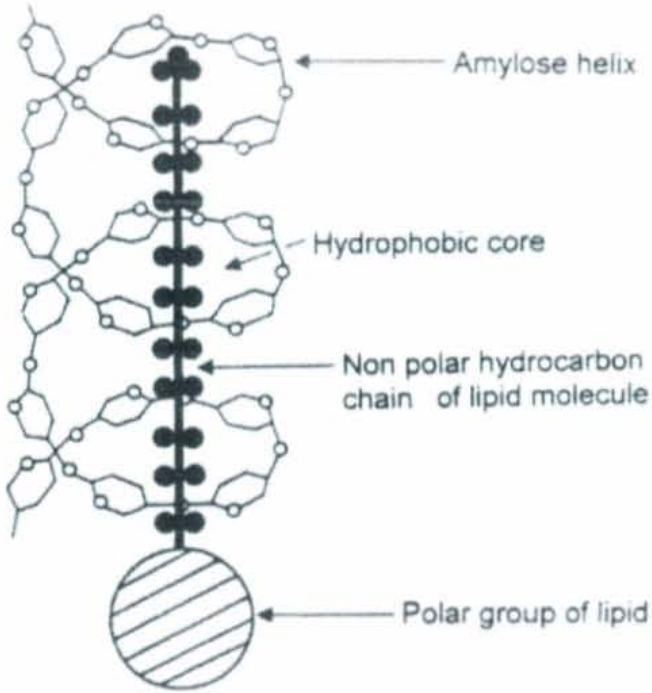
(A) Amylose conformation

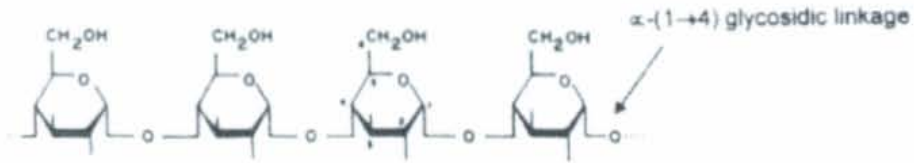
(a)

(b)

(c)

(B) Amylose-lipid complex





(C) Linear-chain structure of amylose

same molecule), or a deformed helix/worm-like coil in aqueous solution (Jayakody *et al.* 2007, Banks & Greenwood 1971, Szejtli & Augustat 1966, Rao & Foster 1963) (Fig. 2.4). Amylose in solid state shows two X-ray diffraction patterns which are similar to the A- and B-type of amylopectin crystallites in native starches (Wu & Sarko 1978a).

2.2.5.1.3.1 Amylose inclusion complexes

Amylose, the essentially linear polymer of starch, has the unique ability to form helical inclusion complexes with several hydrophobic organic and inorganic guest molecules such as lipids, iodine, dimethylsulfoxide, flavor compounds and aliphatic alcohols. The core of the amylose helix consists solely of C-H bonds and is thus hydrophobic (Godet *et al.* 1993). Consequently, the hydrocarbon chain of the fatty acid or lipid has a great affinity for the core of the amylose helix and is stabilized by Van der Waals contacts with adjacent hydrogens of amylose but the polar ends are on the outside of the helix cavity (Snape *et al.* 1998, Godet *et al.* 1993). However, Waduge *et al.* (2006) reported that lipids in starches may also be present trapped in the spaces between amylose and amylopectin. These complexing agents induce the formation of single left-handed amylose helices with a pitch of 0.805nm, also known as V-amylose (Buléon *et al.* 1998). The presence of V-amylose-lipid complexes in native barley, wheat, maize, rice, oat and amylomaize starches has been observed using the technique of ^{13}C cross

polarization magic angle spinning/NMR (^{13}C-CP/MAS-NMR) (Morrison *et al.* 1993a). In the V-form, a single chain of amylase forms a helix with a relatively large cavity, in which these complexing agents can be situated and the size of the ligand determines the number of glucosyl residues per turn (6, 7, or 8) (Snape *et al.* 1998, Figure 2.4 B). It has been reported that lipids and surfactants are required to have a minimum of 8 carbons in the fatty acid chain to form a complex and optimum complexing occurs when the fatty acid chain length is between C-12 &C-14 (Hoover & Hadziyev 1981). The crystalline melting temperature of amylose-lipid complex has been reported to occur in the range ~85-125°C (Waduge *et al.* 2006). Amylose-lipid complex exhibits a V-type X-ray diffraction pattern centered at ~20° 2θ (Evans 1986). The V-type crystalline structure is formed by single helices with 6 anhydroglucose monomer resides per helical turn (Gallant *et al.* 1992). Amylose-lipid complexes have been shown to greatly restrict the hydration capacity, granule swelling of starch, thus greatly influences functional properties of starch (Hoover 2001).

2.2.5.1.3.2 Location of amylose

Although the major component of the amorphous region of the starch granule is amylose, its exact location relative to the amylopectin crystallite is not fully understood. Blanshard (1986) proposed that amylose is present in the amorphous area partially co-crystallized with amylopectin (e.g. potato starch). Kasemsuwan &

Jane (1994) and Jane *et al.* (1992), showed by cross-linking studies on normal maize and normal potato starches, that amylose is randomly interspersed as individual molecules in both the amorphous and crystalline regions of the granule. Jenkins and Donald (1995) showed that an increase in amylose content increases the size of the crystalline portion of the amylopectin cluster and that amylose acts to disrupt the packing of the amylopectin double helices within the crystalline lamella. The authors suggested that the disrupting effect of amylose on amylopectin structure could be due to: (1) co-crystallization of a portion of an amylose chain into a hybrid amylose/amylopectin helx within the crystalline lamellae, (2) penetration of amylose into the amorphous regions where the α-(1→6) branch points are located. Atkin *et al.* (1999) found that the location of amylose within granules differed with different amylose contents. They observed that in starches with low amylose content (e.g. potato), amylose was mainly localized in amorphous growth rings alternating with semicrystalline growth rings, whereas in high-amylose starches (e.g amylomaize), amylose was localized in an independent region between the amylopectin center and the outer surface. Hayashi *et al.* (2004) have shown (using I_2 staining) that amylose is mainly present in the central portion of granules of waxy and normal wheat starches.

2.2.5.1.4 Amylopectin

Amylopectin is a much larger molecule than amylose with a molecular weight of $1x10^7$–$1x10^9$ (Buléon *et al.* 1998, Mua & Jackson 1997) and a highly branched structure built from about 95% (1→4)-α- and 5% (1→6)-α-linkages . The DP_n is within the range 9600–15,900 but comprises three major species with DP_n 13,400–26,500, 4400–8400 and 700–2100 (Takeda *et al.* 2003). The molecular size, shape, structure and polydispersity of the molecule varies with botanical origin. Unlike amylose, however, there is great additional variation with respect to the unit chain lengths and branching patterns.

Amylopectin has a polymodal distribution with A (chain length (CL) 12–16) and B chains, i.e. B_1 (CL 20–24), B_2 (CL 42–48), B_3 (CL 69–75) and B_4 (CL 104–140) chains. A and B_1 chains form a single cluster, whereas B_2, B_3 and B_4 extend into 2, 3 and more than 4 clusters. Moreover, 80–90% of these amylopectin chains constitute a single cluster (A and B_1 chains with CL 14–18), whereas approximately 10% form B_2 chains connecting two clusters, 1-3% form B_3 and very few (0.1-0.6%) form B_4 chains connecting three and four inter-clusters (B_2, B_3 and B_4 chains with CL 45–55) (Vermeylen *et al.* 2004, Jane *et al.* 1999, Hizukuri 1996). Hanashiro *et al.* (2002) suggested that C chains are very similar among botanical sources ranging in size from 10 to 130 glucose units, with most being around 40 glucose units. The average chain length of most amylopectins are

in the range 18 to 24 (Hizukuri 1996). Branch chain-length distributions of amylopectins of wheat, triticale, and barley starches are shown in Table 2.7. In general the average chain length of cereal starches (23–29) are shorter than tuber (30–44) and legume starches (26–29) (Hizukuri 1985).

Granule size has been shown to influence branch chain-length distribution of amylopectin (Ao & Jane 2007, Jane 2006). Studies on the structures of amylopectin molecules isolated from the A- and B-granules of wheat, barley and triticale have shown that the amylopectin molecules of the disk-shaped, large A-granules and the spherical, small B-granules have different structures. The amylopectin molecules of the A-granules consist of substantially more B2 chains that extend through two clusters and lesser A and B1 chains that remain in a single cluster than the amylopectin of the B-granules (Ao & Jane 2007, Takeda *et al.* 1999).The amylopectin molecules that consist of more B2 chains extending through two clusters and carrying other B1 and A chains are likely to have cylindrical-shaped structures (Ao & Jane 2007). The cylindrical-shaped molecules, in turn, can be aligned into a platelet-structure, which agrees with the parallel molecular arrangement in the disk-shaped starch granules revealed by optical map studies (French 1972). In the disk shaped starch granules, starch molecules are perpendicular to the flat surface of the disk-shaped granule. Consequently, there is an equatorial groove on the starch granule where starch molecules are less orderly

packed and are more susceptible to enzyme and chemical attack, whereas, the amylopectin of the B-granule starch consists of more A and Bl chains that remain in one cluster and fewer B2 chains (Jane 2006). Amylopectin molecules consisting of more short chains and larger degrees of branching tend to have cone-shaped molecules and fit into a spherical starch granule. Models of the amylopectin structures and the starch granules are in Fig. 2.5 (Ao & Jane 2007).

Parallel arranged cylindrical amylopectin molecules in A-granule is expected to display larger crystallinity the radial arranged cone-shaped amylopectin in the B-granule, which agrees with the results of crystallinity of waxy barley A-granule starch (36.6%) and waxy barley B-granule starch (33.0%) (Tang *et al.* 2002a). Normal barley A-granule I starch, however, has less crystallinity than normal barley I B-granule starch because the A-granule starch consists of more amylose (28.1%) than the B-granule starch (23.0%) (Jane 2006).

Finally, although far less documented, amylopectins of starches from the same and different botanical origin: (i) may have a different branching pattern and (ii) have non-randomly distributed amylopectin branch points (Dang & Copeland 2003, Thompson 2000).

Table 2.7 Branch chain-length distributions of amylopectins of wheat, triticale, and barley starches

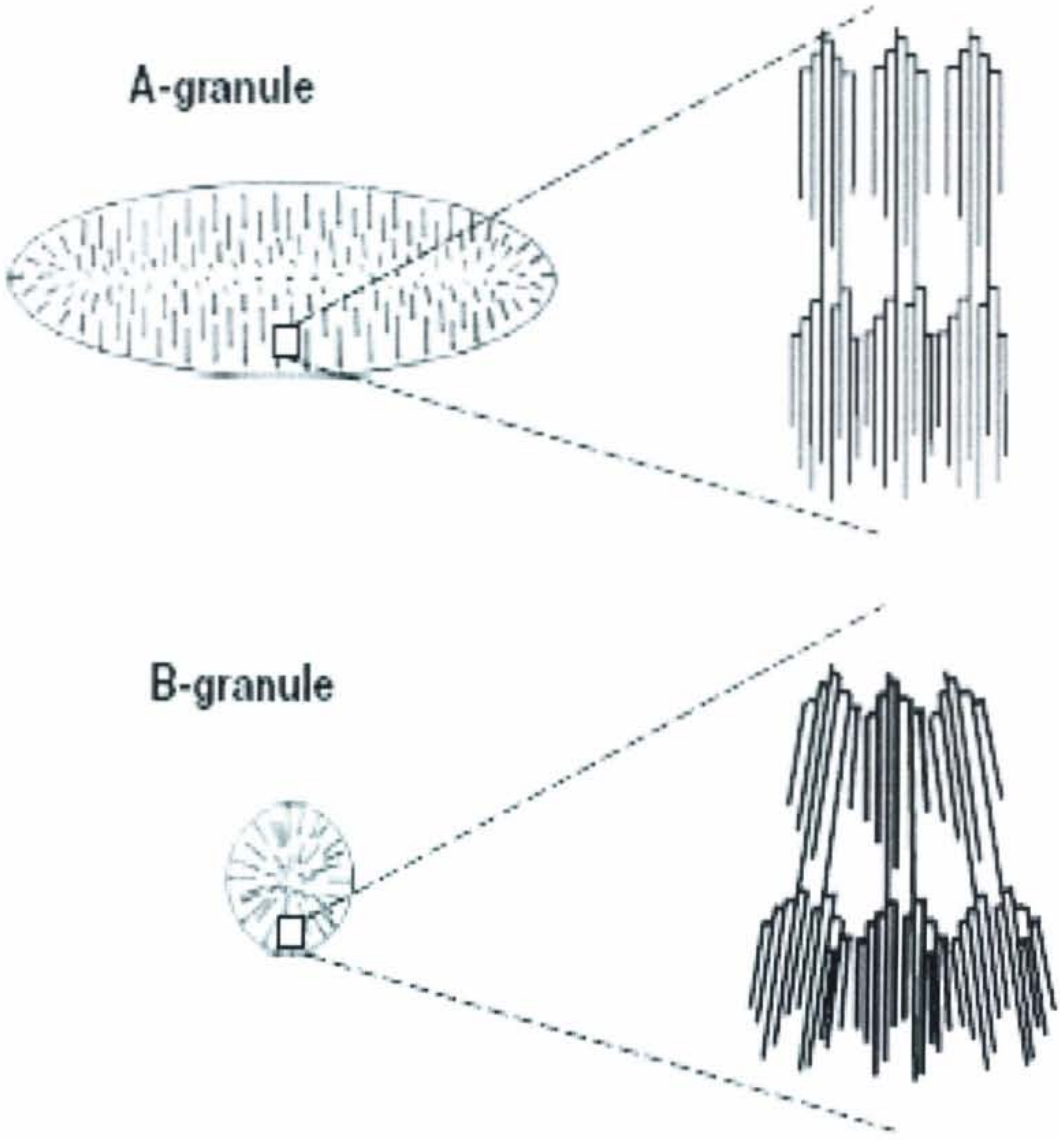
Sample	Peak DP		Average CL	Distribution (%)				
	I	II		DP 6–9	DP6–12	DP13–24	DP25-36	DP≥ 37
Wheat	11	43	23.3	5.6±0.1	23.2±0.3	42.0±0.6	18.0±1.3	16.8±1.6
Wheat A-granule	12	47	24.2	4.9±0.0	21.8±0.0	43.3±0.3	15.1±0.2	19.7±0.1
Wheat B-granule	11	47	21.8	6.9±0.1	25.2±0.1	46.8±0.4	13.5±0.2	14.2±0.5
Triticale	12	47	23.8	5.5±0.1	21.8±0.2	44.4±0.1	16.1±0.2	17.7±0.2
Triticale A-granule	12	50	25.0	4.8±0.1	20.0±0.3	41.9±0.5	18.2±0.5	19.9±0.3
Triticale B-granule	11	50	23.4	6.3±0.2	22.6±0.2	43.8±0.7	17.3±0.5	16.4±0.0
Barley	12	50	25.7	4.0±0.0	16.9±0.1	41.8±0.3	21.0±0.2	20.3±0.2
Barley A-granule	12	50	26.7	3.7±0.0	15.3±0.2	40.3±0.2	21.4±0.2	22.9±0.2
Barley B-granule	12	50	24.9	5.3±0.2	18.9±0.2	42.6±0.1	18.4±0.0	20.0±0.1

Source: Ao & Jane (2007).

Figure 2.5 Proposed granular and molecular structures of the A- and B-granules

The amylopectin branch-structure of the A- and B-granules were constructed on the basis of the molar ratios of short chains (A and B1 chains) to long chains (B2 and longer chains) of amylopectin branch-chain numbers of wheat A- and B-granule starches. Amylopectin molecules of the A-granule starch consisted of more long chains but lesser short chains, which displayed a cylindrical shape and better aligned parallel into a diskshaped A-granule. Amylopectin molecules of the B-granule starch consisted of more short chains but lesser long chains, which displayed a cone shape. The cone-shaped molecules fitted in a spherical B-granule.

Source: Adopted from Ao & Jane (2007) with permission from Elsevier Science.


A-granule
B-granule

2.2.5.1.4.1 Crystallinity and polymorphism

The amylopectin side chains form alternating amorphous and crystalline lamellae (Daniels & Donald 2004). The amylopectin double helical side chain clusters form crystalline lamellae while branching regions of amylopectin molecules form amorphous regions (Fig. 2.6). According to the model proposed by Robin *et al.* (1974), the structural periodiaty of semicrystalline starch granules is formed by repeating layers, which are approximately 9-10 nm thick and consist of crystalline lamellae of 5-6 nm thickness and amorphous lamellae with a thickness of about 4-5nm. The amorphous lamellae contain most of the amylose together with the amylopectin side chains. It has also been suggested that some amylose chain segments co-crystallise with the short side amylopectin chains within the crystalline lamellae or form amylose tie chains (Yuryev *et al.* 1998, Jenkin & Donald 1995, Zobel 1988). As discussed above, double helices are mostly formed by the exterior chains of amylopectin. These form more or less ordered arrays where the ordered structures are crystalline entities. The proportion of double helices within native granules are presented in Table 2.8. It should be emphasised, that not all of the amylopectin fraction in starches form double helices. Recently, Qi *et al.* (2003) showed by studies on rice starches that in fact >80% of the short chains of amylopectin can form double helices.

Native starch granules are characterised by either an A-type (cereal starches of, e.g

Figure 2.6 Schematic diagram of starch granule lamellar structure

Source: Adopted from Daniels & Donald (2004) with permission from ACS Publications.

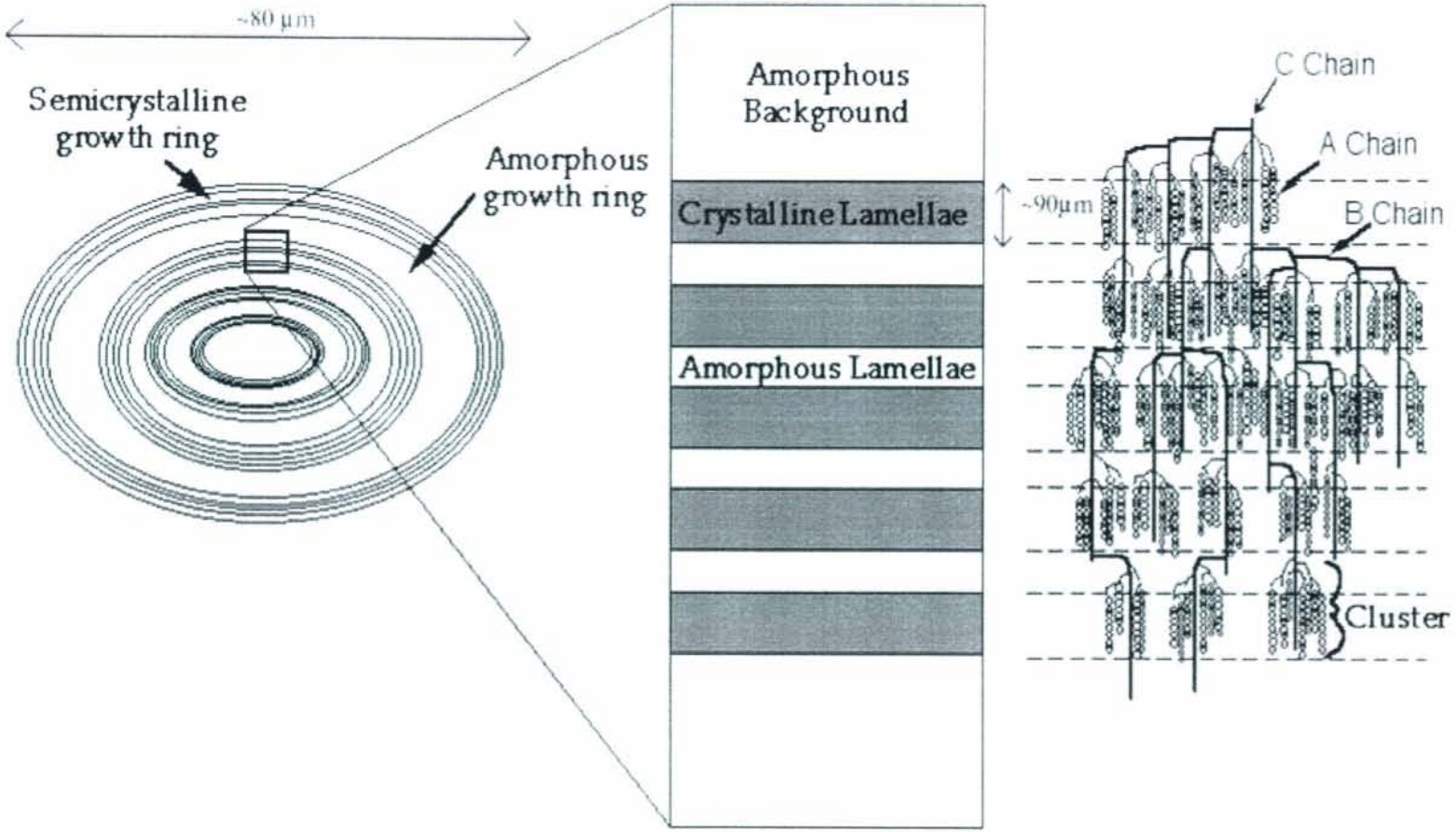

~80 µm
Semicrystalline
growth ring
Amorphous
growth ring
Amorphous
Background
Crystalline Lamellae
Amorphous Lamellae
~90µm
C Chain
A Chain
B Chain
Cluster

Table 2.8 Proportion of double helical material in starches of different botanical origins

Native starch	Amount of double helical amylopectin (%)	References
Wheat		
Normal	32	Bogracheva *et al.*, 2001
Normal	39	Cooke and Gidley, 1992
Normal	46	Morrison *et al.*, 1994
Maize		
High amylose	38	Gidley and Bociek, 1985
Normal	43	Cooke and Gidley, 1992
Waxy	48	Cooke and Gidley, 1992
Potato		
Normal	40-50	Cooke and Gidley, 1992; Gidley and Bociek, 1985
Waxy	51	Bogracheva *et al.*, 2001
Tapioca	44	Cooke and Gidley, 1992
Pea	41–48 (includes mutants)	Bogracheva *et al.*, 2001

Source: Adapted from Tester *et al.* (2004a).

rice, wheat, maize), a B-type (tuber and root starches of, e.g. potato and tapioca, retrograded starches of any botanical origin, high amylose cereal starches such as high amylose maize starch), or a C-type (leguminosae starches of, e.g pea, bean and tropical starches of e.g. cassava starch) X-ray diffraction pattern (Katz 1928). The A- and B-types are believed to be independent. However, the C-type starches are suggested to be mixtures of A-type and B-type starches and C-type is a superposition of the A- and B-types in various proportions (Buléon *et al.*, 1998). The C-type has been further classified into subgroups as C_a (> 95% A-type), C_b, and C_c (50% A-type) based on the extent of their relative resemblance to A-type and B-type or between the two types, respectively (Hizukuri *et al.* 1960). Besides A-, B-, and C-type starches, V-type diffractions patterns exist, which reveal the presence of complexed amylose (Vandeputte & Delcour 2004). Polymorphic transition by thermal treatment has been observed to follow the order: B-, C-, and A-types (Jacobs & Delcour 1998). The A-type crystallinity is more thermodynamically stable and cannot be converted to the B- or C-type by hydrothermal treatment (Kiseleva *et al.* 2004). However, the exact mechanism of polymorphic transformation under hydrothermal treatment is not known.

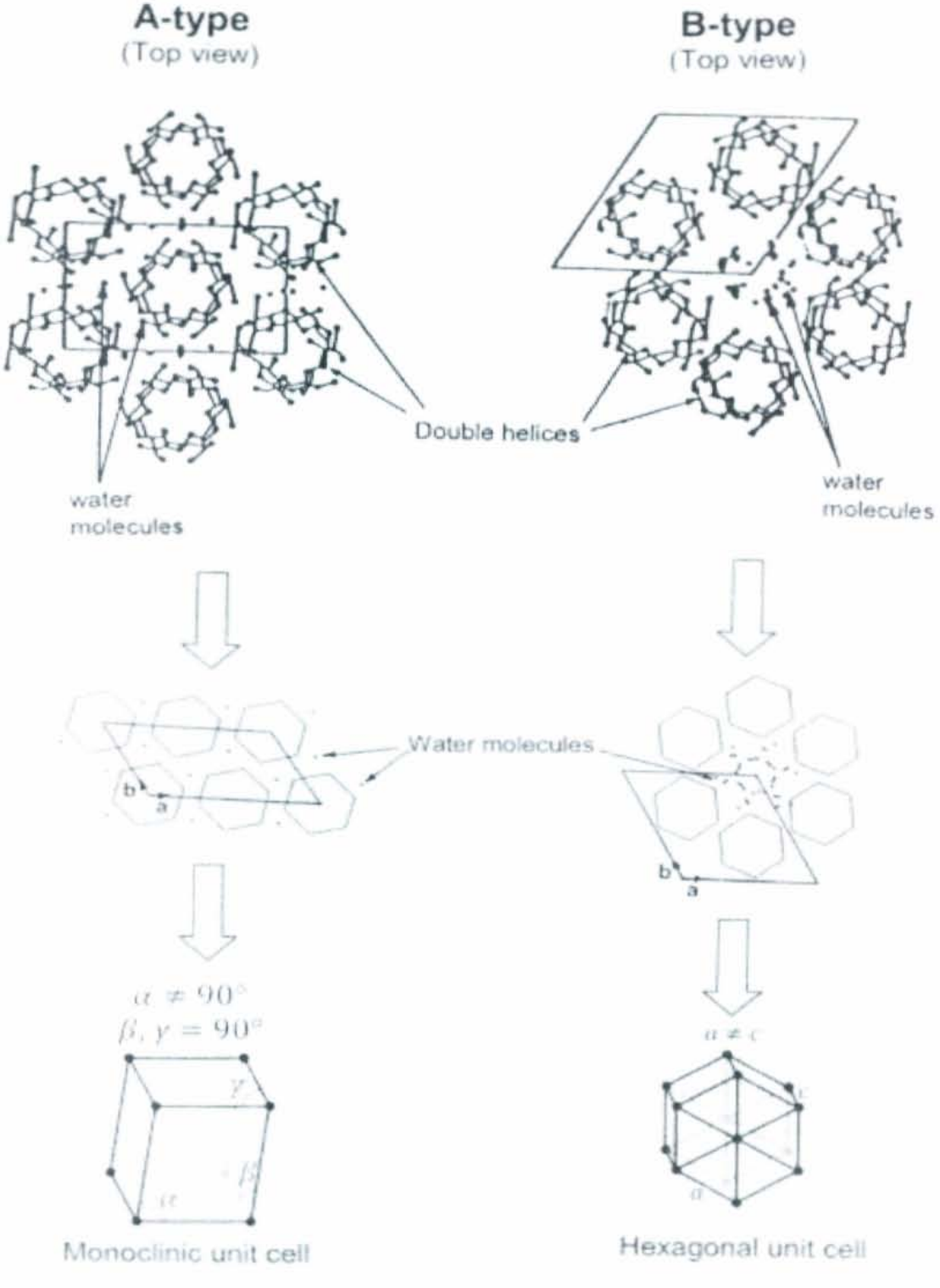
Imberty *et al.* (1988) and Imberty and Pérez (1988) proposed a model for the structure of A-type and B-type starches. Electron diffractograms from micron-sized, needle shaped crystals, revealed a monoclinic (a = 2.12 nm, b = 1.17

nm, c = 1.07 nm, γ = 123.5°) and hexagonal unit cell (a = b = 1.85 nm, c = 1.04 nm, γ = 120°) for the A-type and B-type structures, respectively (Fig. 2.7). A-type structures are densily packed with only 4 water molecules per 12 glucose molecules per A-type unit cell, whereas B-type structures are more open with 36–42 water molecules per unit cell (Fig. 2.7). Moreover, the double helix structure for both types are similar: left-handed parallel stranded double helices with double helices repeats within 2.1 nm (6 glucosyl residues) (Vandeputte & Delcour 2004).

Whether starches are of the A-, B- or C-type, depends on several factors. Firstly, Hizukuri *et al.* (1983) postulated that amylopectin chain length is the major determinant of crystalline polymorphism among native starches. Amylopectins of B-type starches have higher proportions of longer amylopectin chains than A-type starches. Indeed, mild acid hydrolysis of regular A- and B-type starches (e.g. Bogracheva *et al.* 1999, Jacobs *et al.* 1998a, Jane *et al.* 1997) indicates that B-type starches have thicker crystallites, and hence longer external amylopectin chains. Secondly, it was envisaged that, besides the differences in CL, the branching patterns of the different type of starches may also differ. Several studies affirm this reasoning. Lateral distances between various double helices in A- and B-type crystalline unit cells differed (Imberty *et al.* 1991). Moreover, computer simulations on pairs of double helices coupled to one another through an internal

Figure 2.7 Hexagonal packing arrangement of double helices in A-type and B-type starches

(dots indicate water molecules). Source: Wu & Sarko (1978a,b) with permission from Elsevier.


A-type
(Top view)
B-type
(Top view)
Double helices
water molecules
water molecules
Water molecules
b
a
b
a
$\alpha \neq 90^\circ$
$\beta, \gamma = 90^\circ$
$a \neq c$
Monoclinic unit cell
Hexagonal unit cell

α-(1–6) linkage revealed that internal chain lengths determine the lateral distance between the double helices in stable conformations (O'Sullivan & Pérez 1999). Thirdly, analyses on acid resistant starch fractions of A- and B-type starches indeed indicated a different branching pattern for both starch types (Jane *et al.* 1997). Moreover, Gérard *et al.* (2000) suggested that amylopectin clusters with numerous short amylopectin chains and short average internal chain length (ICL) crystallize into the A-type crystal polymorph. In contrast, amylopectin clusters with fewer but longer chains and longer ICL crystallize into a B-type crystal polymorph. The different branching density in amylopectin clusters crystallized into the A- or B-polymorph has recently been confirmed (Vandeputte & Delcour 2004, Takeda *et al.* 2003, Hanashiro *et al.* 2002).

Both wide angle X-ray scattering (WAXS)and small angle X-ray scattering (SAXS) can be used to determine polymorphic patterns and granule crystallinity. WAXS quantifies crystalline order throughout starch granules, but SAXS quantifies differences (periodicity) at the level of amorphous-crystalline lamellae radiating from the hilum to the periphery of starch granules (Tester & Debon 2000). In general, the scanning range of WAXS varies from 3 to 35°2θ. This range encompasses all major diffraction peaks. Cereal starches have been shown to exhibit an A-type X-ray pattern with reflections centered at ~15.3, 17.0, 18.0, 20.0 and 23.4°2θ angles. The B-type starches exhibit reflections at ~5.5, 15.0, 17.0,

19.7, 22.2 and 24°2θ angles. Whereas, C-type starches exhibit major peaks centered at ~5.5, 17.0, 18.0, 20.0 and 23.5°2θ (Jayakody *et al.* 2005, Cheetham & Tao 1998, Shi & Seib 1992, Zobel 1988) (Fig. 2.8).

X-ray determinations of crystallinity include determinations of 'absolute' and 'relative' crystallinity (Buléon *et al.* 1998). The former differentiates between the amorphous and crystalline component (integrated area) of the X-ray diffractogram. The latter relies on calculating the proportion of crystallinity within starch granules using as references, materials with 0 and 100% crystallinity. The '0%' reference representing 'fully amorphous' material (e.g. freeze-dried gelatinised material) with the '100%' reference usually being generated by extensive acid hydrolysis of starch in which all the amorphous (but not crystalline) material has been eroded (Buléon *et al.* 1998). Broad X-ray diffraction peaks indicate either imperfect or relatively small crystallites while sharp peaks indicate more perfect or sufficiently large crystallite (Cooke & Gidley 1992).

Several researchers have shown by studies on potato (Tester *et al.* 1999), rice (Asaoka *et al.* 1985), and sweet potato (Noda *et al.* 1997) starches that crystallinity is influenced by environmental conditions and harvesting time. Starch granule crystallinity is influenced by several factors: the method of sample preparation (Cottrell *et al.* 1995), moisture content (Buléon *et al.* 1998), method of starch drying (Ahmed & Lelivére 1978), granule size (Wong and Lelivére 1982), chain

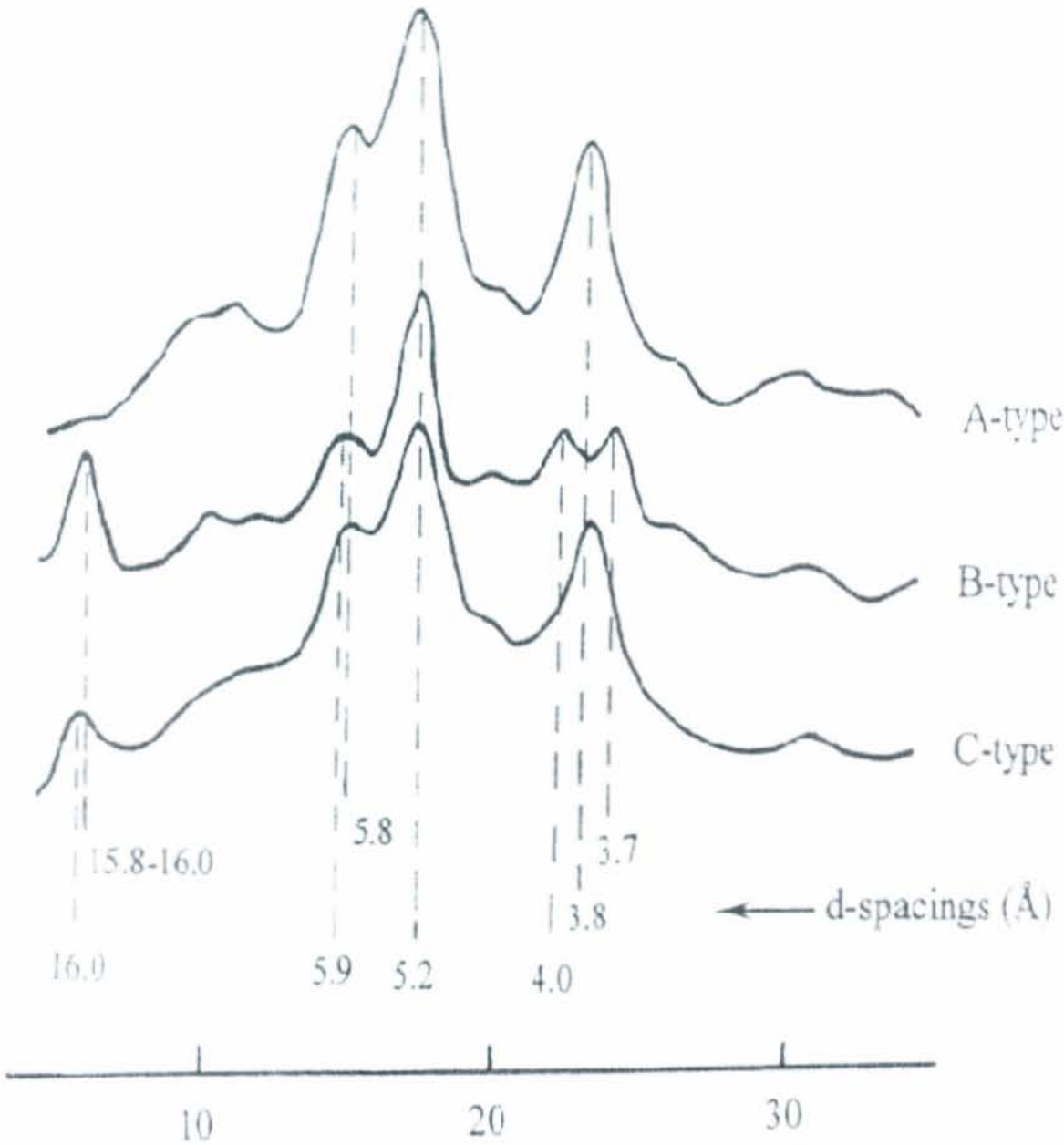
Figure 2.8 X-ray diffraction patterns of A-, B-, and C-type starches with their characteristic d-spacing

A-type: shows strong peaks at 15.27 2θ or with a inter-crystalline spacing d=5.8 Å and 23.4 2θ (d=3.8 Å), and an incomplete doublet *al* 17.05 2θ (d=5.2 Å)and 18.1 2θ (d=4.9 Å). The d-spacing at 4.4 Å is characteristic to amylose-lipid complex.

B-type: shows a peak at 5.52-5.6 2θ (d=15.8-16.0 Å), a broad medium intensity peak at 15.01 2θ (d=5.9 Å), the strongest peak at 17.05 2θ (d=5.2 Å) and medium intensity peaks at 19.72 2θ (d=4.5 Å), 22.22 2θ (d=4.0 Å) and 24.04 2θ (d=3.7 Å). There is a peak at 5.0 2θ (d=17.70 Å) which is characteristic to B-pattern.

C-type: shows the same pattern as A-type except the occurrence of the medium to strong peak at about 5.52 2θ (d=16.0 Å).

Source: Zobel (1988) with permission from Wiley InterScience.


A-type
B-type
C-type
15.8-16.0
5.8
3.7
3.8
d-spacings (Å)
16.0
5.9
5.2
4.0
10
20
30
Diffraction angle, 2θ

length of amylopectin (Biliaderis *et al.* 1981), degree of amylopectin branching (Cottrell 1995), crystal size (Jayakody *et al.* 2005), orientation of the double helices (Jayakody *et al.* 2005), number of crystallites (Jayakody *et al.* 2005), extent of packing of double helices within the crystalline lamella (Jayakody *et al.* 2005), amylose conten (Waduge *et al.* 2006), and extent of disruption of amylopectin crystallites by amylose (Jenkins & Donald 1995). Generally, an increase in moisture content inceases crystallinity. In native dry starch, the double helices are not properly aligned, however, the alignment of double helices improves with increase in moisture content. Consequently, X-ray diffraction is higher in hydrated than in dry starch (Hermans and Weidinger 1948). Crystallinity progressively decreases with increase in amylose content (Waduge *et al.* 2006). The amount of crystallinity within different starch granules has been presented in Table 2.9. Granular crystallinity plays a significant role in the granular architecture and physicochemical properties, such as the susceptibility to acid and enzyme (Tang *et al.* 2002b), insolubility in cold water (Tang *et al.* 2006), granular integrity (Jayakody *et al.* 2005), granule swelling (Jayakody *et al.* 2005), and pasting properties (Jayakody *et al.* 2005). Wong and Lelivére (1982) reported that in wheat starch, small granules are more crystalline than large granules.

Table 2.9 Proportion of crystalline material in starches of different botanical origins

Native starch	Amylose content (%)	Degree of crystallinity(%)	References
Wheat			
Normal	25.6	27.7	Van Hung *et al.* 2007
Waxy	1.0	30.0	Van Hung *et al.* 2007
High-amylose	37.5	9.4	Van Hung *et al.* 2007
Maize			
Normal	28	30	Cheetham and Tao 1998
Waxy	0	42	Cheetham and Tao 1998
High-amylose	65	18	Cheetham and Tao 1998
Potato	29	26	Yusuph *et al.*2003
Lentils	24	19	Hoover and Ratnayake 2002

2.2.6 Physicochemical properties

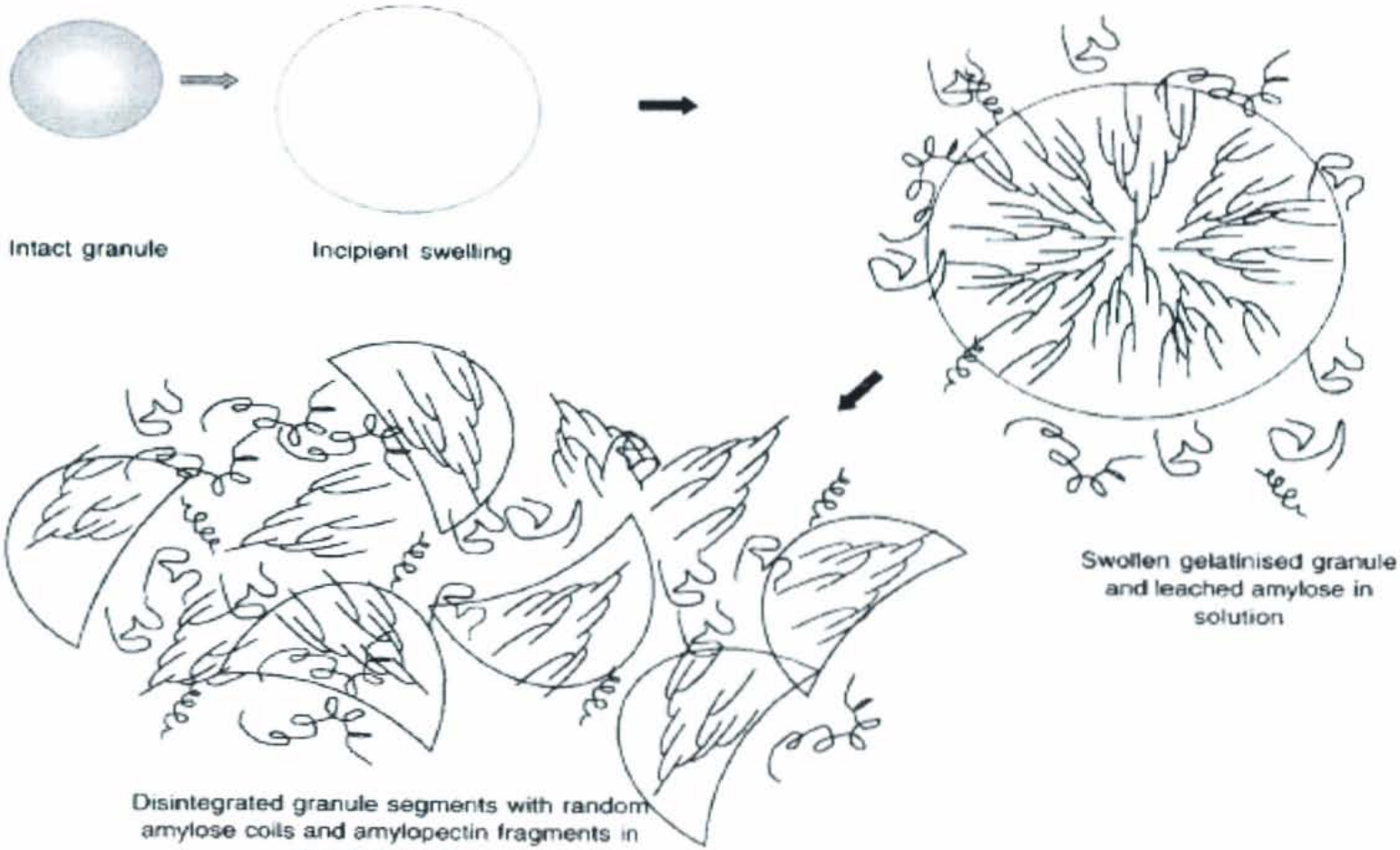
2.2.6.1 Granular swelling

When starch is heated in excess water, the crystalline structure is disrupted (due to dissociation of hydrogen bonds) and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of the starch components. This leads to an increase in granule swelling (Fig. 2.9, Tester *et al.* 2004b) and amylose leaching (Liu *et al.* 1999, Tester & Morrison 1990a,b). Until a certain temperature (the onset of gelatinization) is reached, the water uptake is reversible, but after this point, the changes are irreversible (Eliasson & Gudmundsson 1996). The irreversible swelling starts at a temperature corresponding to the onset temperature (To) in DSC measurements. Debet and Gidley (2006) have shown that although the temperatures of structural disorganization (as monitored by loss of birefringence or by DSC) are relatively similar, yet swelling profiles show major differences. This illustrates the fact that starch swelling rate and extent cannot be predicted directly from a knowledge of the thermally induced loss of granular order.

The extent of granular swelling has been measured by gravimetry (Leach *et al.* 1959), colorimetry (Tester & Morrison 1990), and laser light scattering (Ziegler *et al.* 1993) expressed as swelling power [SP] and as swelling factor [SF] (Tester & Morrison 1990). The SP is defined as weight of a sedimented starch gel, relative to

Figure 2.9 Idealised diagram of the swelling and gelatinisation of a starch granule in the presence of water

Source: Tester *et al.* (2004b) with permission from Cambridge University Press.


Intact granule
Incipient swelling
Swollen gelatinised granule and leached amylose in solution
Disintegrated granule segments with random amylose coils and amylopectin fragments in aqueous solution.

its dry weight, obtained after gelatinizing starch in excess water at a given temperature for specified time followed centrifugation (Crosbie 1991). SP is expressed on a weight basis (g/g). The SP measures both the intergranular and intragranular water (Tester & Morrison 1990a,b). The SF is defined as the ratio of the swollen volume to the initial volume of air dried starch and has no units (Tester & Morrison 1990a,b). The measurement of SF was based on the observation that blue dextran dye (molecular weight 2x106 Da) will dissolve in the supernatant and interstitial water but not in the intragranular water. Hence, SF measures only the water that enters the granule and hence contributes to volume expansion on heating (Tester & Morrison 1990a,b).

In general, starches from legumes, roots and tubers exhibit a single-stage swelling (Hoover & Sosulski 1986, Hoover 2001), whereas, normal cereal starches show a two-stage swelling (Leach *et al.* 1959, Langton & Hermansson 1989). Single stage swelling pattern indicates relaxation of bonding forces within starch granules over one temperature and not at multiple temperature ranges because bonding forces are more uniform and stronger, whereas, two stage swelling indicates that there are two types of forces within granule structure which requires different energy inputs to weaken starch chain interactions (Soni & Agarwal 1983). Among different starches, the extent of granular swelling follows the order: waxy >normal >high amylose (Debet & Gidley 2006). This is also true in wheat starches at

heating temperatures higher than 50°C (Van Hung 2007).

Granular swelling is primarily a property of intact amylopectin, and amylose acts as a diluent (Tester & Morrison 1990). Jenkins *et al.* (1994) showed that the initial absorption of water and the location of swelling has been shown to be influenced by: 1) botanical source (Debet & Gidley 2006), 2)amylose content (Tester & Morrison 1990), 3) amylose- lipid complex (Tester & Morrison 1990, Gunaratne & Hoover 2002), 4) starch chains interaction (Hoover & Sosulski 1986), 5) amylopectin molecular structure (Qi *et al.* 2003), 6) phosphorous content (Swinkels 1985, Gunaratne & Hoover, 2002), 7) growth temperature (Myllärinen *et al.* 1998), 8) starch damage (Karkalas *et al.* 1992), 9) hydrothermal modifications (Hoover & Vasanthan 1994a,b), 10) granule integrity (Sandhya Rani & Bhattacharya 1989), 11) crystallinity (Jayakody & Hoover 2002), 12) amylopectin unit-chain length distribution (Srichuwong *et al.* 2005a), 13) chemical modification (Landerito & Wang 2005), 14) surface proteins and lipids (Debet & Gidley 2006), and 15) granule size (Vasanthan & Bhatty 1996, Tang *et al.* 2002).

2.2.6.2 Amylose leaching (AML)

During heating, at the same time as the absorption of water, material is leached out from the starch granule (Fig. 2.9, Tester *et al.* 2004b). This material is mainly amylose, although amylopectin might be leached out, depending on the type of

starch and conditions (Tester & Morrison, 1990a). Studies on AML is important because the soluble fraction provides information on the extent of interaction between amylose-amylose and/or amylose-amylopectin in the granule interior.

The extent of AML has been shown to be influenced by: 1) total amylose content (Nakazawa & Wang 2003), 2) the extent of interaction between amylose chains (AM-AM) and/or between amylose and outer branches of amylopectin (AM-AMP) (Ratnayake *et al.*, 2001; Zhou *et al.*, 2004), 3) the amount of lipid-complexed amylose chains (Gunaratne & Hoover, 2002; Nakazawa & Wang, 2004), 4) phosphate content (Gunaratne & Hoover, 2002), 5) granular size (smaller granules leach more amylose than larger granules) (Lindeboom *et al.*, 2004), and 6) heating temperature (Hoover & Vasanthan, 1994b; Vasanthan & Bhatty, 1996; Gunaratne & Hoover, 2002). The leaching of amylose is necessary for gel formation but in many cases, the leaching of amylose causes problems during the manufacture of pasta, and potato flakes (Eliasson & Gudmundsson, 1996).

2.2.6.3 Gelatinization

Gelatinization has been defined as an irreversible change of granular swelling and melting of starch crystallites when native starch is heated in water under specific temperature ranges and certain moisture levels (Jacobs & Delcour 1998, Eliasson & Gudmundsson 1996, Cooke & Gidley 1992, Biliaderis 1990, French 1984).

There are actually two processes occurring during the gelatinization phase transition: first, the melting of the starch crystallites, which is an endothermic process and, second, the formation of the amylose-lipid complexes, which is an exothermic process (Eliasson & Gudmundsson 1996). This phase transition is associated with the diffusion of water into the granule, water uptake by the amorphous background region, hydration and radial swelling of the starch granules, leaching of amylose into the solution, increase in viscosity, loss of optical birefringence, loss of crystalline order, unraveling and dissociation of double helices (in the crystalline regions) and starch solubilization (Biliaderis 1998, Jenkins 1994, Atwell *et al.* 1988, Hoover & Hadziyev 1981, Stevens & Elton 1971). Jenkins (1994) showed by means of small angle neutron scattering studies, that the mechanisms proposed by Evans and Haismann (1982), Blanshard (1987), Biliaderis *et al.* (1986) were not compatible with his results, but were in broad agreement with the gelatinization mechanism proposed by Donovan (1979). Jenkins (1994) has postulated that, in excess water, gelatinization is a primarily swelling driven process. Water uptake by the amorphous background regions is accompanied by swelling within these region. This swelling acts to destabilize the amylopectin crystallites within the crystalline lamellae, which are ripped apart. This process occurs rapidly for an individual crystallite, but over a wide range for the whole granule. Smaller crystallites are less soluble and destroyed first. In

conditions of limiting water, initial crystallite disruption occurs by the same swelling driven mechanism. However, there is insufficient water for this process to proceed to completion. At higher temperatures the remaining crystallites slowly melt. At the molecular level, gelatinization involves the uncoiling of external chains of amylopectin that are packed together as double helices in clusters. Hydrogen bonds stabilizing the structure of the double helices are broken during uncoiling. The sequence of events during starch gelatinization at low, intermediate and excess water are illustrated in Fig 2.10.

Gelatinization parameters of starches are influenced by: 1) botanical source (Peroni *et al.* 2006), 2) amylose content (Yoshimoto *et al.* 2002, Hoover & Manuel 1996a), 3) lipid-complexed amylose chain (Morrison 1995), 4) phosphorous content (Blennow *et al.* 2000), 5) method of starch extraction (Waigh *et al.* 2000a,b), 6) size of the granule (Singh & Kaur 2004), 7) granule morphology (Stevens & Elton 1971), 8) starch : water ratio (Farhat *et al.* 1999), 9) lipid content (Russell 1987), 10) starch damage (Waduge *et al.* 2006), 11) crystallite size (Singh *et al.* 2006), 12) crystallite perfection (Perera *et al.* 2001), 13) double helical content (Jacobs *et al.* 1998a), 14) amylopectin chain length (Wang *et al.* 2006), 15) stability of amorphous region (Biliaderis 1990), 16) heating rate (Freitas *et al.* 2004, Ziegler *et al.* 1993), 17) maturity of starch granule (Karlsson & Eliasson 2003), and 18) growth temperature (Kohyama *et al.* 2004, Kiseleva *et al.* 2003,

Figure 2.10 Gelatinization mechanism at different starch: water ratios

A-C indicates the helix to coil transformation in presence of excess water (A), intermediate water (B) and low water (C) contents

D-F indicates the DSC endotherm in presence of excess water (D), intermediate water (E) and low water (F) contents

Source: Waigh *et al.* (2000b) with permission from Elsevier.

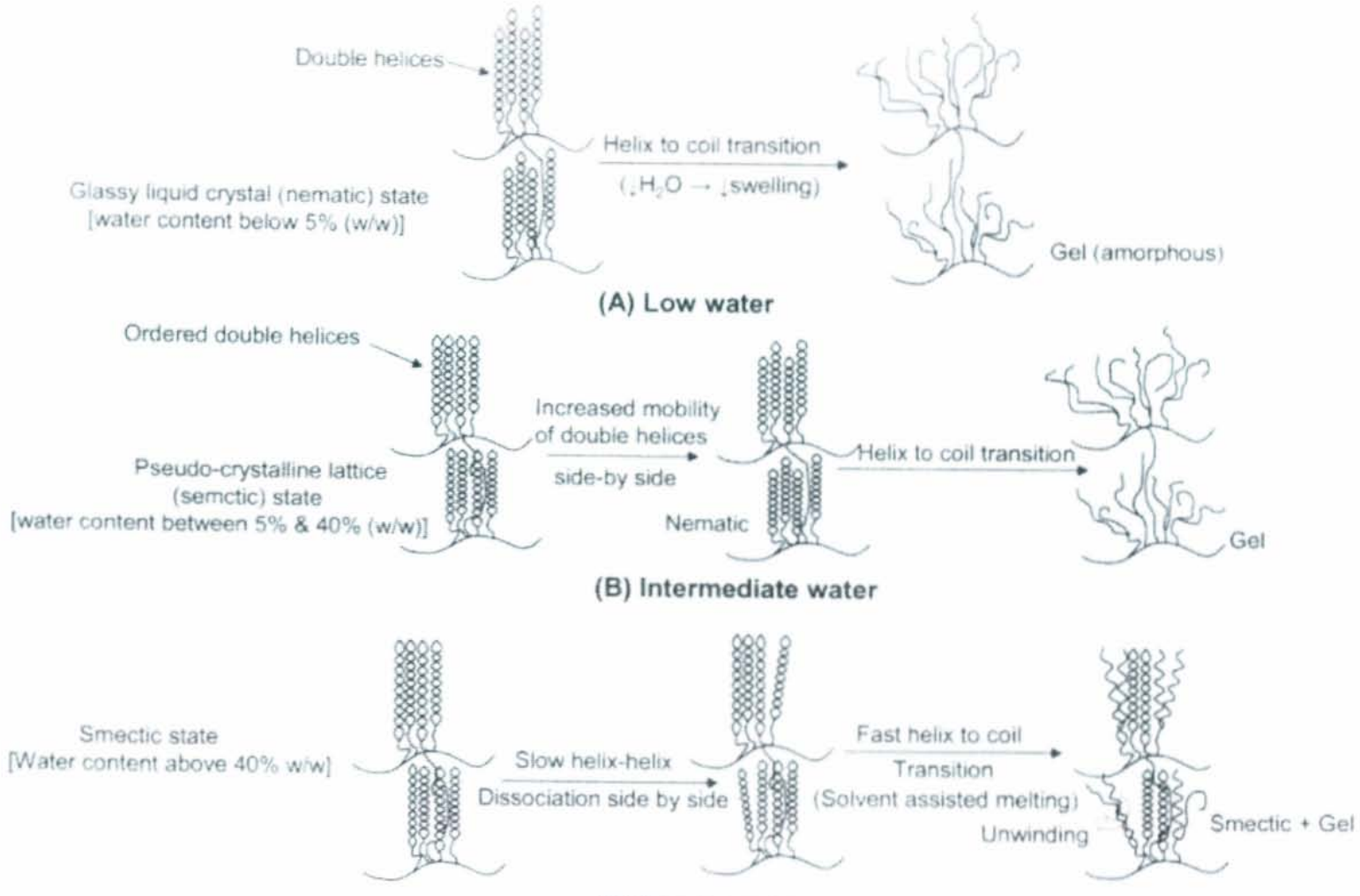

Double helices
Glassy liquid crystal (nematic) state
[water content below 5% (w/w)]
Helix to coil transition
(↓H2O → ↓swelling)
Gel (amorphous)
(A) Low water
Ordered double helices
Pseudo-crystalline lattice
(semctic) state
[water content between 5% & 40% (w/w)]
Increased mobility
of double helices
side-by side
Nematic
Helix to coil transition
Gel
(B) Intermediate water
Smectic state
[Water content above 40% w/w]
Slow helix-helix
Dissociation side by side
Fast helix to coil
Transition
(Solvent assisted melting)
Unwinding
Smectic + Gel
(C) Excess water

Table 2.10 Gelatinization parameters of wheat starches

Source	Amylose content(%)	Methodology	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_{gel} (J/g)	ΔH_{gel} (J/g)
Wheat							
Normal	25.6	DSC:S:W1:3	55.1	61.3	66.7	6.8	Van Hung *et al.* 2007
Waxy	1.0	DSC:S:W1:3	58.9	63.3	68.2	9.8	Van Hung *et al.* 2007
High-amylose	37.5	DSC:S:W1:3	47.2	51.8	56.1	1.7	Van Hung *et al.* 2007
Normal	29.2	DSC:S:W1:3	61.7	65.3	69.3	12.4	Ao & Jane 2007
Normal wheat							
A-granule	30.9	DSC:S:W1:3	61.2	64.3	68.1	11.7	Ao & Jane 2007
B-granule	25.5	DSC:S:W1:3	57.9	64.7	67.5	12.1	Ao & Jane 2007
Barley							
Normal	32.3	DSC:S:W1:3	61.4	65.3	72.8	10.0	Waduge *et al.* 2006
Waxy	0	DSC:S:W1:3	59.3	64.9	81.8	13.0	Waduge *et al.* 2006
High-amylose	55.3	DSC:S:W1:3	61.0	67.9	76.6	6.0	Waduge *et al.* 2006
Maize							
Normal	22.4	DSC:S:W1:3	65.3	71.3	80.9	11.0	Liu *et al.* 1999
Waxy	3.3	DSC:S:W1:3	62.9	72.8	84.3	13.6	Liu *et al.* 1999
High-amylose	66.0	DSC:S:W1:3	69.9	92.3	104.5	13.7	Liu *et al.* 1999
Rice							
Normal	19.9	DSC:S:W1:2	67.7	71.8	83.4	12.2	Park *et al.* 2007
Waxy	0.9	DSC:S:W1:2	57.6	64.4	84.6	12.7	Park *et al.* 2007
Triticale	26.9	DSC:S:W1:3	60.7	64.2	68.4	12.6	Ao & Jane 2007

DSC-differential scanning calorimetry. Starch:water ratio is expressed as S:W (S-starch; W-water). T_o=onset temperature, Tp=peak temperature; Tc=final temperature; ΔH_{gel} =Enthalpy of gelatinization, enthalpy values are expressed in J/g of the dry starch (dsb, based on dry starch weight).

1987). Although, amylopectin can retrograde upon cooling, linear amylose molecules have a greater tendency to reassociate and form hydrogen bonds than the larger amylopectin molecules (Thomas & Atwell 1999). During retrogradation, amylose forms double helical associations of 40-70 glucose units, whereas amylopectin crystallization occurs by association of the outermost short branches (DP 15) (Hoover 2001). The retrograded starch, which shows a 'B'-type X-ray diffraction pattern (Zobel 1988), contains both crystalline and amorphous regions (Hoover 2001). Shi and Seib (1992) showed that the retogradation of waxy starches was directly proportional to the mole fraction of branches with degree of polymerisation (DP) 14-24, and inversely proportional to the mole fraction of branches with DP 6-9. The low degree of retrogradation for waxy starches has been attributed to the high proportion of short chain branches of DP 6-9 (Lu *et al.* 1997b).

2.2.6.5 Acid hydrolysis

Acid hydrolysis has been used to modify starch granule structure and produce 'soluble' starch for over a century (Kirchoff 1811). Starch treated with sulfuric acid (15% v/v, 25°C) is referred to as Nageli amylodextrins, while starch treated with hydrochloric acid (7.5% v/v, 30-40°C) is referred to as lintnerized starch (Rohwer & Klem 1984). Other acids such as nitric and phosphoric have also been used for starch degradation (Singh & Ali 2000). Acid hydrolyzes both α-(1→4)

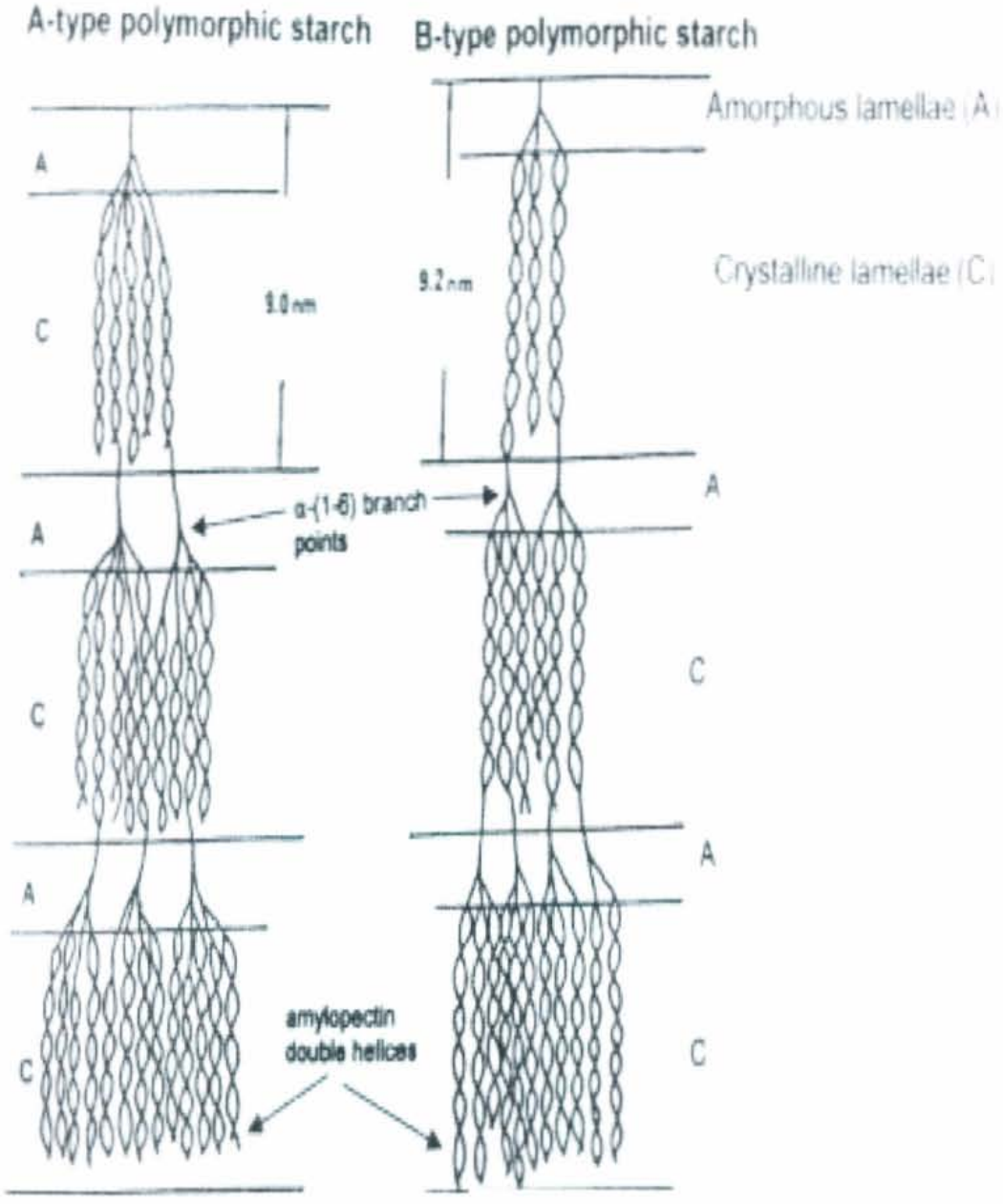
and α-(1→6) linkages. All starches exhibit a two-stage hydrolysis pattern (Hoover 2000). A relatively fast hydrolysis rate during the first 8 days followed by a slower rate between 8 and 12 days has been reported for various cereal, tuber and root and legume starches (Shi & Seib 1992, Hoover & Vasanthan 1994a, Vasanthan & Bhatty 1996, Li *et al.* 2001, Gunaratne & Hoover 2002). The first stage of hydrolysis mainly corresponds to the hydrolysis of the amorphous region of the starch granule, whereas the second stage corresponds to the hydrolysis of the crystalline region within the granule (Hoover 2000). To account for the slower hydrolysis rate of the crystalline domains of the starch granule, two hypotheses (French 1984, Kainuma & French 1971) have been proposed: 1) the dense packing of starch chains within the starch crystallites does not readily allow the penetration of H_3O^+ into the regions, and 2) sterically hindered conformational transformation (chair → half chair) in the crystalline region (Hoover 2000).

Many researchers have shown that B-type (tuber and root)starches are more resistant to acid hydrolysis than A-type (cereal) starches (Srichuwong *et al.* 2005a, Vermeylen *et al.* 2004, Jane *et al.* 1997, Jayakody & Hoover 2002) (Fig 2.11). Jane *et al.* 1997 reported that A-type starches contain more shorter chains than B-type starches and most of their branch α-(1→6) linkages are located in the crystalline areas. Whereas, B-type starches contain a larger proportion of long B chains with most of the branch linkages in the amorphous area (Fig 2.11). The

Figure 2.11 Proposed models for branching patterns of A- and B-type starches

(a) the A-type starches, and (b) the B-type starches. 'A' and 'C' stand for the amorphous and crystalline regions, respectively; 9.0 nm and 9.2 nm are the repeating distances of A- and B-type starches, respectively. The chain length between the arrows stands for the internal long-B chain.

Source: Jane *et al.* (1997) with permission from Elsevier.


A-type polymorphic starch
B-type polymorphic starch
Amorphous lamellae (A)
Crystalline lamellae (C)
A
C
9.0 nm
9.2 nm
α-(1-6) branch points
amylopectin double helices

above authors have also shown that branch linkages inside the crystalline area are protected from acid hydrolysis. Resistance of B-type crystallites to acid hydrolysis has also been shown to be due to their higher stability and three dimensional size (Vermeylen *et al.* 2004). Srichuwong *et al.* (2005a) have shown by studies on cereal and tuber starches that, although the characteristics of the amorphous lamella is a critical factor influencing hydrolysis rates, the amylopecin chain length distribution also plays a significant role in influencing the extent of acid hydrolysis. These authors also showed that very short chains (DP 6-8) of amylopectin are readily hydrolyzed together with amorphous material by acid, since they are not long enough to form stable double helices.

The extent and rate of acid hydrolysis has been shown to be influenced by: 1) starch source (Hoover 2001, Hoover & Vasanthan 1994a), granule size (Jayakody & Hoover 2002), type of unit cell (Jane 2006), proportion of B-type crystallites (Srichuwong *et al.* 2005a), presence of pores on the granule surface (Jayakody & Hoover 2002), amylopectin structure (Srichuwong *et al.* 2005a), crystallinity (Jayakody *et al.* 2005), amylopectin unit chain-length distribution (Srichuwong *et al.* 2005a), characteristics of amorphous lamellae (Srichuwong *et al.* 2005a), amylose content (Jayakody & Hoover 2002), lipid complexed amylose chains (Waduge *et al.* 2006, Jayakody & Hoover 2002, Hoover 2000), phosphorus content (Hoover 2000), granular swelling (Jayakody *et al.* 2005), and extent of

starch damage (Tester *et al.* 1998).

2.2.6.6 Alpha-amylase hydrolysis

Enzymic and acid hydrolyses have been used traditionally to modify native starches and to create products with altered solubility, viscosity, and/or gelation properties that find broad applications in food, paper, textile, and other industries (You & Izydorczyk 2007). Compared to acid hydrolysis, α-Amylase, on the other hand (E.C. 3.2.1.1., 1,4-α-D-glucan glucanohydrolase), due to its relatively large size (diameter of 6 nm), cannot easily diffuse into the granule and its action is confined to localized regions only, where both amorphous and crystalline regions are hydrolyzed simultaneously. Furthermore, the extent of starch polymer degradation has been shown to be higher during enzyme hydrolysis (You & Izydorczyk 2007, Colonna *et al.* 1988, Leach & Schoch 1961). α-Amylase hydrolyzes starch by randomly cleaving the internal α-1,4 glucosidic bonds but not α-1,6 glucosidic bonds, thus giving rise to varing smaller chains of oligosaccharides having the α-configuration at C1 of the reducing glucose unit, hence the name α- amylase (Ao *et al.* 2007).

α-amylases have been produced from various sources (animals, plants and microbes) and have different properties. The most abundant microbial source of this enzyme is the genus *Bacillus*, especially *B. subtilis*, *B. amyloliquefaciens* and *B. licheniformis* (Sujka *et al.* 2006). *B. licheniformis* α-amylase is one of the most

efficient enzymes among bacterial α-amylases (Liakopoulou-Kyriakides *et al.* 2001). Scanning and transmission electron microscopy studies on the susceptibility of starches towards α-amylases (e.g. bacterial, fungal and porcine pancreatic) have shown the presence of successive strong and weak radial internal layers (Zhou *et al.* 2004). Hydrolysis of native wheat (Jacobs *et al.* 1998b), potato (Leach & Schoch 1961) and sago (Wang *et al.* 1995) starches with bacterial α-amylase has shown that hydrolysis occurs granule by granule. In contrast acid hydrolysis occurs throught the entire granular population with preferential attack on the amorphous parts (Jayakody *et al.* 2007). Valetudie *et al.* (1993) have shown that the hydrolysis rate is higher for potato, sweet potato, and cassava starches with porcine pancreatic amylase than with bacterial amylase (*B. subtilis*).

The enzymatic reaction of α-amylase with starch granules occurs *via* several steps: diffusion to the solid surface, adsorption (prerequisite step for subsequent catalytic activity) and finally catalysis. In the case of most starch granules initial hydrolysis occurs at the surface, depending on the type of starch. In general, enzymes either erode the entire granule surface or sections of it (exocorrosion) or digest channels from selected points on the surface towards the center of the granule (endocorrosion) (Gallant *et al.*1992, French 1984).

The following factors have been shown to influence α-amylase hydrolysis: 1) botanical source, 2) amylase source, 3) granule morphology, 4) granule size, 5)

surface pores, 6) presence of channels, 7) differences in type and proportion of polymorphic forms, 8) degree of gelatinization, 9) extent of starch damage, 10) extent of molecular association between starch components (Dreher *et al.* 1984), 11) double helical content (Tester *et al.* 2004b), 12) extent of crystallite perfection (Zhang *et al.* 2006a), 13) amylose/amylopectin ratio (Hoover & Sosulski 1985), 14) amylopectin chain length distribution (Srichuwong *et al.* 2005b,c), 15) degree of crystallinity (Hoover & Sosulski, 1985), 16) amylose-lipid complexes (Nebensy *et al.* 2002, Hoover & Manuel 1995), 17) starch protein interaction (Valetudie *et al.* 1993), 18) phosphate content (Slaughter *et al.* 2001), 19) antinutrients (Thompson & Gabon 1987), and 20) physical (Hoover & Vasanthan 1994a,b) and chemical (Wolf *et al.* 1999) modification.

2.2.6.7 Pasting characteristics

Use of starch in the textile, paper, adhesive and food industries depends on the viscosity of the starch paste (Moorthy 2002). In food systems, the cooking procedures for many foods and their cooked characteristics are related to starch gelatinization and pasting (Li *et al.* 2008). When starch is heated in excess water, the crystalline structure is disrupted due to the breakage of hydrogen bonds, water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin, and substantial swelling occurs, together with melting of crystallites, loss of birefringence and leaching out of polysaccharides,

predominantly amylose, from the starch granule (Bao & Bergman 2004). The leaching out of amylose and amylopectin molecules has been found to be highly linked to the swelling of the starch granules, and both the swelling of starch and the leaching of amylose have been related to the pasting and rheological properties of starch solutions during heating (Noisuwan *et al.* 2007). Pasting refers to the changes that occur after gelatinization upon further heating and these include further swelling of granules, leaching of molecular components from the granules, and eventual disruption of granules and an increase in viscosity, especially with the application of shear forces (Tester and Morrison 1990a,b). Viscosity changes in starch suspensions result from a combination of granule swelling and solubilization.

The pasting behavior is usually studied by observing changes in the viscosity of a starch system based on rheological principles (Zaidul *et al.* 2007). The Brabender visco-amylograph, rapid visco-analyser (RVA) and rotational viscometers have been extensively used for measuring starch paste viscosity. Many researchers have also used the dynamic rheometer for studying the viscoelastic or rheological properties of starches (Singh *et al.* 2003). The Brabender visco-amylograph can be restrictive because of the large amount of flour or starch required and the long analysis time, limiting its application (Panozzo & McCormick 1993). The Rapid Visco-analyser (RVA) has several advantages over the viscoamylograph. These

include small sample sizes, a short analysis time and the ability to set temperature profiles.

2.2.7 Starch annealing

2.2.7.1 Overview

Annealing of starch is a physical treatment of starch granules in the presence of heat and water. Tester *et al.* 2000 have shown that annealing can be initiated at room temperature when the moisture content exceeds 22% on a total weight basis, but is restricted (in terms of its effect on increasing the gelatinization temperature) unless it exceeds 60% by weight. During annealing, starch granules in excess (>60% [w/w]) or at an intermediate water content (40% [w/w]) are held at a temperature above the glass transition temperature (T_g) but below the onset (T_o) temperature of gelatinization for a set period of time (Tester & Debon 2000, Jacobs & Delcour 1998, Hoover & Vasanthan 1994a). T_g is the temperature at which the amorphous domains of the starch granule are transformed from a rigid glassy to a mobile rubbery state when heated in the presence of solvents such as water or glycerol. These solvents are referred to as plasticizers (Tester & Debon 2000). The plasticizing effect of water increases glucan chain mobility within the amorphous lamellar regions of the semicrystalline growth ring (Perry & Donald 2000). Several authors (Tester & Debon 2000, Jacobs & Delcour 1998, Muhrbeck & Svensson 1996, Seow & Teo 1993, Larsson & Eliasson 1991, Tester &

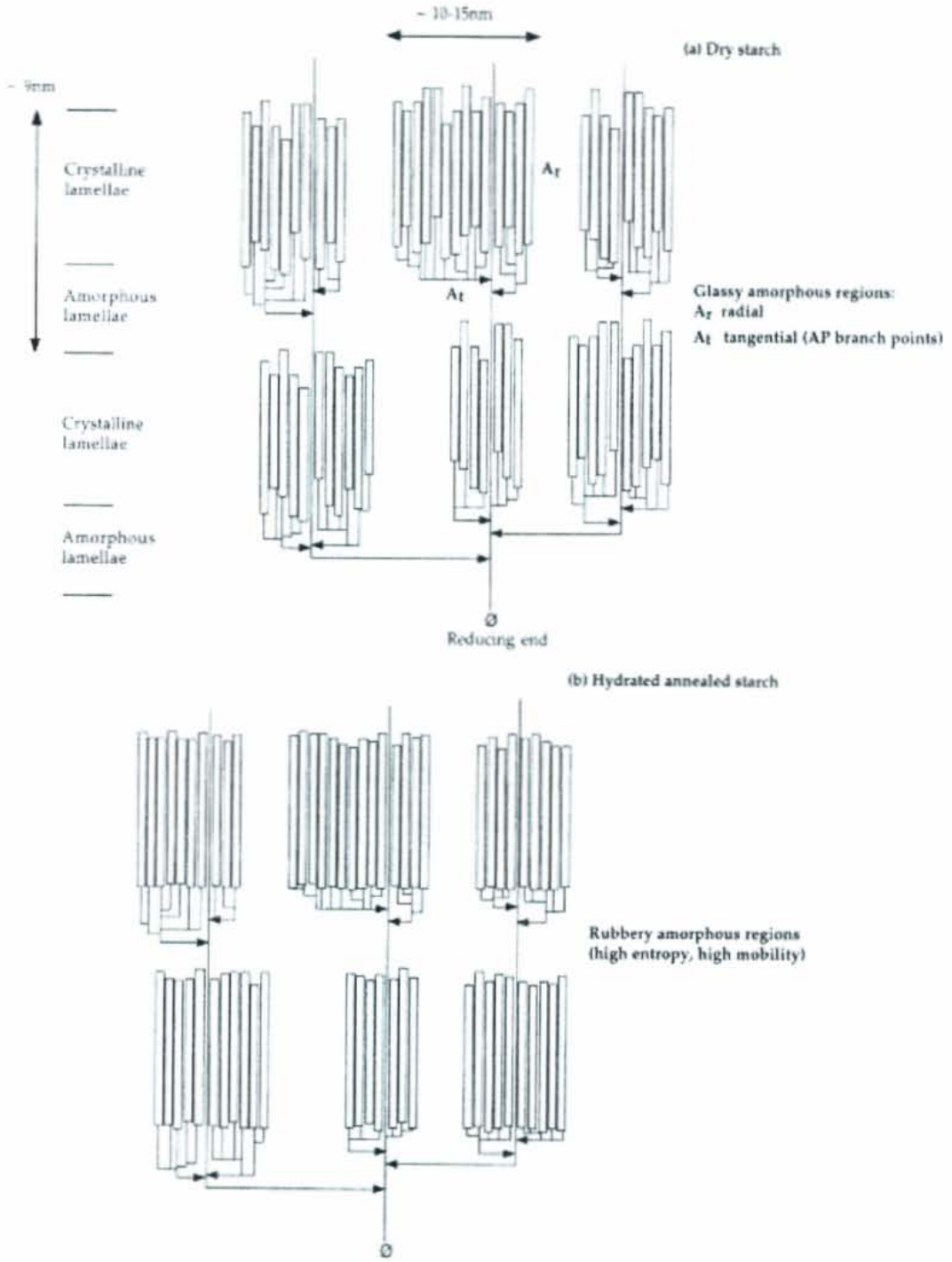
Morrison 1990a, Slade & Levine 1987, Lorenz and Kulp 1984) have described starch annealing as a crystal growth/perfection, diffusion controlled non-equilibrium process.

2.2.7.2 Mechanism of annealing

Perry and Donald (2000) and Waigh *et al.* (1996) have proposed that double helices of the unhydrated form of starch are intact, but are not arranged regularly side by side (Fig. 2.12a), due to the differing lengths of radial and tangential branches. This state is known as a nematic, collapsed or 'withered state'. The amorphous region of the granule is the area most vulnerable to the initial water absorption and plasticization. Before hydration the amorphous area is more glassy and immobile. Hydration of the starch granule increases the mobility of the amorphous regions. This induces vibrational movement of tangential and radial chains in both amorphous and crystalline domains. Simultaneously hydration causes limited but reversible granule swelling, allowing mobility of crystalline domains. An increase in annealing temperature ($> T_g$ but $< T_o$) and excess water accelerates the rate of hydration and increases glucan chain mobility. This dynamic nature allows limited side by side movement of the double helices (Waigh *et al.* 2000, Perry and Donald 2000) resulting in the formation of a smectic-type structure. An increase in the incubation temperature, enhances

Figure 2.12 Pictorial representation of mechanism of annealing on the semi-crystalline lamellae

(amylopectin double helices are represented as rectangles): (a) dry starch with glassy amorphous regions; (b) hydrated annealed starch with rubbery amorphous regions. Source: Tester & Debon 2000, reproduced with permission from Elsevier Science.


~ 10-15nm
(a) Dry starch
~ 9nm
Crystalline lamellae
Amorphous lamellae
Crystalline lamellae
Amorphous lamellae
A_r
A_t
Glassy amorphous regions:
A_r radial
A_t tangential (AP branch points)
Ø
Reducing end
(b) Hydrated annealed starch
Rubbery amorphous regions
(high entropy, high mobility)
Ø

initially the order of the amorphous lamellae and, subsequently, the order of double helices of amylopectin (Tester *et al.* 1999). At this stage, molecules are closely aligned in a distinct series of layers, with their axes lying perpendicular to the plane of the layers. With the progress of annealing, the initially weaker or imperfect crystallites gradually disappear, while the rest of the crystallites become more perfect due to fusion and re-crystallization. The native starch (*in vivo*) contains crystallites of varying stabilities. However, annealing decreases the variations in crystalline stabilities resulting in more homogenous crystallites (Tester & Debon 2000, Tester *et al.* 1998, Jacobs *et al.* 1998b, Hoover & Vasanthan 1994a, Larsson & Eliasson 1991, Paredes-Lopez & Hernández-Löpez 1991, Tester & Morrison 1990a, Yost & Hoseney 1986). Jacobs *et al.* (1998a) and Hoover & Vasanthan (1994a) postulated that amylose chain mobility could increase on annealing, resulting in the formation of double helices arising from interactions between amylose-amylose and/or amylose-amylopectin chains.

2.2.7.3 Starch to water ratio on annealing treatment

Annealing of starches has been studied at various starch:water ratios (1:1, 1:3, 1:5) and at temperatures ranging from 40 to 75°C (Hormdok & Noomhorn 2007, Kozlov *et al.* 2007, Tukomane *et al.* 2007, Kohyama & Sasaki 2006, Vermeylen *et al.* 2006, Kiseleva *et al.* 2005 & 2004, Lawal 2005, Genkina *et al.* 2004a & b, Gomez *et al.* 2004, Kiseleva *et al.* 2004, Nakazawa and Wang 2004 & 2003, Qi *et*

al. 2004, Ozcan & Jackson 2003, Atichokudomchai *et al.* 2002, Tester *et al.* 2000, Andreev *et al.* 1999, Jacobs *et al.* 1998b & c, Tester *et al.* 1998, Wang *et al.* 1997, Hoover & Manuel 1996, Muhrbeck & Svensson 1996, Jacobs *et al.* 1995, Seow and Vasanti-Nair 1994, Hoover and Vasanthan 1994a & b, Seow and Teo 1993, Stute 1992, Cameron and Donald 1992, Larsson and Eliasson 1991, Lopez and Lopez 1991, Knutson 1990, Krueger *et al.* 1987a & b, Yost and Hoseney 1986, Kuge and Kitamura 1985).

2.2.7.4 Effect of annealing on granule morphology

Several authors (Waduge *et al.* 2006, Jacobs *et al.* 1998c, Hoover & Vasanthan 1994a, Stute 1992, Wiegal 1933) have found no changes to granule morphology on annealing of wheat, oat, lentil, barley (certain cultivars) and potato starches. However, Kiseleva *et al.* (2005) observed that the lens shaped granules of high amylose and waxy wheat starches were slightly deformed on annealing, the extent of this deformation being greater in the latter. Waduge *et al.* (2006) reported that in some cultivars of barley, pore size increased slightly on annealing. Kiseleva *et al.* (2003) reported that the Maltese-cross and concentric growth rings remain unchanged on annealing, however, concentric growth rings were much denser after annealing.

2.2.7.5 Effect of annealing on starch structure

The following changes have been shown to occur on annealing: Polymorphic

transformation of the A+B X-ray pattern to the A-pattern (Waduge *et al.* 2006, Genkina *et al.* 2004c), increase in granule stability (Hoover and Vasanthan 1994a), crystallite growth and perfection/optimization (Tester & Debon 2000, Tester *et al.* 1998, Jacobs *et al.* 1998b, Hoover & Vasanthan 1994a, Larsson & Eliasson 1991, Paredes-Lopez & Hernández-Löpez 1991, Tester & Morrison 1990a, Yost & Hoseney 1986), increase in granule rigidity (Jacobs *et al.* 1995), twisting of unordered ends of double helices (Tester *et al.* 1999, Tester *et al.* 1998), glucan chain interactions within the amorphous and crystalline domains of the granule (Jacobs & Delcour 1998, Hoover and Vasanthan 1994a, Stute 1992), increase in order within the amorphous domain without increase in crystallinity (Tester & Debon 2000, Jacobs & Delcour 1998), development of crystallinity in the amorphous regions of the granule (Krueger *et al.* 1987a & b), formation of double helices and compartmentalization of amylose-amylose, amylopectin-amylopectin, and amylose-amylopectin helices (Atichokudomchai *et al.* 2002, Tester *et al.* 2000, Jacobs *et al.* 1998a & b, Shi *et al.* 1998, Hoover & Vasanthan 1994a, Seow & Vasanti-Nair 1994, Morrison *et al.* 1993a, Knutson 1990), amylose-lipid interactions (Jacobs *et al.* 1998b), extra reinforcing of the α-D-(1→6) linkages (Jacobs *et al.* 1998a), polymer chain realignment within granules and partial crystallite melting (Marchant & Blanshard 1980), mobility differences in amorphous or crystalline regions (Stute 1992, Nakazawa *et al.* 1984), and an

increase in the glassy nature (more rigid and less mobile) of the amorphous material (Tester & Debon 2000). However, annealing has been shown to have no influence on the wide angle X-ray diffraction pattern of maize (Tukomane *et al.* 2007, Waduge *et al.* 2006, Qi *et al.* 2005, Muhrbeck & Wischmann 1998), legume (Hoover & Manuel 1996), and potato (Stute 1992) starches.

2.2.7.6 Effect of annealing on gelatinization characteristics

Annealing has been shown to increase the gelatinization temperatures (T_o, T_p, T_c) and decrease the gelatinization temperature range (T_c-T_o) in all starches (Genkina *et al.* 2007, Kohyama & Sasaki 2006, Liu & Shi 2006, Vermeylen *et al.* 2006, Waduge *et al.* 2006, Lawal 2005, Tsutsui *et al.* 2005, Tester *et al.* 2005, Kiseleva *et al.* 2004, Tester *et al.* 2000, Jacobs & Delcour 1998, Tester *et al.* 1998, Jacobs *et al.* 1998c &1995, Wang *et al.* 1997, Muhrbeck & Svensson 1996, Hoover & Vasanthan 1994a, Stute 1992, Larsson & Eliasson 1991, Muhrbeck & Eliasson 1991, Liu & Lelièvre 1991, Paredes-Lopez & Hernández-Löpez 1991, Knutson 1990, Tester & Morrison 1990b, Krueger *et al.* 1987a & b, Slade & Levine 1987, Yost & Hoseney 1986, Kuge & Kitamura 1985, Lorenz *et al.* 1984 & 1980, Lorenz & Kulp 1978a, b). However, gelatinization enthalpies (ΔH) have been reported to increase (Waduge *et al.* 2006, Kiseleva *et al.* 2005, Kiseleva *et al.* 2004, Genkina *et al.* 2004b, Nakazawa and Wang 2003 & 2004, Atichokudomchai *et al.* 2002, Jacobs *et al.* 1998b & c, Hoover & Manuel 1996, Muhrbeck & Svensson 1996,

Jacobs *et al.* 1995, Hoover & Vasanthan 1994a, Larsson & Eliasson 1991, Knutson 1990, Krueger *et al.* 1987a & b, Slade & Levine 1987), or remain unchanged (Waduge *et al.* 2006, Qi *et al.* 2005, Jacobs *et al.* 1998c, Muhrbeck & Wischmann 1998, Eerlingen *et al.* 1996, Wang *et al.* 1997, Shi & Seib 1995, Seow & Teo 1993, Stute 1992, Larsson & Eliasson 1991, Yost & Hoseney 1986) or decrease (Kohyama & Sasaki 2006, Larsson & Eliasson 1991) on annealing. The increase in gelatinization temperature has been shown to be most pronounced for T_o and least for T_c. Annealing has a greater influence on T_o, since T_o represents melting of the weakest crystallites (Nakazawa and Wang 2003, Wang *et al.* 1997, Larsson & Eliasson 1991) which are more susceptible to crystallite perfection on annealing than crystallites that have higher stability (represented by T_c) (Jacobs *et al.* 1998b). The decrease in T_c-T_o on annealing indicates greater homogeneity and cooperative melting of crystallites (Jacobs & Delcour 1998). Increase in starch mobility within the amorphous regions leads to a molecular re-organization which involves interaction between amylose-amylose and/or amylose-amylopectin chains (Atichokudomchai *et al.* 2002, Tester *et al.* 2000, Jacobs *et al.* 1998a & b, Shi *et al.* 1998, Hoover & Vasanthan 1994a, Seow & Vasanti-Nair 1994, Morrison *et al.* 1993, Knutson 1990). This interaction together with crystallite perfection increases T_g. Consequently, this increases T_o, T_p and T_c in the annealed starches. Qi *et al.* (2005), Genkina *et al.* (2004b), Kiseleva *et al.* (2004), Tester *et al.* (2000) and

Tester *et al.* (1998) have postulated that changes to the gelatinization transition temperatures on annealing could also be due to lengthening of the double helices that were not optimized during biosynthesis. Kiseleva *et al.* (2004) have postulated that the lengthening of amylopectin pre-existing double helices on annealing could occur due to twisting of uncoiled ends resulting from an increase in glucan chain mobility. This twisting leads to the formation of additional intrahelical hydrogen bonds resulting in an increase in crystalline lamella thickness, which in turn elevates the melting temperature of amylopectin double helices. For such a mechanism to have a significant impact on gelatinization temperatures, the free ends of the double helices in the native starch should be long enough to intertwine and form strong intrahelical hydrogen bonds.

Cooke and Gidley (1992) have shown by using ^{13}C cross polarization magic angle spinning/NMR (^{13}C-CP MAS/NMR) and DSC that ΔH is a reflection of the number of double helices that unravel and melt during gelatinization. Thus, starches in which ΔH remains the same pre- and post-annealing, suggests that the only molecular reorganization that occurs in these starches is crystalline perfection, and that the double helical order (number of double helices and stabilizing hydrogen bonds) is not influenced by annealing. Evidence for the constancy of ΔH pre- and post-annealing in normal wheat (Tester *et al.* 1998) and normal corn (Tester *et al.* 2000) starches was shown by ^{13}C-CP MAS/NMR. However,

significant increase in ΔH has been shown to occur in high amylose barley (Waduge *et al.* 2006) and corn starches (Tester *et al.* 2000). Waduge *et al.* (2006), Tester *et al.* (2000) and Knutson (1990) hypothesized, that when amylose content reaches a certain threshold, amylose chains may be in close proximity to each other and/or with amylopectin chains. Consequently, on annealing, interactions could occur between amylose-amylose and/or amylose-amlopectin chains resulting in the formation of new double helices. Tester *et al.* (2000) showed using ^{13}C-CP MAS/NMR that the amount of double helices in amylomaize starch (63.1% amylose) increased by 11% on annealing (single step). The corresponding increase in ΔH was ~5%. Waduge *et al.* (2006) showed by studies on barley starches of varying amylose content (0-55.3%) that a particular cultivar (SB 94893) having the highest amylose content (55.3%) exhibited the largest increase in ΔH (~28%) on annealing. However, its unit amylopectin chain length distribution was higher (DP5-17:56.9%) than the barley cultivar SB 948907 (DP5-17: 52.6%)[amylose content 43.7%] in which ΔH remained unchanged on annealing. This clearly demonstrates that the increase in ΔH is influenced by the interplay of: 1) amylose content, 2) location of amylose and amylopectin within the starch granule interior, and 3) amylopectin unit chain length distribution.

2.2.7.7 Impact of annealing temperature, moisture content and annealing time on gelatinization parameters

Several studies (Tester & Debon 2000, Hoover and Vasanthan 1994a, Larsson and Eliasson 1991, Knutson 1990, Krueger *et al.* 1987a, Slade & Levine 1987, Lorenz *et al.* 1984) have shown that the effect of annealing on starch structure is more pronounced if the annealing temperature is set (close) to, but below, T_o. However, if the annealing temperature is set very close to T_o, then it would trigger starch gelatinization. Therefore, annealing temperatures are generally kept at about 5 to 15°C below T_o (Tester & Debon 2000, Eliasson & Gudmundsson 1996). However, annealing temperatures (15 to 28°C) below To have also been shown to have a significant impact on the gelatinization parameters of starches (Nakazawa & Wang 2003, Tester *et al.* 1998). Kruger *et al.* (1987a) showed that on annealing T_o and T_p of maize starch increased gradually up to a moisture content of 67% (w/w), after which excess water had no further effect. Hoover and Vasanthan (1994a) reported steep increase in T_o, T_p and T_c at an annealing moisture of 50% in wheat and lentil starches and at 10% and 70%, respectively, in potato and oat starches. For ΔH, a steep increase occurred at moisture contents of 40 and 50% in potato and wheat starches, respectively. Whereas changes in ΔH for oat and lentil starches were gradual (Hoover and Vasanthan 1994a). Interaction between amylose- amylose and/or amylose-amlopectin chains allows enthalpically driven assembly of the lamellar structure to be initiated with amylopectin double helices moving into

alignment (Figure 2.12b). This would then explain the increase in gelatinization parameters with increase in moisture content.

The impact of annealing time on T_o, T_p, T_c and ΔH was studied by Kiseleva *et al.* 2005, Genkina *et al.* 2004a & b, Jacobs *et al.* 1998a, Muhrbeck & Wischmann 1998, Hoover and Vasanthan 1994a, Seow & Vasanti-Nair 1994, Seow & Teo 1993, Larsson and Eliasson 1991, Knutson 1990, Krueger *et al.* 1987a). In general, T_o, T_p and T_c increases with annealing time. The increase being more pronounced in T_o and least in T_c. Hoover and Vasanthan (1994a) showed that in oat, wheat, potato and lentil starches, increases in T_o, T_p, T_c and ΔH do not begin simultaneously during the time course of annealing (at 50°C). The rates of increase in T_o, T_p, and T_c were gradual in wheat and oat starches, but rapid in potato (during the first 30 min). Annealing beyond 24h, did not significantly increase T_o, T_p, and T_c of oat, potato and lentil starches. However, those of wheat starch became more pronounced as the annealing time exceeded 24h. Increases in ΔH were slower and were evident in wheat, oat, potato and lentil starches only after annealing had been in progress for 48, 6, 2 and 1h, respectively. Genkina *et al.* (2004a) showed that To of sweet potato starches increased rapidly during the first 60 min of annealing (at 45°C). After, that increments were much lower tending towards constant value after 8h. Larsson and Eliasson (1991) reported that for wheat starch, the largest changes in T_o, T_p, T_c and T_c-T_o occurred during the first

4h of annealing (at 50°C). No changes were observed after 6h.

2.2.7.8 Impact of annealing on amylose-lipid complex formation

Morrison *et al.* (1993b & c) have shown by means of ^{13}C-CP MAS/NMR, DSC and X-ray studies the presence of amylose-lipid complexes in native starch granules of barley, maize, rice and oat starches. DSC studies have shown that the amylose-lipid complex transition occurs in the range 85-115°C (Slade & Levine 1988, Russell 1987, Billaderis *et al.* 1986 & 1985, Kugimiya & Donovan 1981), 96-125°C (Karkalas *et al.* 1995), ~110°C (Nakazawa & Wang 2004) and 93.2-96.8°C (Andreev *et al.* 1999). Tester *et al.* (2005) postulated that since amylose-lipid complexes are distinct entities, they are unlikely to be formed during annealing (Tester *et al.* 2005). Several reports have indicated that new amylose-lipid complexes are not formed during single or double step annealing (Kiseleva *et al.* 2005, Nakazawa & Wang 2004, Jacobs *et al.* 1998c, Larsson & Eliasson 1991). This was based on the observation that the amylose-lipid complex melting endotherm (Kohyama & Sasaki 2006, Kiseleva *et al.* 2005, Nakazawa and Wang 2004, Wasserman *et al.* 2002, Jacobs *et al.* 1995 &1998a, Larsson and Eliasson 1991), the ^{13}C-CP MAS/NMR signal at 31 ppm (Jacobs *et al.* 1998a), and the apparent amylose content (Kohyama & Sasaki 2006) remained unchanged on annealing. Tester and Debon (2000), Jacobs *et al.* (1998c), Morrison *et al.* (1993c), Larsson and Eliasson (1991) have postulated that this may be due to the fact that

the annealing temperature (35-50°C) is much lower than the melting temperature range (85-125°C) of the amylose-lipid complex. Andreev *et al.* (1999) have shown by DSC studies on (maize, wheat, barley, and rye) and high amylose (barley) starches, that only maize starch has the ability to form additional amylose-lipid complexes on annealing. Wasserman *et al.* (2002) have postulated that the ability of maize starch to form additional amylose-lipid complexes on annealing may be due to the entry of surface lipids (*via* the channels on the granule surface) into the granule interior. It is likely, that once inside the granule interior, the thermal energy imparted to the fatty acid chain during annealing may increase its mobility, thereby facilitating its interaction with the amylose helix. Waduge *et al.* (2006) and Lorenz *et al.* (1984) have shown increases in the intensity of V-amylose lipid complex (2θ ~20°) in barley starches on annealing. However, the enthalpy of the melting of amylose-lipid complex remained unchanged on annealing (Waduge *et al.* 2006). Waduge *et al.* (2006) have proposed that the increased intensity of the amylose-lipid peak on annealing was not due to formation of additional amylose-lipid complexes, but to enhanced ordering of lipid molecules that were present as amylose-lipid complexes within granules of native barley starches.

2.2.7.9 Impact of annealing on X-ray diffraction pattern and crystallinity

Muhrbeck and Wischmann (1998) reported that the effect of annealing is more pronounced in B-type starches than on A-type starches. Annealing of barley

(Waduge *et al.* 2006), potato (Vermeylen *et al.* 2006, Jacobs *et al.* 1998a, Hoover & Vasanthan 1994a), new cocoyam (Laval 2005), cassava (Tukomane *et al.* 2007), wheat (Qi *et al.* 2005, Jacobs *et al.* 1998a, Hoover & Manuel 1996b, Hoover & Vasanthan 1994a, Stute 1992, Gough & Pybus 1971), oat and lentil (Hoover & Vasanthan 1994a), pea (Hoover & Manuel 1996b), and maize (Qi *et al.* 2005, Ozcan & Jackson 2003) starches have shown no effect on their polymorphic pattern. However, in some varieties of barley (Waduge *et al.* 2006), cassava (Gomez *et al.* 2004), and sweet potato (Genkina *et al.* 2004c), the A+B X-ray diffraction pattern changed to an A-type pattern on annealing. The X-ray intensities have been shown to increase slightly on annealing in potato, lentil, oat, wheat (Hoover & Vasanthan 1994a), and barley starches (Waduge *et al.* 2006, Jacobs *et al.* 1998b). X-ray crystallinity has been shown to increase in high amylose barley (Waduge *et al.* 2006), wheat (Hoover & Vasanthan 1994a), and to decrease in potato (Vermeylen *et al.* 2006), or remain unchanged in potato (Jacobs & Delcour 1998), pea (Jacobs & Delcour 1998), wheat (Jacobs & Delcour 1998, Slade & Levine 1987), maize (Ozcan & Jackson 2003), and in normal and waxy barley (Waduge *et al.* 2006) starches on annealing. The increase in crystallinity on annealing was attributed to the interplay of the following factors: 1) amylopectin content (Waduge *et al.* 2006), 2) changes in orientation of the starch crystallites (Tester & Debon 2000), 3) crystallite perfection(Tester & Debon 2000, Jacobs &

Delcour 1998, Muhrbeck & Svensson 1996, Seow & Teo 1993, Larsson & Eliasson 1991, Tester & Morrison 1990b, Slade & Levine 1987, Lorenz *et al.* 1984), 4) enhanced ordering of the V-amylose–lipid complex (Lorenz *et al.* 1984) and 5) formation of amylose crystallites (Kruger *et al.* 1987a & b). The unchanged crystallinity observed in some starches on annealing is indicative that changes in factors 2 to 5 may have been of a low order of magnitude. The slight decrease in crystallinity reported by Vermeylen *et al.* (2006) may be crystallite disruption or crystallite reorientation. However, the authors have not provided any explanation for this phenomenon.

2.2.7.10 Impact of annealing on granular swelling

Annealing has been shown to reduce granular swelling in potato(Nakazawa & Wang 2004, Debon & Tester 2000, Hoover & Vasanthan 1994a), breadfruit (Adebowale *et al.* 2005a), wheat (Tester *et al.* 1998, Hoover & Vasanthan 1994a, Lorenz & Kulp 1978a), cassava (Nakazawa & Wang 2004), corn (Qi *et al.* 2005, Nakazawa & Wang 2004), oat (Hoover & Vasanthan 1994a), lentil (Hoover & Vasanthan 1994a), pea (Hoover & Manuel 1996), and barley (Waduge *et al.* 2006) starches. The decrease in granular swelling has been attributed to the interplay of the following factors: 1) increased crystalline perfection and decreased hydration (Waduge *et al.* 2006, Tester *et al.* 1998), 2) AM-AM and/or AMP-AMP interaction (Jacobs *et al.* 1998c), 3) increased intragranular binding forces and reinforcement

of the granule (Hizukuri 1996, Jacobs *et al.* 1995), and 4) V-amylose-lipid complex formation (Waduge *et al.* 2006, Jacobs *et al.* 1998c, Hoover & Vasanthan 1994a).

2.2.7.11 Impact of annealing on amylose leaching (AML)

Annealing [single, two and multi-step] treatments reduce amylose leaching at all temperatures below 100°C in potato (Nakazawa & Wang 2004, Jacobs *et al.* 1995, Hoover & Vasanthan 1994a, Kuge & Kitamura 1985), wheat (Hoover & Vasanthan 1994a, Lorenz & Kulp 1978a), cassava (Gomez *et al.* 2004), oat (Hoover & Vasanthan 1994a), lentil (Hoover & Vasanthan 1994a), pea (Jacobs *et al.* 1995), rice (Jacobs *et al.* 1995), and certain cultivars of barley (Waduge *et al.* 2006) starches. However, an increase in AML has been reported for wheat (Jacobs *et al.* 1995) and certain cultivars of barley starches (Waduge *et al.* 2006) on annealing. The reduction in AML on annealing has been attributed to the interplay of the following: 1) interaction between amylose-amylose and/or amylose-amylopectin (Waduge *et al.* 2006, Hoover & Vasanthan 1994a), 2) decrease in granular swelling (Tester *et al.* 2000), and 3) increase in V-amylose–lipid content (Waduge *et al.* 2006, Tester *et al.* 2000) and the molecular size of amylose (Waduge *et al.* 2006).

2.2.7.12 Impact of annealing on pasting properties

Generally, annealing has been shown to increase the pasting temperature, thermal stability and decrease peak viscosity and the viscosity at the end of the cooling cycle (Hormdok & Noorhorm 2007, Adebowale *et al.* 2005a & b, Adebowale & Lawal 2002, Jacobs *et al.* 1995 & 1996, Hoover & Vasanthan 1994a, Stute 1992), the exceptions being, pea (Jacobs *et al.* 1995), rice (Jacobs *et al.* 1995), and wheat (Jacobs *et al.* 1995, Hoover and Vasanthan 1994a) starches which exhibit a higher peak viscosity (wheat>pea>rice) on annealing. The RVA profile of annealed [single step] rice starch shows an increase in pasting temperature. However, this parameter remains unchanged in the Brabender viscoamylograph (Jacobs *et al.* 1995). The reduced viscosity and improved shear stability on annealing has been attributed to reduced granular swelling and amylose leaching, and increased interaction between starch chains during annealing (Jacobs *et al.* 1995, Hoover & Vasanthan 1994a, Stute 1992). The increase in viscosity exhibited by wheat starch on annealing was attributed by Jacobs *et al.* (1995) to higher rigidity and resistance to shear. Hoover and Vasanthan (1994a) have shown by DSC studies that, on annealing, wheat starch exhibits a greater decrease in T_c-T_o than potato starch. They attributed this to interaction between double helices (in the crystalline domain) which is more extensive in wheat than in potato starch. Thus, although, the extent of granular swelling is reduced (wheat>potato) as a result of annealing,

the increase in granular stability of wheat starch on annealing is so high that it negates the effect of decreased granular swelling on peak viscosity. This would then explain why the viscosity of wheat starch increases on annealing, whereas that of potato starch decreases.

2.2.7.13 Impact of annealing on acid hydrolysis

The impact of annealing on acid hydrolysis has been shown to be influenced by the method used for annealing (single step, double step, multistep), annealing temperature and starch source (Waduge *et al.* 2006, Qi *et al.* 2005, Nakazawa & Wang 2003, Jacobs *et al.* 1998a, Tester *et al.* 1998, Hoover & Vasanthan 1994a). Waduge *et al.* (2006) reported that in starches extracted from different cultivars of barley, the difference in acid hydrolysis [single step, 0.25g starch/10 mL 2.2M HCl, at 35°C/18 days] between native and annealed starches was only marginal. No difference in hydrolysis was observed between native and annealed wheat and pea starches subjected to double step annealing [0.167g starch/10 mL 2.2M HCl, at 35°C/20 days] (Jacobs *et al.* 1998a). However, Nakazawa and Wang (2003) showed by studies on potato, wheat, cassava, maize, waxy maize and high amylose maize starches that annealing increased acid susceptibility [multi-step, 15.3%H_2SO_4, 0.5g starch/10mL, at 38°C/30 days] in all starches, with potato starch showing the greatest and high amylose maize starch showing the smallest changes. Tester *et al.* (1998) reported that during the rapid phase of acid hydrolysis

[single step, 0.1g starch/10 mL 2M HCl, at 35°C/10 days], annealed wheat starch was more extensively degraded than its native counterpart while, during the slow phase of hydrolysis, there was no difference in the extent of hydrolysis. Hoover & Vasanthan (1994a) reported that in potato, lentil, oat, and wheat the differences in acid hydrolysis [single step, 0.25g starch/10 mL 2.2M HCl, at 35°C/20 days] between native and annealed starches were ~5%.

The decrease in acid hydrolysis on annealing has been attributed to: 1) perfection of starch crystallites (Waduge *et al.* 2006), 2) formation of double helical structures between amylose chains (Jacobs *et al.* 1998a), 3) increased embedding of α-(1→6) branch points within the crystalline structure (Jacobs *et al.* 1998a), and 4) formation of V-amylose-lipid complexes (Waduge *et al.* 2006, Jacobs *et al.* 1998a, Hoover & Vasanthan 1994a). The increase in acid hydrolysis on annealing has been attributed to: 1) an increase in the concentration of α-glucan in the amorphous region as a consequence of crystalline perfection (Tester *et al.* 2000) and 2) formation of void spaces in the crystalline lamellae due to crystalline perfection (Nakazawa & Wang 2003). Similarity in hydrolysis between native and annealed starches during the slow phase of hydrolysis has been attributed to: 1) limited crystallite perfection and 2) unchanged double helical content pre- and post-annealing (Nakazawa & Wang 2003, Tester *et al.* 2000).

2.2.7.14 Impact of annealing on α-amylase hydrolysis

Annealing increases the susceptibility of wheat starch towards fungal α-amylase (Lorenz *et al.* 1980) and bacterial α-amylase [*Bacillus subtilis*] (Gough & Pybus 1971). However, Jacobs *et al.* (1998c) showed by using pancreatin (a mixture of α-amylase from porcine stomach mucosa, lipids and protease) that during the early stages of hydrolysis (<20h), susceptibility of one step and double step annealed wheat starches is lower than that of its native counterpart. However, during the latter stages (>20h) this trend is reversed. Hoover & Vasanthan (1994a) reported that the susceptibility of annealed (single step) wheat starch was lower than its native counterpart throughout the time course of hydrolysis by porcine pancreatic α-amylase. Both single step (Jacobs *et al.* 1998c, Hoover & Vasanthan 1994a) and double step (Jacobs *et al.* 1998c) annealing has been shown to decrease the susceptibility of potato starch towards porcine pancreatic α-amylase (Hoover & Vasanthan 1994a) and pancreatin (Jacobs *et al.* 1998c). Legume starches such as pinto bean, black bean, lentil and field pea starches have been shown (Hoover & Manuel 1996) to exhibit increased susceptibility towards porcine pancreatic α-amylase on annealing (single step). A similar finding was also reported by Jacobs *et al.* (1998c) for pancreatin hydrolyzed single and double step annealed pea starch. However, the extent of hydrolysis of the single and double step annealed pea starches were nearly similar.

The decrease in α-amylase susceptibility on annealing has been attributed to the

interplay of the following factors: 1) crystallite perfection and double helical content, 2) crystal type, 3) annealing steps, 4) interaction between amylose-amylose and/or amylose-amylopectin chains and 5) amylose-lipid complex formation on annealing (Jacobs *et al.* 1998c, Hoover & Vasanthan 1994a).

2.2.7.15 Uses of annealed starches

Annealing has been shown to improve thermal stability and decrease the extent of set-back (Adebowale *et al.* 2005a, Jacobs *et al.* 1995, Hoover and Vasanthan 1994a, Stute 1992). This suggests that annealed starches could be utilized in the canned and frozen food industries, for their respective advantages. Rice noodles prepared from rice flour are widely consumed in South East Asia. Traditionally, rice noodles are prepared from long-grain rice which has been stored for a period of time. This process limits starch granule swelling and improves the paste or gel quality (Zhou *et al.* 2003), making the rice flour suitable for preparing good quality noodles. The decrease in granular swelling and amylose leaching, and the increase in heat and shear stability that occur on annealing are all desirable properties for noodle manufacture. Hormdok and Noomhorm (2007) evaluated rice starch (native & annealed), fresh rice flour, aged flour and compound rice flours with 50/100g native rice starch or annealed rice starch as replacement ingredients for the manufacture of noodles of acceptable quality. The study showed that the textural (adhesiveness, chewiness, tensile strength) quality of the

rice noodle prepared using annealed rice starch was comparable to that of commercial noodles. Annealing has been used to enhance resistant starch (sum of starch and starch containing products not absorbed in the small intestine) levels while maintaining granule structure (Brumovsky & Thompson 2001, Haralampu & Gross 1998). In these studies, high amylose maize starch (HAMS) has been used for enhancing resistant starch, since HAMS is digested very slowly (Wolf *et al.* 1977). Brumovsky & Thompson (2001) showed that partial acid (HCl) hydrolysis of HAMS (hylon VII) for 6h at 25°C, followed by annealing (24h, 70°C, moisture content 67%) gave a resistant starch content of ~32% by total dietary fiber (TDF) analysis. The authors have postulated, that limited acid hydrolysis enhances the mobility of the starch chains, to allow more efficient rearrangement of starch chains during annealing. Haralampu & Gross (1998) showed that the resistant starch content of HAMS can be increased by heating to 95°C for 1h, followed by debranching with pullalanase and storage at 57°C overnight. The retrograded starch was then annealed at 90°C for 2h, leading to about 32% resistant starch by TDF analysis. Resistant starch can be incorporated in foods without altering the appearance and texture, partly due to its bland taste, white color and microparticulate structure. It can thus be used as a fat mimetic or to increase the dietary fiber content of food (Würsch 1999).

615G, Pittsburgh, PA, USA) at 130 ± 1°C for 1h. The samples were then removed and cooled in a desiccator. The moisture content was calculated as the percentage weight loss of the sample.

3.2.3.2 Ash content

Ash content was determined by the standard AACC method (2000). Pre-weighed (~5g) samples were transferred into a clean, dry porcelain crucible, and charred using a flame. The sample was then placed in a pre-heated (550°C) muffle furnace (Lab Heat-Blue M model M30A-1C, Blue M Electric Co., Blue Island, IL, USA) and allowed to stand until it became a cotton–like substance and free of carbonaceous matter (~12h). The sample was cooled to room temperature in a desiccator and weighed. The ash content was calculated as the percentage weight loss of the sample.

3.2.3.3 Nitrogen content

The nitrogen content was determined according to the micro-Kjeldahl method (AACC 2000). Samples (0.3g, db) were weighed on nitrogen-free papers and placed in the digestion tubes of a Buchi 430 digester (Buchi Laboratorimus-Technik AG, Flawill/Schweiz, Switzerland). The catalyst (two Kjeltab M pellets) and 20mL of concentrated sulfuric acid were added to each tube and the sample was digested until a clear yellow solution was obtained. The

digested samples were then cooled, diluted with 50mL of distilled water, 100mL of 40% (w/v) NaOH was then added, and the released ammonia was steam distilled into 50mL of 4% (w/v) boric acid (H_3BO_3) containing 12 drops of end point indicator (N-point indicator, EM Science, NJ, USA) using a Buchi 321 distillation unit until 150mL of distillate was collected. The amount of ammonia in the distillate was determined by titrating against 0.05N sulfuric acid. Percentage nitrogen was calculated as follows:

$$\text{Nitrogen (\%)} = \frac{\text{(Volume of acid - Blank) x Normality of acid x 14.0067 x 100}}{\text{Sample weight [db] (g)}}$$

3.2.3.4 Total phosphorous

Total starch phosphorous was determined according to the method of Jayakody *et al* (2005). Dry starch sample (5mg, db) was placed into screw-capped tubes (calibrated at the 5mL level) and gently heated with concentrated sulfuric acid (0.3mL) for 12h at room temperature before charring. The partially-digested samples were heated using a micro-Bunsen burner until charring was completed, and the climbing film of acid on the walls of the tubes was no longer viscous with partially charred organic matter. After the contents of the tubes had cooled, hydrogen peroxide (30µL, 30% [w/v]) was added (15µL at a time) to hit the walls of the tubes just above the acid, and the tubes were well shaken. The tubes were then gently boiled for 1 min. The solutions were allowed to slowly cool to room temperature, and the volume was made up to 3.6mL with distilled water. For assay,

anhydrous sodium sulfite solution (0.1mL, 33% [w/v]) was added with stirring followed by the addition of ammonium paramolybdate (0.1mL, 2% [w/v]) and ascorbic acid (0.01g), The contents of the tubes were adjusted to 5.0mL with distilled water, and the absorbance read at 822nm using a UV-visible spectrophotometer (Milton Roy, Spectronic-601, Rochester, NY, USA). A standard curve was prepared using known amounts of NaH_2PO_4.

3.2.3.5 Starch Lipids

3.2.3.5.1 Surface lipids

Surface lipids were determined according to the procedure outlined by Vasanthan & Hoover (1992b). the lipids were extracted at room temperature (25-27 °C) by mixing starch (5g, db) with 100mL of 2:1 (v/v) chloroform/methanol under vigorous agitation in a wrist action mechanical shaker (Burrell, Model 75, Burrell Corporation, Pittsburg, PA, USA) for lh. The solution was then filtered (Whatman No.4 filter paper) into a 250mL round bottom flask and the residue was washed thoroughly with a small amount of the chloroform/methanol solution. The solution was then evaporated to dryness using a rotary evaporator (Rotavpor R110, Buchi Laboratorimus- Technik AG, Flawill/Schweiz, Switzerland). The crude lipid extracts were purified by the method of Bligh and Dyer (1959) before quantification. The starch residue was saved for bound lipid extraction.

3.2.3.5.2 Bound lipids

Bound lipids were determined according to the procedure described by Vasanthan and Hoover (1992b). Bound lipid was extracted using the residue left after surface lipid extraction. The residue was refluxed with 3: 1 (v/v) n-propanol/water in a Soxhlet apparatus for 7h (Vasanthan & Hoover 1992b). The extracted solvent was evaporated using the rotary evaporator (Rotavpor R110, Buchi Laboratorimus-Technik AG, Flawill/Schweiz, Switzerland) and the remaining crude lipid residue was purified using the method of Bligh and Dyer (1959) before quantification.

3.2.3.5.3 Lipid purification (Bligh & Dyer 1959)

The crude lipid extracts (surface and bound) were purified by extraction in a separatory funnel with chloroform/methanol/water(1:2:0.8, v/v/v) and forming a biphasic system (chloroforrn/methanol/water, 1:1:0.9, v/v/v) by addition of chloroform and water at room temperature. The heavy chloroform layer was withdrawn into a pre-weighted 25mL round bottom flask and evaporated to dryness on the rotary evaporator followed by drying at 60°C for lh in a forced air oven. The dried lipid was cooled to room temperature in a desiccator.

3.2.3.6 Determination of amylose content

Amylose content was determined by a colorimetric procedure and by high performance size-exclusion chromatography.

Apparent and total amylose contents were determined by a colori- metric method described by Chrastil (1987)

3.2.3.6.1.1 Apparent amylose

Starch (20 mg, dry basis) was weighed in a screw cap tube and suspended in deionized water (4mL). The contents of the tubes were vortexed and mixed for 30s. Sodium hydroxide (2 mL, 1M) or urea-dimethylsuphoxide (0.6M urea in 90% Me_2SO)(2mL)was then added and the mixture was vortexed. The tube was capped and heated at 95°C for 30 min in a water bath with occasional mixing. The solution was then cooled to room temperature and an aliquot (0.1mL) was added to 5mL of 0.5% trichloroacetic acid (TCA) in a separate test tube. The solutions were mixed and 0.05mL of 0.1N I_2-KI solution (1.27g of I_2 per L + 3g of KI per L) was added and mixed immediately. The resulting blue color was read at 620nm after 30min against a reference prepared without starch.

3.2.3.6.1.2 Total amylose

Total amylose content was also was determined by the above procedure, but with prior defatting with hot n-propanol/water (3:1, v/v) for 7h. In order to correct for over- estimation of apparent and total amylose content, amylose content was

calculated from a standard curve (Y=0.3189X + 0.1634, R_2=0.9977) prepared using mixtures of pure potato amylose and amylopectin (over the range 0-100% amylose) (Appendix A.1).

3.2.3.6.2 Total amylose content by high performance size-exclusion chromatography

A high performance size-exclusion chromatography (HP-SEC) method was used to determine amylose concentration (Demeke *et al.* 1999). A 5 mg starch sample was suspended in 5 mL distilled water in a glass tube and incubated at 130°C for 30 min. To one mL of gelatinized starch solution mixed vigorously, 55µL of 1M sodium acetate (pH 4.0) was added and mixed vigorously. Four units of isoamylase was added to the solution to de-branch the starch. After four hours of incubation at 40°C, the debranching reaction was stopped by boiling for 20 min to inactivate isoamylase. The debranched starch solution was freeze dried. The freeze-dried sample was dissolved in 200µL of DMSO (99% v/v) and then centrifuged (15,000 g.min) in a microfuge. Supernatant (40µL) was injected into a PLgel MiniMix-C guard column attached to a PLgel Minimix 4.6 mm i.d. column (Polymer Laboratories, Inc. Amherst, MA) to separate amylose and amylopectin using an HPLC system (Waters 600 controller, Waters 610 fluid unit, Waters 717 plus autosampler, Waters 410 differential refractometer). The data were collected

and analyzed using Millenium software. Starch samples, column, and detector were maintained at 40, 100 and 45°C, respectively. DMSO (99%) was used as an eluent at a flow rate of 0.2 mL/min. The amylose concentration was calculated by integration of the peak area corresponding to amylose to that of the peak area corresponding to both amylose and amylopectin.

3.2.4 Starch damage

The starch damage was estimated following the method of Jayakody *et al.* (2005) Starch samples (lg, db) in phosphate buffer (40 mL, 0.02M, pH 6.9) in a 125 mL Erlenmeyer flask was incubated with fungal a-amylase from *Aspergillus oryzae* (2500 Sigma units, 39.3 units/mg solid) in a water bath at 37°C for 15 min. At the end of incubation, the enzyme action was terminated by adding 10 mL of anhydrous trichloroacetic acid (10%, w/v). The mixture was allowed to stand for 2min and then centrifuged at 2000rpm for 10 min. The supernatants were then neutralized to pH7.0. The amount of reducing sugars present in the supernatants (2.0 mL) was determined using the Somogyi-Nelson method (Nelson (1944), Somogyi (1952)). The percentage starch damage was catculated as follows:

$$\text{Starch Damage (\%)} = \frac{M}{Wx1.05} \times 100$$

Where; M -mg maltose equivalents in the digest (50 mL)

W -mg of starch (db)

1.05 -molecular weight conversion of starch to maltose

3.2.4.1 Determination of reducing sugar

Reducing sugar was determined by the method of Nelson (1944) & Somogyi(1952).

Materials:

Alkaline reagent: Anhydrous sodium carbonate (25.0g), sodium potassium tartrate (25.0g), sodium bicarbonate (20.0g), and anhydrous sodium sulfate (200.0g) were dissolved in 800mL of distilled water, and made up to one liter.

Copper reagent: Cupric sulfate.5H2O (30.0g) was dissolved in 200.0mL of water and two drops of concentrated sulfuric acid were added.

Arsenomolybdate reagent: Ammonium molybdate (25.0g) was dissolved in water (450.0mL) to which concentrated sulfuric acid (21.0mL) was added; sodium arsenate.$7H_20$ (3.0g) was dissolved separately in 25.0 mL of water and added slowly to the above solution with constant stirring. The whole solution was diluted to 500mL and incubated for 24 to 48 h at 37°C.

Method:

A freshly prepared 1 mL aliquot of alkaline copper reagent (mixture of 25 parts of alkaline reagent and one part of copper reagent) was added to 2mL of the starch supernatant and heated for 20 min in a boiling water bath. The tubes were cooled rapidly in cold water, and 1 mL of arsenomolybdate reagent was added to each test tube, mixed gently, and the resulting solution was kept for 5min at room

temperature to develop the color. The solution was then diluted with distilled water (6 mL)and the absorbance was measured at 510nm. A reagent blank was prepared using 2 mL water instead of starch solution. The standard curve was established in order to calculate the glucose ($Y=0.0441\ X$, $R^2=0.9998$) and maltose ($Y=0.0232X$, $R^2=0.9994$) equivalents in the sample(Appendix A.2& A.3).

3.2.5 Starch structure determination

3.2.5.1 Amylopectin branch chain length distribution

Isoamylase debranching of whole starch accompanied by high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was used to determine the branch chain length distribution of native and annealed starches (Jayakody *et al.*, 2005).

Starch was dispersed in 2 mL of 90% DMSO at a concentration of 5 mg/mL by stirring in a boiling water bath for 20 min. After cooling, methanol (6 mL) was added with vortex mixing, and the tube placed in an ice bath for 30 min. The pellet, which was recovered by centrifugation (1,000 x g for 12 min), was dispersed in sodium acetate buffer (2 mL, 50 mM, pH 3.5) by stirring in a boiling water bath for 20 min. Following equilibration of the tube at 37°C, isoamylase (5µL, 68,000µ/mg protein) was added. The sample was incubated at 37°C with slow stirring for 22 h. The enzyme was inactivated by boiling for 10 min. An aliquot (200µL) of the cooled debranched sample was diluted with NaOH (2 ML, 150

mM). The sample was filtered (0.45μm nylon syringe filter) and injected into the HPAEC-PAD system (50μL sample loop).

The HPAEC-PAD system consisted of a Dionex DX 600 equipped with an ED50 electrochemical detector with a gold working electrode, GP50 gradient pump, LC30 chromatography oven, and an AS40 automated sampler (Dionex Corporation, Sunnyvale, CA, USA). The standard triple potential waveform was employed, with the following periods and pulse potentials: $T_1 = 0.40$ s, with 0.20s sampling time, $E_1 = 0.05$ V; $T_2 = 0.20$ s, $E_2 = 0.75$ V; $T_3 = 0.40$ s, $E_3 = -0.15$ V. Data were collected using Chromeleon software, version 6.50 (Dionex Corporation, Sunnyvale, CA, USA). Eluents were prepared in distilled deionized water with helium sparging; eluent A was 50 mM sodium acetate in 150 mM NaOH, and eluent B was 150 mM NaOH. Linear debranched were separated on a Dionex CarboPac ™ PA1 analytical 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 was installed in front of the analytical column.

3.2.5.2 X-ray diffraction and relative crystallinity

3.2.5.2.1 X-ray diffraction

Starches for X-ray diffraction measurements were kept in a desiccator over saturated K_2SO_4 (25°C, aw=0.98) for 1 week to adjust their moisture contents to

about 19%. The hydrated samples (0.5 g dry basis) were packed tightly into an elliptical aluminum holder. X-ray diffractograms of native and annealed starches were obtained by a Rigaku D/MAX-2200V-PC X-ray diffractometer (Rigaku-Denki, Co. Tokyo, Japan) with operating conditions of target voltage 40kV; current 100mA; scanning range 3-35°; scan speed 2.00°/min; step time 0.9sec; divergence slit width 1.0°; scatter slit width 1.0° and receiving slit width 0.6mm. The moisture content of the samples was determined before and after scanning.

3.2.5.2.2 Starch crystallinity

Crystallinity of the native and annealed starches was quantitatively estimated following the method of Nara and Komiya (1983) by using the origin software (Origin-version 6.0, Microcal Inc., Northampton, MA, USA). A smooth curve, which connected peak baselines was computer plotted on the diffractogram. The area above the smooth curve was considered as the crystalline portion, and the lower area between the smooth curve and a linear base line was taken as the amorphous portion. The ratio of the upper area to the total diffraction area was calculated as the crystallinity (Appendix A.4).

The following equation was used to determine the percent crystallinity:

$$\text{Crystallinity (\%)} = \frac{Ac}{Ac + Aa} \times 100$$

Where: Ac and Aa are the crystalline and amorphous area of the X-ray diffractogram.

3.2.6 Swelling factor (SF)

The SF of the starches, when heated to 50-90°C in excess water was measured according to the method of Tester and Morrison (1990 b). Starch samples (50mg, db) were weighed into screw-capped tubes, 5 mL of distilled water was added and the mixture was heated in the range of 50-90°C in a constant temperature water bath for 30min (The tubes were shaken by hand every 5min to resuspend the starch slurry). The tubes were then cooled to 20°C rapidly on ice, 0.5mL of blue dextran (Pharmacia, MW 2 x 106,5mg/mL) was added and the contents mixed well. The tubes were then centrifuged at 2,000g for 5min and the absorbance of the supernatant (As) was measured at 620nm using a UV-visible spectrophotometer (Milton Roy, Spectronic-601, Rochester, NY, USA) against a reference without starch. The method measures only intragranular water and hence the true SF at the given temperature. The SF is reported as the ratio of the volume of swollen starch granule to the volume of the dry starch (Tester and Morrison, 1990a). Calculation of SF was based on starch weight corrected to 12% moisture, assuming a density of 1.4 mg/mL.

Free or interstitial plus supernatant water (FW) is given by;

$FW = 5.5 (Ar/As) - 0.5$

Where Ar and As are the absorbance of the reference and sample, respectively.

The initial volume of starch (V0) of weight W (in mg) is;

$V0 = W/1,400$

And the volume of absorbed intragranular water (V1) is thus;

$V1 = 5.0\text{-}FW$

Hence the volume of the swollen starch granule (V2) is;

$V2 = Vo + V1$

And $SF = V2/ Vo$

This can also be expressed as follows:

$SF=1 + \{(7700/ W) \times [(As - Ar) / As]\}$

The coefficient of variation of the method was generally less than 1 %.

3.2.7 Extent of amylose leaching (AML)

Starches (20mg, db) in water (10 mL) were heated at 50-90°C in volume calibrated sealed tubes for 30min (tubes were shaken by hand every 5 min to resuspend the starch slurry). The tubes were then cooled to room temperature and centrifuged at 2,000g for 10min. The supernatant liquid (1mL) was withdrawn and amylose content determined as described by Chrastil (1987). Amylose leaching was expressed as percentage of amylose leached per 100g of dry starch. Three replicate samples were used in this determination.

3.2.8 Acid hydrolysis

Native and annealed starches were hydrolyzed in triplicate with 2.2 M HCl at 35°C (1g starch (db) / 40mL acid) for periods ranging from 1 to 20 days. The starch slurries were vortexed daily to resuspend the deposited granules. At the

relevant time intervals, aliquots of the reaction mixture were neutralized and centrifuged (2000xg) and the supernatant liquid was assayed for total carbohydrate (Nelson, 1944, Somogyi, 1952). The extent of hydrolysis was detennined by expressing the solubilized carbohydrates as a percentage of the initial starch. Results used for calculation are means of triplicate measurements. The extent of hydrolysis was calculated as follows:

$$\text{Hydrolysis (\%)} = \frac{\text{Reducing sugar(as glucose) x 0.9 x 100}}{\text{Initial starch weight db (g)}}$$

3.2.9 Enzymatic hydrolysis

Enzymatic hydrolysis was determined as described by Jayakody *et al.*(2007). Enzymatic hydrolyses of native and annealed starches were conducted using a crystalline suspension of porcine pancreatic α-amylase in 2.9 M sodium chloride containing 3mM calcium chloride (Sigma Chemical Co., St. Louis, MO, USA) in which the concentration of α-amylase was 32mg protein/mL and the specific activity was 1,122 units/mg protein.

Starch (20mg, db) was suspended in phosphate buffer (10mL, 0.02 M, pH 6.9) containing 0.006M NaCl. A 5.5μL of α-amylase was added, the mixture gently mixed and digested at 37 °C in a water bath for periods ranging from 24 to 72h. The hydrolysate was vortexed on a daily basis to resuspend the deposited granules. The reaction was terminated by adding 5mL of absolute ethanol to the digestion

mixture and then centrifuged (2000xg). The supernatant liquid was assayed for reducing sugar content (Nelson, 1944, Somogyi, 1952). Controls without enzyme but subjected to the above experimental conditions were run concurrently. The reported values are the means of three replicates. The extent of hydrolysis was calculated as shown below:

$$\text{Hydrolysis (\%)} = \frac{\text{Reducing sugar(as maltose) x 0.95 x 100}}{\text{Initial starch weight db (g)}}$$

3.2.10 Pasting properties

A rapid VisoTM Analyser RVA-4 (Newport Scientific Pty, Ltd, Warriewood, NSW, Australia) was used to determine the pasting properties of starches (7% db, 27 g total weight). Native and annealed starch slurries were equilibrated at 50°C for 1 min, heated at 6°C /min to 95°C, held at 95°C for 5 min, cooled at 6°C /min to 50°C, and held at 50°C for 2 min. the spindle speed was 960 rpm for the first 10s (to disperse the sample) and then at 160 rpm for the remainder (~ 23 min) of the experiment. The reported values are the means of duplicate measurements.

3.2.11 Starch gelatinization

Gelatinization characteristics of the starches were determined by differential scanning calorimetry (DSC). Gelatinization parameters of native and annealed starches were measured using a Seiko differential scanning calorimeter (DSC 210, Seiko Instruments Inc., Chiba, Japan) equipped with a thermal analysis data

station and data recording software. Deionized Water (11μL) was added with a micro-syringe (MICROLITER®, #702, Hamilton Co. Reno, NV, USA) to starch (3.0mg) in the DSC pans and the contents were stirred (with micro-needle). The pans were then sealed, reweighed and allowed to stand overnight at room temperature before DSC analysis. The scanning temperature range and the heating rates were 25-135°C at a rate of 10°C/min, then held at 135°C for 5 min. In all measurements, a thermogram was recorded with an empty aluminum pan as a reference. During the scans, the space surrounding the sample chamber was flushed with dry nitrogen at rate of 100 mL/min to avoid condensation. The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c). The enthalpy of gelatinization (ΔH) were estimated by integrating the area between the thermogram and a base line under the peak and was expressed in terms of Joules per gram (J/g) of dry starch.

3.2.12 Retrogradation

Retrogradation characteristics were determined by DSC and by turbidity.

3.2.12.1 DSC

Retrogradation characteristics of the starches were determined by DSC according to the method of Jayakody *et al.* (2005). The samples were prepared with a starch to water ratio of 1:1. Starch was mixed with water using a micro-needle in order to facilitate even distribution of water in the mix. The pan was then hermetically

sealed, reweighed and kept in a glass vial at room temperature for 12h for moisture equilibration. Sealed pans were reweighed prior to scanning. After the initial DSC run, sample pans containing the gelatinized starch were covered in a single thin layer with Teflon® film and then with a double layer of saran film. The covered pans were first immersed in a water bath at 4°C for 24h and then immersed in a water bath (PolyScience, Model 2L-M PolyScience Niles, IL, USA) at 40°C for periods ranging from 0 to 168 h. At the end of each time period, the covering films were carefully removed and the stored samples were equilibrated at 25°C for 1h in a desiccator before reweighing and rescanning. The scanning temperature range and heating rate were identical to that used for the study of the above gelatinization parameters.

3.2.12.2 Turbidity measurements

Turbidity was determined using the method described by Perera and Hoover (1999). A 2% aqueous suspension (pH 7.0) of native and annealed starches was heated at 95°C for 1h. The samples were then stored at 4°C for 24h (to increase nucleation), followed by 1 to 19 days at 40°C. The development of turbidity at the relevant time intervals was followed by measuring the absorbance at 640nm against a water blank in a UV-visible spectrophotometer (Milton Roy, Spectronic-601, Rochester, NY, USA).

3.2.13 Annealing

Starches were subjected to one step annealing. Native starch samples (30g, db) were weighed into glass containers. The starch slurries were prepared with a starch to water ratio of 1:3. The sealed samples were incubated at approximately 10°C below the onset temperature of gelatinization (50°C for CDC teal, 46°C for 99WAX27 and 40°C for 11132) for 72h in water bath (New Brunswick Scientific, G76D, Edison, NJ, USA). At end of the incubation period, samples were centrifuged (2,000g) and supernatant was decanted (no amylose or soluble carbohydrates were detected in the supernatant). The annealed starches were washed once with deionized water and air-dried at room temperature (25°C).

3.2.14 Statistical analysis

All determinations were replicated three times, and mean values and standard deviations were reported. Analysis of variance (two way ANOVA) was performed by Tukey's HSD test ($P < 0.05$) using Statistical Software SPSS version 14.0 for Windows (SPSS Inc. Chicago, IL, USA).

Chapter 4. Results and Discussion

4.1 Isolation and chemical composition

The data on yield and composition are presented in Tables 4.1 &4.2. The purity of the starches was judged on the basis of composition [low ash (0.03-0.15%), low nitrogen content (0.02-0.07%)] and microscopic examination [Fig. 4.1]. The average yield of starch from CDC teal, 11132 and 99WAX27 was 45.0%, 38.5% and 48.0%, respectively. The total phosphorus content and non-lipid phosphorus content ranged from 0.007 to 0.058% (CDC teal >11132 >99WAX27) and 0.007 to 0.034% (CDC teal > 11132 > 99WAX27), respectively (Table 4.1). The decrease in total phosphorus content of CDC teal and 11132 starches on defatting suggests the presence of lipid phosphorus (in the form of phospholipids) in the above starches (Table 4.2). The phosphorus remaining in defatted CDC teal and 11132 starches are mainly in the form of phosphate monoesters and/or inorganic phosphorus. In 99WAX27 starch, phosphorus mainly occurs as non-lipid phosphorus, since the total phosphorus content remained unchanged on defatting (Table 4.1). The total phosphorus content of CDC teal (0.056%) and that of 11132 (0.058%) were higher than those reported by Franco *et al.* (2002) for wheat starches (0.047-0.049%), but was within the range reported by Raeker *et al.* (1998) for starches from soft wheat cultivars. Amylose concentration was determined colorimetrically (Chrastil, 1987) and by high performance size exclusion

Table 4.1 Chemical composition of native and annealed wheat starches[1]

Wheat Cultivar	Moisture (%)	Ash (%)	Nitrogen (%)	Total phosphorous (%)[2]	Non-lipid phosphorus (%)[3]	Lipid (%)	
						CM[4]	PW[5]
CDC Teal							
Native	9.11±0.05[a]	0.15±0.01[a]	0.04±0.01[a]	0.056±0.03[a]	0.034±0.06[a]	0.03±0.01[a]	0.69±0.06[a]
Annealed	10.56±0.01[b]	0.15±0.01[a]	0.04±0.01[a]	0.056±0.03[a]	0.034±0.06[a]	0.01±0.01[a]	0.72±0.08[a]
11132							
Native	9.27±0.02[a]	0.15±0.02[a]	0.07±0.02[a]	0.058±0.07[a]	0.033±0.1[a]	0.07±0.02[a]	0.77±0.05[a]
Annealed	10.75±0.02[b]	0.15±0.02[a]	0.07±0.02[a]	0.058±0.07[a]	0.033±0.1[a]	0.03±0.01[a]	0.82±0.06[a]
99WAX27							
Native	10.44±0.03[a]	0.03±0.01[a]	0.02±0.02[a]	0.007±0.10[a]	0.007±0.02[a]	0.02±0.01[a]	0.24±0.01[a]
Annealed	10.50±0.04[a]	0.03±0.01[a]	0.02±0.02[a]	0.007±0.10[a]	0.007±0.02[a]	0.01±0.01[a]	0.25±0.02[a]

[1] All data reported on dry basis and represents the mean of three determinations. Means of native and annealed starches of a particular cultivar with different superscripts are significantly different ($p<0.05$). The average yield of starch isolated from CDC Teal, 11132 and 99WAX27 was 45.0%, 38.5% and 48.0%, respectively.

[2] Determined before removal of free and bound lipids.

[3] Determined after removal of free and bound lipids.

[4] Lipids extracted from native starch by chloroform-methanol (CM) 2:1 (v/v) at 25°C (mainly free lipids).

[5] Lipids extracted by hot 1-propanol-water (PW) 3:1 (v/v) from the residue left after CM extraction (mainly bound lipids).

Table 4.2 Amylose concentration of native and annealed wheat starches determined by colorimetry and high performance size exclusion chromatography[1]

Wheat Cultivar	Amylose concentration (%)[2]		LCA[6]	Amylose concentration (%)[3]		LCA[6]	Amylose concentration (%)[4]
	apparent[5]	total[5]		apparent[5]	total[5]		total[5]
CDC Teal							
Native	23.3±0.1[a]	26.9±0.2[a]	13.9±0.4[a]	23.2±0.1[a]	26.7±0.1[a]	13.4±0.1[a]	26.9±0.2[a]
Annealed	21.5±0.2[b]	24.6±0.3[b]	12.8±0.3[b]	21.4±0.2[b]	24.6±0.3[b]	12.5±0.1[b]	26.8±0.1[a]
11132							
Native	26.3±0.1[a]	32.3±0.2[a]	18.4±0.5[a]	26.4±0.2[a]	32.4±0.1[a]	18.7±0.3[a]	32.2±0.1[a]
Annealed	24.5±0.2[b]	29.3±0.2[b]	16.3±0.1[b]	24.3±0.1[b]	29.2±0.1[b]	16.7±0.5[b]	32.7±0.1[a]
99WAX27							
Native	–	–		–	–		–
Annealed	–	–		–	–		–

[1] All data reported on dry basis and represents the means of three determinations. Means of native and annealed starches of a particular cultivar with different superscripts are significantly different ($p<0.05$).

[2] Amylose concentration determined by Chrastil (1987) colorimetric method in which starch was solubilized with NaOH.

[3] Amylose concentration determined by Chrastil (1987) colorimetric method in which starch was solubilized with urea-dimethylsuphoxide.

[4] Amylose concentration determined by high performance size exclusion chromatography.

[5] Apparent and total amylose determined by iodine binding before and after removal of free and bound lipids, respectively.

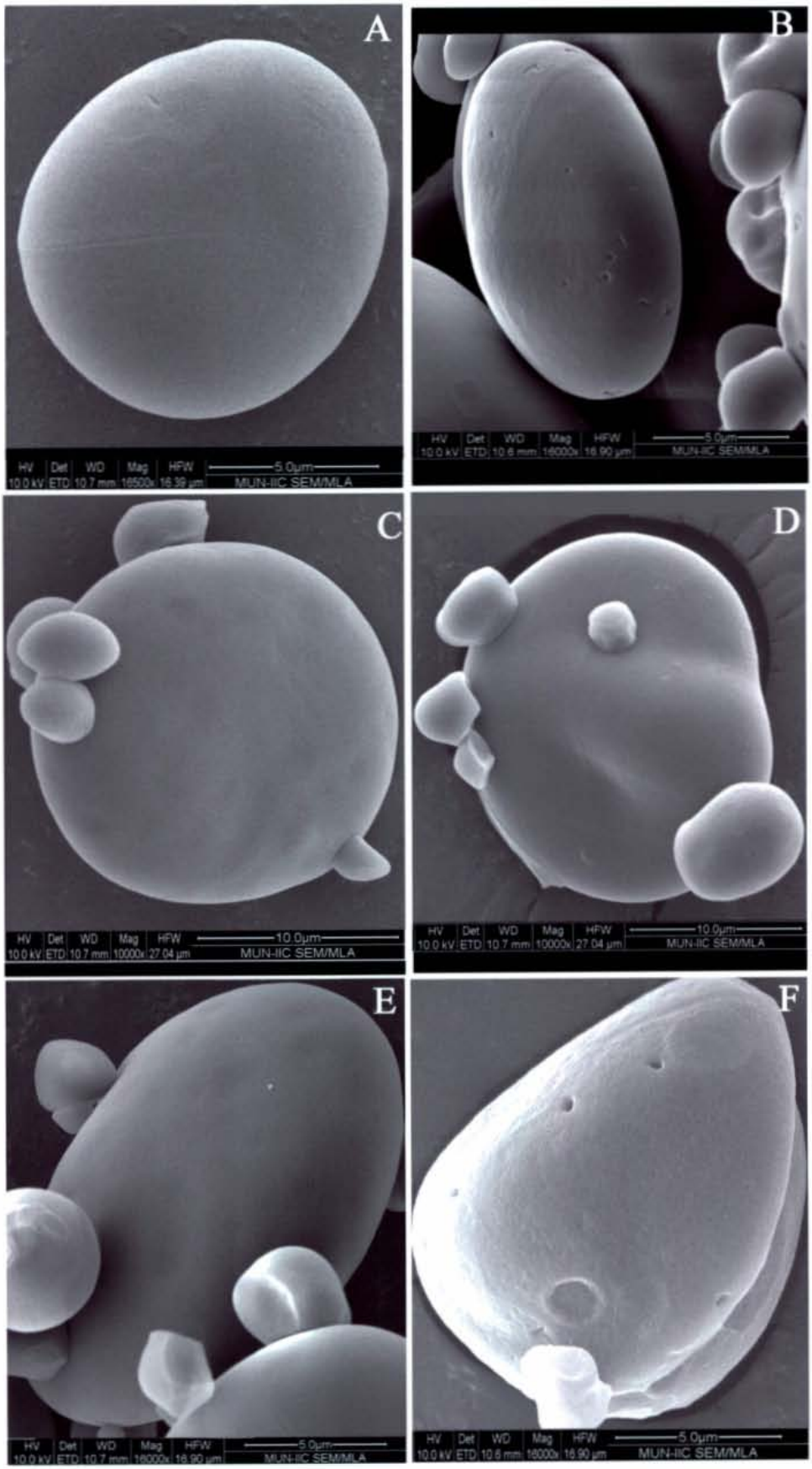
[6] Lipid complexed amylose chains (%)= $\frac{\text{total-apparent}}{\text{total}} x100$

Figure 4.1 Scanning electron micrographs of native and annealed starches

A) Native CDC Teal, B) Annealed CDC Teal

C) Native 11132, D) Annealed 11132

E) Native 99WAX27, F) Annealed 99WAX27


A
HV Det WD Mag HFW
10.0 kV ETD 10.7 mm 16500x 16.39 µm
5.0µm
MUN-IIC SEM/MLA
B
HV Det WD Mag HFW
10.0 kV ETD 10.6 mm 16000x 16.90 µm
5.0µm
MUN-IIC SEM/MLA
C
HV Det WD Mag HFW
10.0 kV ETD 10.7 mm 10000x 27.04 µm
10.0µm
MUN-IIC SEM/MLA
D
HV Det WD Mag HFW
10.0 kV ETD 10.7 mm 10000x 27.04 µm
10.0µm
MUN-IIC SEM/MLA
E
HV Det WD Mag HFW
10.0 kV ETD 10.7 mm 16000x 16.90 µm
5.0µm
MUN-IIC SEM/MLA
F
HV Det WD Mag HFW
10.0 kV ETD 10.6 mm 16000x 16.90 µm
5.0µm
MUN-IIC SEM/MLA

chromatography [HPSEC] (Demeke *et al.*, 1999), since the amylose concentration of wheat starch has been found to vary depending upon the method used in its determination (Demeke *et al.*, 1999, Yamamori *et al.*, 1995). The apparent and total amylose concentration determined by Chrastil's method (1987) in which starch is solubilized by sodium hydroxide were 23.3 (CDC teal), 26.9 (11132) and 0% (99WAX27), and 26.3% (CDC teal) 32.3% (11132) and 0% (99WAX27) (Table 4.2). The apparent and total amylose concentrations determined by Chrastil's method (1987) in which starch is solubilized by urea-dimethyl sulfoxide were 23.2% (CDC teal), 26.4% (11132) and 0% (99W AX27), and 26.7% (CDC teal), 32.4% (11132) and 0% (99WAX27) (Table 4.2). There was no significant difference between the total amylose concentration determined by high perfonnance size exclusion chromatography (26.9% CDC teal, 32.2% [11132], 0% (99WAX27) and that by colorimetry (Table 4.2). The total amylose concentration of CDC teal and 1132 starches was within the range reported for other varieties of normal (25.7-28.8%) [Yamamori *et al.*, 1995] and high amylose (30-37%) [Van Hung et a1. 2006, Yamamori et a1., 1995] wheat starches, The free and bound lipid contents (Table 4.1) ranged from 0.02 to 0.07% (11132 >99WAX27~CDC tea1) and 0.24 to 0.77% (11132 >CDCtea1 > 99WAX27). An increase in bound lipid content with increase in amylose concentration has also been reported (Suh *et al.*, 2004, Li *et al.*, 2001a,b) for barley starches. There was no significant changes

in phosphorus (total and non-lipid), nitrogen, ash, and lipid (free and bound) contents on annealing (Table 4.1). However, the amylose concentration (apparent and total) determined colorimetrically using NaOH and/or urea-DMSO decreased on annealing (Table 4.2). The extent of this decrease for apparent amylose concentration was in the range 1.87 -1.97% (CDC teal) and 1.81-2.06% (11132), whereas, for total amylose concentration this was in the range of 1.91 to 2.25% and 1.91-3.24% for CDC teal and 11132, respectively. Determination of total amylose concentration by HPSEC, showed no decrease on annealing in both CDC teal and 1132 starches (Table 4.2). The iodine-based colorimetric determination of amylose concentration is based on the ability of the amylose helix to interact with pentaiodide (I_5^-) ions. The I_5^- has been shown to be present in the central tunnel of the helix (Teitelbaum *et al.*, 1978). The decrease in total amylose concentration after annealing suggests decreased iodine binding to the amylose helix. Decreased iodine binding could occur if the thennal energy imparted to helical amylose chains had triggered a change in conformation (helix to coil) and/or facilitated interaction (mainly by hydrogen bonding) between amylose-amylose (AM-AM) and/or amylose-amylopectin (AM-AMP) chains. McGrance *et al.* (1998) have shown that one turn of the helix accommodates one pentaiodide (I_5^-) anion. This suggests, that a decrease in the number of helical turns as a result of a change in amylose conformation would decrease the iodine complexing ability of annealed

starches. Furthermore, if all the interactions formed between AM-AM and/or AM-AMP chains on annealing were disrupted by sodium hydroxide or by urea-dimethylsulfoxide (reagents used for starch solubilization prior to amylose determination in the Chrastil's [1987] colorimetric method), then the same extent of iodine binding should have occurred in both native and annealed wheat starches. The results suggest, that some of the above interactions (especially those between AM-AM chains) may not have been disrupted during starch solubilization. Morrison & Laignelet (1983) and Teitelbaum *et al* 1978) have shown that the blue amylose-polyiodide color development is due to rapid and complete formation of the I_5^- complex in short helical segments of amylose which slowly adjust into longer or more ordered helical segments. It is likely that AM-AM and/or AM-AMP interactions formed on annealing could restrict the ability of amylose to form longer or more ordered helical segments, thereby decreasing the color intensity of the amylose-polyiodide complex Kohyama & Sasaki (2006) have shown by studies on wheat, maize and potato starches, that the total amylose concentration remains the same pre and post annealing. However, their method of amylose determination was based on complex formation between concanavalin A (ConA) and amylopectin. In this procedure, the amylopectin in a solubilized lipid free starch sample is precipitated by reaction with Con A and removed by centrifugation. The amylose remaining in the supernatant is then determined after

amylolytic hydrolysis to glucose and expressed as a proportion (%) of the glucose derived from amylolytic hydrolysis of the total starch in a separate aliquot of the solubilized sample (prior to Con A treatment).

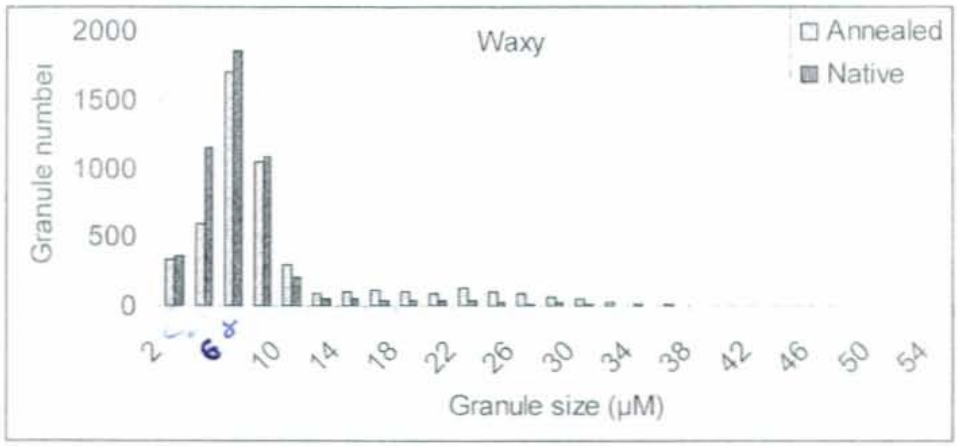
In our study, HPSEC also showed that amylose concentration remained the same pre and post annealing (Table 4.2). This was not surprising, since the Con A and HPSEC methods of amylose estimation do not rely on iodine binding to the amylose helix. It was interesting to observe, that the percentage of lipid complexed amylose chains (Table 4.2) also decreased on annealing. This lends support to our postulate, that a change in amylose conformation occurs on annealing. A helix to random coil transition would decrease lipid binding. One could then argue, that if less amylose-lipid complexes are present after annealing, then the apparent amylose concentration should have theoretically increased after annealing. The results (Table 4.2) suggest that iodine binding to annealed starches is influenced to a greater extent by changes to amylose conformation and/or to starch chain (AM-AM and/or AM-AMP) interactions, rather than by the decreased ability of amylose chains to complex lipids.

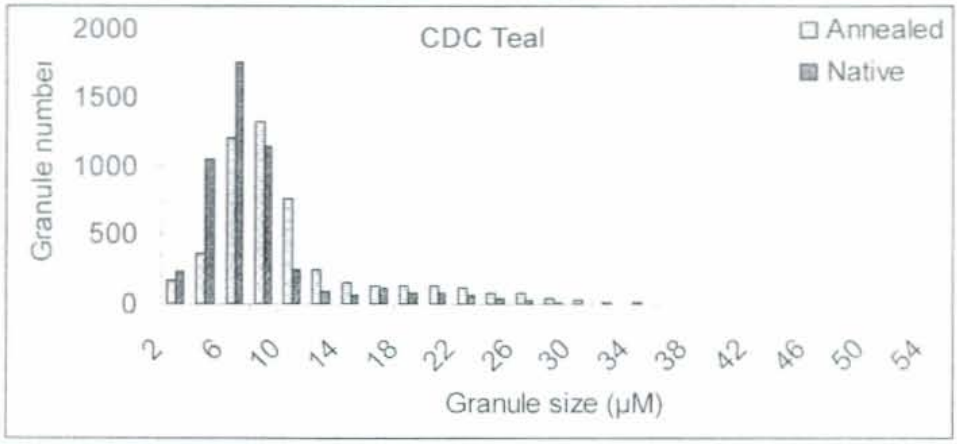
4.2 Morphology and granule size distribution

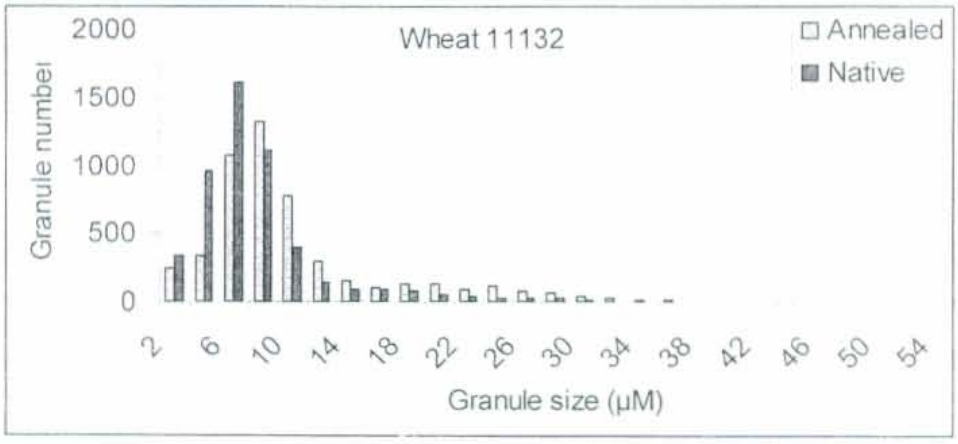
Scanning electron micrographs of the shape and surface characteristics of the native and annealed starches are presented in Fig 4.1 (a,c,e) and Fig. 4.1 (b,d,f), respectively. The shape of the large (A-type) and small (B-type) granules of both

native and annealed starches were oval to elliptical to round in shape. The granule surfaces of all native starches appeared smooth with no evidence of pores, cracks or indentations (Fig. 4.1, a,c,e) . The shape of all granules of CDC teal (Fig. 4.1b), 11132 (Fig. 4.1d) and 99WAX27 (Fig. 4.1f) remained unchanged on annealing. However, the surfaces of many granules of annealed CDC teal (Fig. 4.1b) and 99WAX27 (Fig. 4.1f) starches appeared rough and were covered with pores and indentations. The extent of these changes were more pronounced in the latter. However, the granule surface of 11132 starch remained unchanged on annealing (Fig. 4.1d). Waduge *et al*., 2006 and Hoover & Vasanthan, 1994a) have shown that the granule morphology (shape and size) and surface characteristics of normal wheat, oat and barley (starches) remains unchanged on annealing. It is difficult to make any comparison between our results and those of the above author's, since the magnification used in their studies (xl000 to x3000) were much lower. In this study, at the above magnifications, no differences were observed in the granule morphology and surface characteristics of native and annealed wheat starches. All three native wheat starches exhibited a bimodal granule size distribution (Fig. 4.2), the two size populations-large and small being termed type A (>10μm) and type-B (<10μm), respectively. The granule diameters ranged from 2 to 38μm in 99WAX27 and 11132 starches, and from 2 to 36μm in CDC teal starch. Annealing decreased the proportion of granules having diameters in the range 2 to 8 μm

Figure 4.2 Granule size distribution of native and annealed wheat starches


Waxy
Annealed
Native
Granule number
2000
1500
1000
500
0
2
6
10
14
18
22
26
30
34
38
42
46
50
54
Granule size (µM)


CDC Teal
Annealed
Native
Granule number
2000
1500
1000
500
0
2
6
10
14
18
22
26
30
34
38
42
46
50
54
Granule size (µM)


Wheat 11132
Annealed
Native
Granule number
2000
1500
1000
500
0
2
6
10
14
18
22
26
30
34
38
42
46
50
54
Granule size (µM)

(CDC teal, 1132) and 2 to 10μm (99W AX27). Van Hung and Morita (2005) have shown by studies on isolated A and B-type granules from normal wheat starch, that at low temperatures (<50°C), the swelling power of B-type granules is higher than that of A-type granules This suggests that the decrease in the proportion of small B-types granules on annealing (Fig. 4.2), may have been due to an increase in granular swelling under the annealing conditions (excess water, <50°C) used in this study. The final granule diameter of the swollen granules may have been set by the annealing temperature.

4.3 Amylopectin branch chain length distribution

The branch chain length distributions of isoamylase -debranched amylopectins of native and annealed starches analyzed by HPAEC-PAD are presented in Table 4.3. The chains were classified into four groups (dP 6-12, dP 13-24, dP 25-36, dP 37-50). The chain length distribution and the average chain length ($\overline{CL}$) of CDC teal was similar to that of waxy wheat (99WAX27) starch. This suggests that the waxy character has no influence on the chain length distribution profiles of amylopectin molecules (Yasui *et al.*, 1996). However, high amylose (11132) wheat starch exhibited a higher proportion of short chains (dP 6-12) and lower proportions of medium (dP 13-24) and long chains (dP 37-50) than CDC teal and 99WAX27 starches. This suggests that the lack of starch synthase II polypeptide

Table 4.3 Amylopectin chain length distribution of native and annealed wheat starches[1]

Wheat cultivar	Distribution (%)[2]				$\overline{CL}$[4]
	DP 6-12[3]	DP 13-24[3]	DP 25-36[3]	DP 37-50[3]	
CDC Teal					
Native	36.51±2.05[a]	48.89±1.35[a]	11.65±0.68[a]	2.95±0.02[a]	16.76±0.29[a]
Annealed	37.13±1.57[a]	48.65±0.79[a]	11.79±0.53[a]	2.43±0.25[a]	16.59±0.27[a]
11132					
Native	39.57±1.94[a]	45.33±1.66[a]	12.53±0.46[a]	2.57±0.18[a]	16.49±0.22[a]
Annealed	40.65±1.49[a]	45.27±0.64[a]	11.61±0.55[a]	2.42±0.29[a]	16.25±0.30[a]
99WAX27					
Native	35.53±2.31[a]	48.85±1.34[a]	12.54±0.99[a]	3.08±0.02[a]	17.00±0.33[a]
Annealed	36.01±2.24[a]	48.48±1.42[a]	12.41±0.60[a]	3.11±0.18[a]	16.94±0.33[a]

[1] All data reported on dry basis and represents the means of three determinations.

[2] DP_n: Indicates degree of polymerization. Total relative area was used to calculate percent distribution.

[3] Means of native and annealed starches of a particular cultivar with different superscripts are significantly different ($p<0.05$).

[4] Average chain length ($\overline{CL}$) calculated by $\sum(DP_n \times \text{peak area}_n) / \sum(\text{peak area}_n)$

from A and B genomes in 11132 results in shorter A chains (dP 6-12) being synthesized from B_1 chains (dP 13-24) and B_3 chains (dP 37-50). The proportion of chains with dP 6-12 (36.5-39.5%) and dP 37-50 (2.5-3.1 %) in the wheat starches used in this study was significantly different from that reported (Franco et al., 2002, Yoo & Jane, 2002, Yasui et al., 1996, Sasaki et al., 2002) for other wheat cultivars (dP 6-12 [18.9-25.8%], dP >37 [15.9-23.4%]). Sasaki *et al.* (2002) have also shown by studies on wheat starches with amylose contents in the range 18.5 to 28.6, that starches with high amylose content tended to have a higher proportion of short chains (dP 6-12). The amylopectin branch chain length distribution remained unchanged in all three starches on annealing. This suggests that chain length elongation, hydrolysis or debranching of amylopectin chains did not occur on annealing. A similar finding was reported by Kohyama & Sasaki (2006) for potato, corn and normal wheat starches subjected to one step annealing at 20 and 55 °C for 22h and by Tester *et al.* (2005) for starches extracted from potato tubers that were stored at 15, 25 and 55°C for 7 days (*in vivo* annealing).

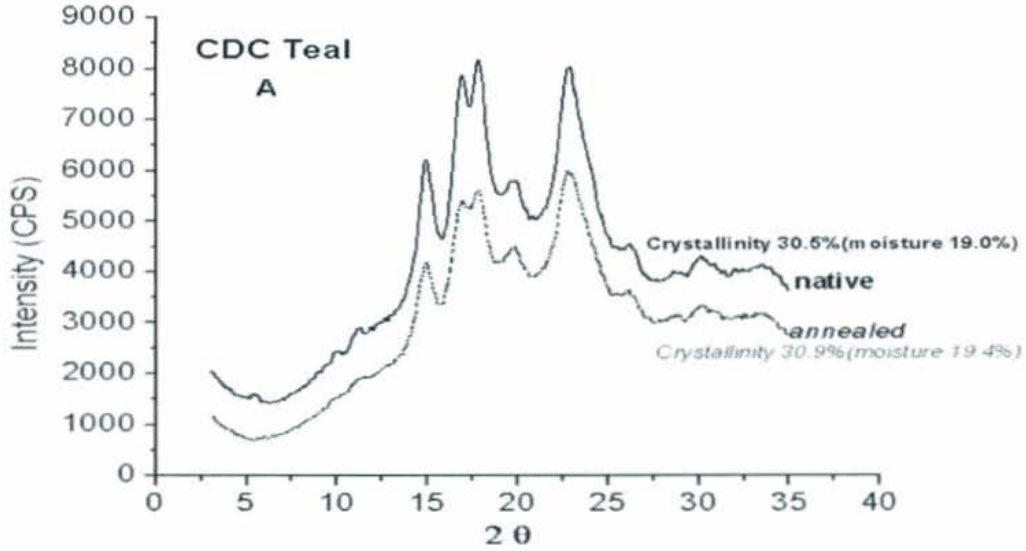
4.4 X-ray pattern and crystallinity

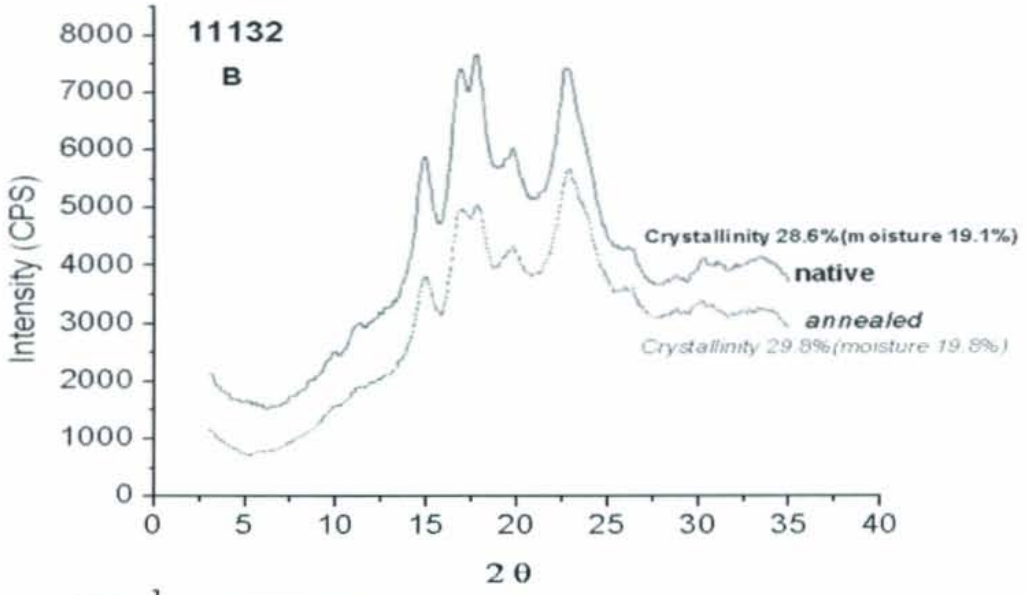
The X-ray diffraction patterns of starch granules result from parallel packing of left-handed co-axial double helices in extended regular arrays. Granule crystallinity is believed to result from clustered amylopectin chains of DP 13-15.

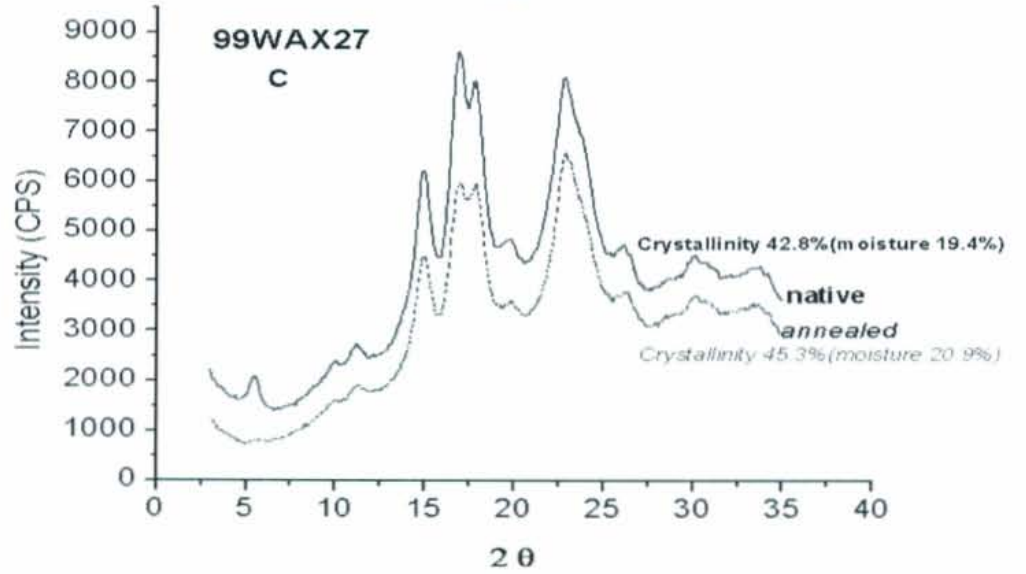
The X-ray diffraction pattern and crystallinity of native and annealed wheat starches

Figure 4.3 X-ray patterns and crystallinity of native and annealed wheat starches

CDC Teal (A), 11132 (B) and 99WAX27 (C) starches.


CDC Teal
A
Intensity (CPS)
2 θ
Crystallinity 30.5%(moisture 19.0%)
native
annealed
Crystallinity 30.9%(moisture 19.4%)


11132
B
Intensity (CPS)
2 θ
Crystallinity 28.6%(moisture 19.1%)
native
annealed
Crystallinity 29.8%(moisture 19.8%)


99WAX27
C
Intensity (CPS)
2 θ
Crystallinity 42.8%(moisture 19.4%)
native
annealed
Crystallinity 45.3%(moisture 20.9%)

determined at their maximum water absorption capacities are presented in Fig. 4.3. All three native wheat starches exhibited a predominant A-type X-ray pattern. However, CDC teal and 99WAX27 starches exhibited a peak at 5.5° 2θ (Fig. 4.3) which is typical for B-type crystallites(Hizukuri et al., 1983). Jane (2006) and Hizukuri (1986) have shown that B-type crystallinity is found in starches with long amylopectin chains. This suggests that the variation in intensity of the peak at 5.5° 2θ (99WAX27 > CDC teal) and the absence of this peak (Fig. 4.3) in the high amylose (11132) cultivar, reflects differences in average amylopectin chain length (99WAX27 > CDC teal > 11132), and in the proportion of chains with DP > 37 (99WAX27 > CDC teal > 1132). It is highly unlikely that the peak at 5.5° 2θ is influenced by amylose concentration (11132 > CDC teal > 99WAX27), since Vermeylen *et al.* (2006) have shown the presence of the 5.5° 2θ peak in wheat starches in which the apparent amylose concentration (37.0-37.5%) was much higher than that of 11132 starch (26.3%). Crystallinity of the above starches followed the order: 99WAX27 > CDC teal > 11132 (Fig. 4.3). Starch crystallinity has been shown to be influenced by: 1) amylopectin content, 2) average amylopectin chain length ($\overline{CL}$), 3) orientation of the double helices (within the crystallites) to the X-ray beam, 4) crystallite size and 5) starch moisture content (Abdel-Aal et al., 2002, Fujita et al., 1998, Jayakody et al., 2005). The higher crystallinity of native 99WAX27 (Fig. 4.3) starch could be attributed to its higher

amylopectin content (Table 4.2) and longer $\overline{CL}$ (Table 4.3). The difference in crystallinity (Fig. 4.3) between native CDC teal and 11132 starches (CDC teal > 11132) is influenced by the higher amylose content (Table 4.2), shorter $\overline{CL}$ (Table 3) and the presence of amylose chains within amylopectin crystallites (this would disrupt the packing of amylopectin crystallites, thereby changing their orientation to the X-ray beam) of 11132 starch. All native starches exhibited a peak at 19.5° 2θ (Fig. 4.3), which is characteristic of amylose-lipid complexes (Hoover & Hadziyev, 1981). The intensity of this peak followed the order: CDC teal ~ 11132 > 99WAX27. The nearly similar intensities for CDC teal and 11132 starches was rather surprising, since the amount of lipid complexed chains (Table 4.2) was much higher in 11132 (18.42-18.70%) than in CDC teal (12.9-13.3%) starch. This suggests that the organization of the amylose-lipid complexes into three dimensional structures (long range order) is probably of a higher order of magnitude in CDC teal starch. The weak amylose-lipid peak exhibited by 99WAX27 starch (Fig. 4.3) is indicative of weak interaction of lipids with the outer branches of amylopectin. This seems plausible, since 99WAX27 starch is devoid of amylose (Table 4.2). Annealing changed the X-ray pattern from A + B to a pure A-type pattern in CDC teal and 99WAX27 starches (Fig. 4.3). However, the A-type X-ray pattern of 11132 starch remained unchanged (Fig. 4.3). A similar transformation (A + B → A) pattern has also been observed in some varieties of

barley (Waduge *et al.*, 2006) and sweet potato (Genkina *et al.*, 2004a,b,c) starches. Annealing increased crystallinity (99WAX27 > 11132 > CDC teal) in all starches (Fig.4.3). An increase in crystallinity on annealing has also been shown to occur in high amylose barley (Waduge *et al.*, 2006) and normal wheat starches (Hoover & Vasanthan, 1994a). An increase in crystallinity on annealing could arise due to the interplay of several factors: 1) An increase in crystal perfection, 2) formation of new crystallites formed by interactions between (AM-AM, AM-AMP, AMP-AMP) starch chains, 3) increase in crystallite size and 4) crystallite reorientation. Consequently, it is difficult to explain the order of increase in crystallinity observed among the wheat starches on annealing. Annealing decreased the intensity of the amylose-lipid complex peak (centered at 19.5°,2θ) in all starches. This lends credence to our earlier suggestion (based on the data in Table 4.2) that a change in amylose conformation (helix→ coil) occurs on annealing.

4.5 Gelatinization

Starch gelatinization is the disruption of molecular order manifested in irreversible changes in granular properties such as granular swelling, crystallite melting, loss of birefringence and starch solubilization. The gelatinization transition temperatures (onset [T_o], mid-point [T_p], conclusion [T_c]), gelatinization transition temperature range (T_c- T_o) and gelatinization enthalpy (ΔH) of native and annealed

Table 4.4 Gelatinization parameters of native and annealed wheat starches[1]

Starch source	Gelatinization transition parameters[1]				
	To (°C)[2]	Tp (°C)[2]	Tc (°C)[2]	(Tc-To) (°C)[3]	ΔH (J/g)[4]
CDC Teal					
Native	58.3±0.2[a]	63.5±0.1[a]	72.2±0.2[a]	13.9±0.2[a]	12.3±0.2[a]
Annealed	68.1±0.1[b]	71.5±0.1[b]	78.5±0.1[b]	10.4±0.1[b]	12.5±0.1[a]
11132					
Native	52.6±0.1[a]	57.2±0.1[a]	65.4±0.1[a]	12.7±0.1[a]	11.3±0.2[a]
Annealed	61.2±0.2[b]	64.3±0.5[b]	71.6±0.3[b]	10.5±0.3[b]	11.0±0.1[a]
99WAX27					
Native	59.6±0.1[a]	65.5±0.2[a]	73.9±0.3[a]	14.3±0.1[a]	13.3±0.2[a]
Annealed	65.0±0.1[b]	69.4±0.1[b]	76.9±0.2[a]	11.9±0.2[b]	13.7±0.3[a]

[1] All data reported on dry basis and represent the mean ± SD of three determinations. Means within each column with different superscripts for native starch and its annealed counterpart are significantly different ($P<0.05$) by Tukey's HSD test.

[2] To, Tp, and Tc represent the onset, peak, and conclusion temperature, respectively.

[3] (Tc-To) represents the gelatinization temperature range

[4] ΔH represents the gelatinization enthalpy

This would also then explain the lower gelatinization parameters for 11132 starch (Table 4.4). The difference in T_o, T_p, T_c and ΔH between native CDC teal and 99WAX27 starches could be attributed to the presence of crystalline defects in CDC teal starch. This seems plausible, since differences in the proportion of dP 6-12 chains between the two starches was not significant (Table 4.3). The variation in crystalline stability (T_c- T_o) among the native starches (99WAX27 >CDC teal >11132) could be attributed to differences in their amylopectin chain ($\overline{CL}$) length (99WAX27 >CDC teal >11132) (Table 4.3). In all starches, annealing increased T_o, T_p, T_c and decreased T_c -T_o (Table 4.4). These changes have been attributed to perfection of pre-existing crystallites (Hoover & Vasanthan, 1994a, Jacobs & Delcour, 1998, Kiseleva *et al.*, 2004, Waduge et a1. 2006, Knutson, 1990, Tester et a1. 1998,2000). The extent of increase in T_o, T_p, and T_c ($T_o > T_p > T_c$) followed the order: CDC teal >11132 >99WAX27. Whereas, the extent of decrease in T_c- T_o followed the order: CDC teal >99WAX27 >11132 (Table 4.4). The above orders suggest that crystallites in native CDC teal starch are less perfect than in the other starches. Consequently, the impact of crystalline perfection on T_o, T_p, T_c, and T_c –T_o would be more pronounced in CDC teal starch. The ΔH of all starches remained unchanged on annealing. This suggests that no new double helices were fonned on annealing. The constancy of ΔH pre and post annealing has also been reported for maize and barley starches of varying amylose content (Tester *et al.*,

2000, Waduge *et al.*, 2006).

4.6 Amylose leaching (AML) and Swelling factor (SF)

Studies on AML and SF provide information on the extent of interaction between starch chains in the amorphous and crystalline domains of the native granule.

The extent of AML and SF at various temperatures are presented in Fig. 4.4 and Fig. 4.5, respectively. AML has been shown to be influenced by: 1) Total amylose content, 2) extent of interaction between amylose-amylose (AM-AM) and/or amylose-amylopectin (AM-AMP) chains within the native granule, and 3) amount of lipid complexed amylose chains (Jayakody *et al.*, 2005, Nakazawa & Wang, 2003, Hoover & Vasanthan, 1994a). In both native CDC teal and 11132 starches, AML, was detected only at temperatures exceeding 70°C and was most pronounced in the temperature range 80-85°C. A similar trend has also been reported in barley (Waduge *et al.*, 2006) and maize (Jayakody & Hoover, 2002) starches. The higher amount of leached amylose in native 11132 starch could be attributed to its higher total amylose content (Table 4.2) and/or to weaker interaction between AM-AM and/or AM-AMP chains. It is likely, that the small difference in the extent of AML between native CDC teal and 11132 starches (Fig. 4.4) is due to the higher content of lipid complexed amylose chains in the latter (Table 4.2). In both CDC teal and 11132 starches, AML decreased on annealing (Fig. 4.4). The decrease in AML on annealing has been attributed to: (1) additional

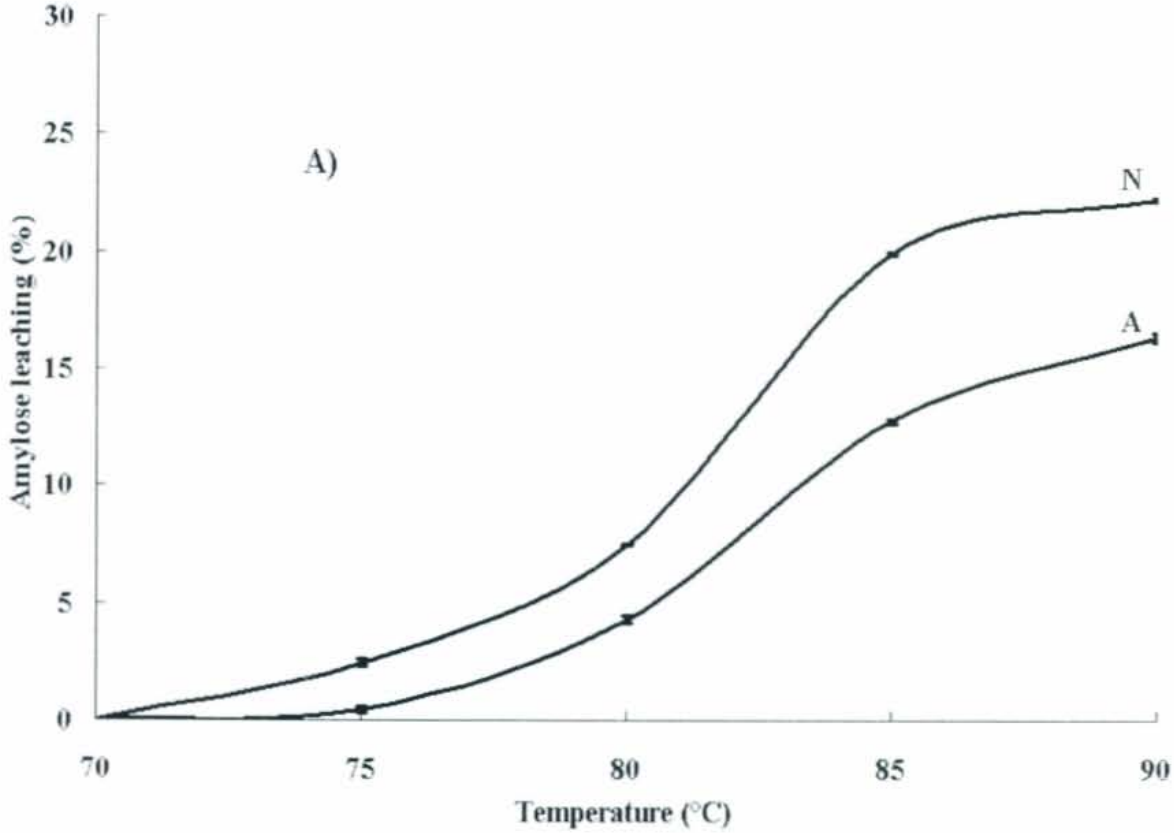
Figure 4.4 Amylose leaching in the temperature range 70°C to 90°C

A) CDC Teal native [N]

CDC Teal annealed [A]

B) 11132 native [N]

11132 annealed [A]


A)
N
A
Amylose leaching (%)
30
25
20
15
10
5
0
70
75
80
85
90
Temperature (°C)

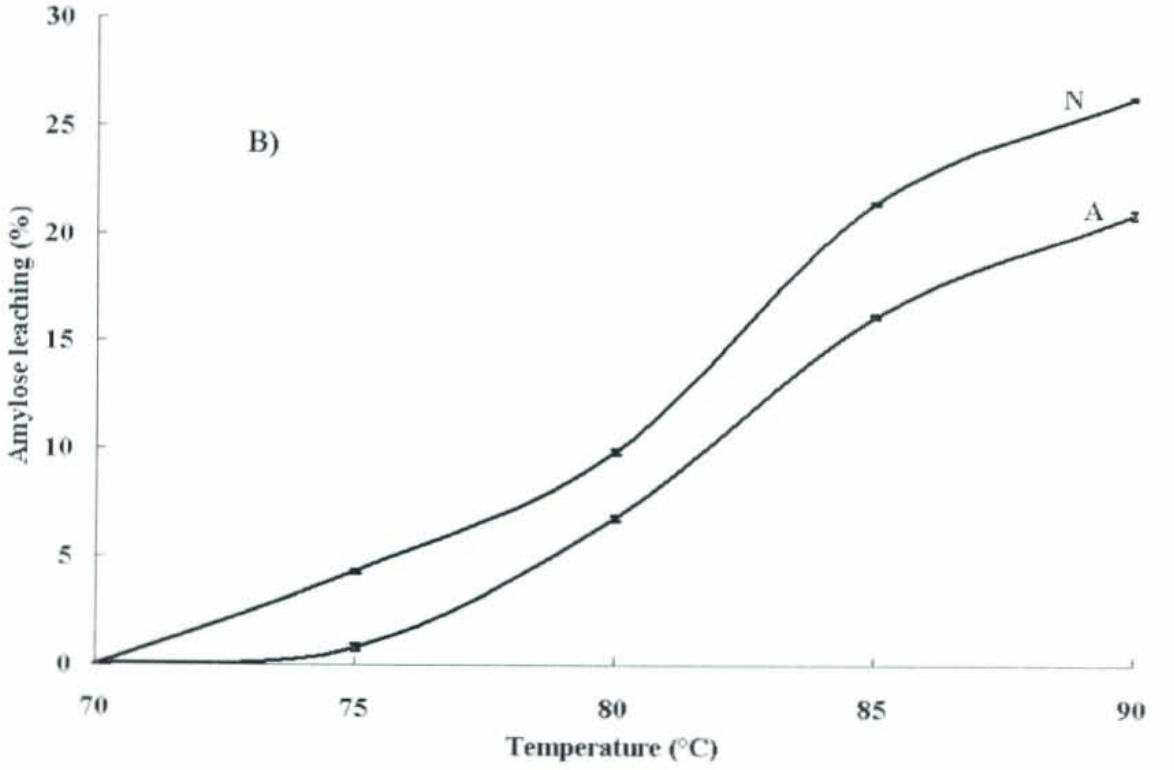

B)
N
A
Amylose leaching (%)
30
25
20
15
10
5
0
70
75
80
85
90
Temperature (°C)

Figure 4.5 Swelling factor for native and annealed wheat starches in the range 50°C to 90°C.

A) CDC Teal native [N]

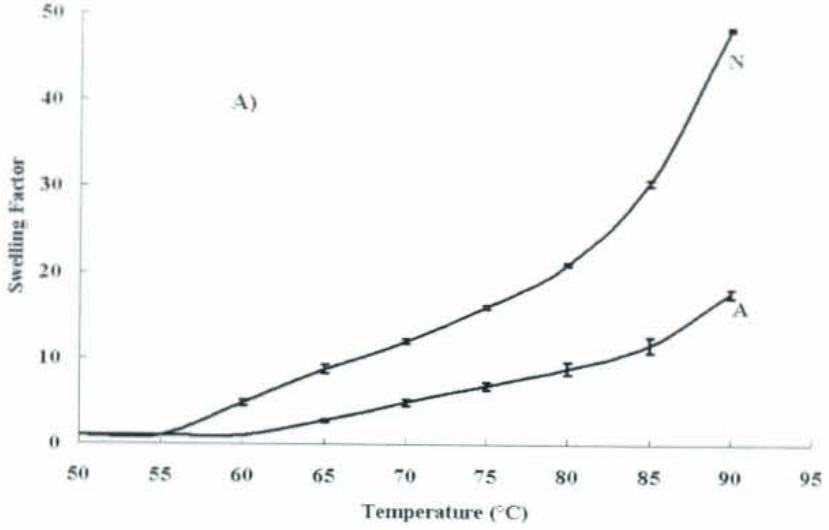
CDC Teal annealed [A]

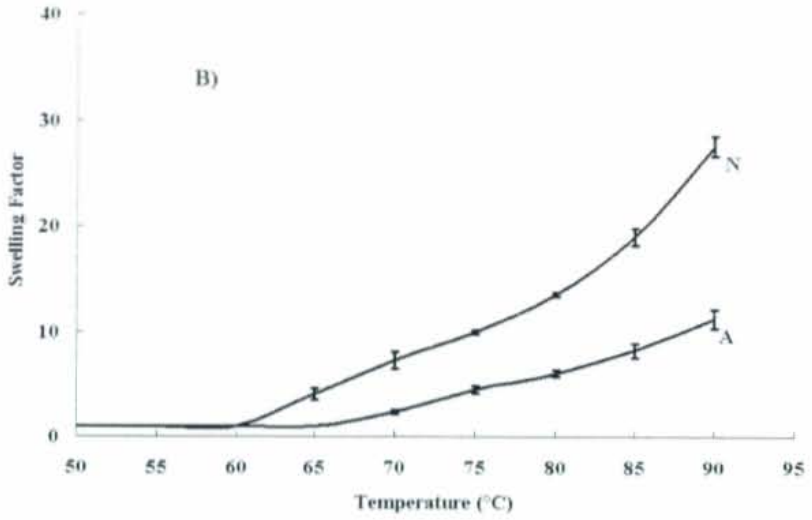
B) 11132 native [N]

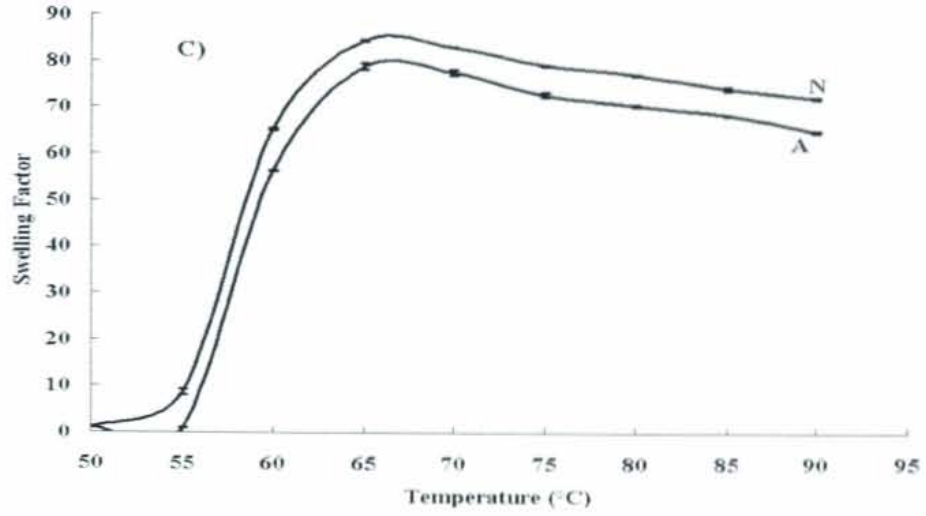
11132 annealed [A]

C) 99WAX27 native [N]

99WAX27 annealed [A]


A)
Swelling Factor
Temperature (°C)
N
A


B)
Swelling Factor
Temperature (°C)
N
A


C)
Swelling Factor
Temperature (°C)
N
A

interaction between AM-AM and/or AM-AMP chains, and 2) increase in the amount of lipid complexed amylose chains (Hoover & Vasanthan, 1994a, Tester *et al.*, 2000). In this study, the decreased AML reflects mainly AM-AM and/or AM-AMP interactions, since annealing decreased the amount of lipid complexed amylose chains in both starches (Table 4.2). The higher extent of AML reduction in CDC teal (Fig. 4.4) starch is indicative that amylose chains in native CDC teal starch are probably more compacted than in 11132 starch, and are therefore able to interact with other amylose chains more strongly on annealing.

The swelling factor (SF) of native starches followed the order: 99WAX27 >CDC teal >11132 (Fig. 4.5). A similar trend has also been reported for maize (Jayakody & Hoover, 2002, Tester *et al.*, 2000) and barley (Waduge *et al.*, 2006) starches. The SF of native 99WAX27 starch increased rapidly in the temperature range 55 to 65°C. Thereafter, the SF decreased gradually. The SF of 99WAX27 starch over the temperature range 55 to 70°C (Fig. 4.5) was much higher than that reported for native waxy barley (0% amylose) (Waduge *et al.*, 2006) and waxy maize (0% amylose) (Hoover & Manuel, 1996a) starches. For instance, at 70°C, the SF of native waxy barley (Waduge *et al.*, 2006) and native waxy maize starch (Hoover & Manuel, 1996a) have been reported to be 48 and 22, respectively. In both native CDC teal and 11132 starches, SF increased gradually until 75 °C. Thereafter, the increase in SF (CDC teal > 1132) was more rapid (Fig. 4.5). A similar trend has

also been reported in native normal and high amylose barley (Waduge *et al.*, 2006) and maize (Hoover & Manuel, 1996a) starches. The SF has been shown to be influenced by: 1) amylopectin structure (Sasaki & Matsuki, 1998, Shi & Seib, 1992, Tester *et al.*, 1993), 2) V-amylose-lipid complexes (Tester & Morrison, 1990a,b; Tester *et al.*, 1993), 3) amylose content (Morrison *et al.*, 1993, Tester *et al.*, 2000), and 4) extent of interaction between AM-AM and/or AM-AMP chains (Hoover & Manuel, 1996a, Tester *et al.*, 2000). Among the native wheat starches, the higher SF of 99WAX27 can be attributed to its higher crystallinity (Fig. 4.5) and to the absence of amylose-lipid complexes (Table 4.2). The difference in SF between native CDC teal and 11132 starches (CDC teal >11132) can be attributed to the lower crystallinity (Fig. 4.5), a more disrupted crystalline structure and to a higher content of lipid complexed amylose chains (Table 4.2) in the latter.

The SF of all three wheat starches decreased (Fig. 4.5) on annealing (CDC teal >11132 >99WAX27). The SF reduction in the 99WAX27 starch is mainly influenced by the increase in crystalline perfection (Table 4.4), whereas, in CDC teal and 11132 starches, in addition to crystalline perfection (Table 4.4), AM-AM and/or AM-AMP interactions (Fig. 4.5) or annealing may have also been responsible for the observed reduction in SF. The extent of this reduction is more pronounced in CDC teal than in 11132 starch, due to greater changes to crystalline perfection (Table 4.4) and to stronger interaction between AM-AM and/or

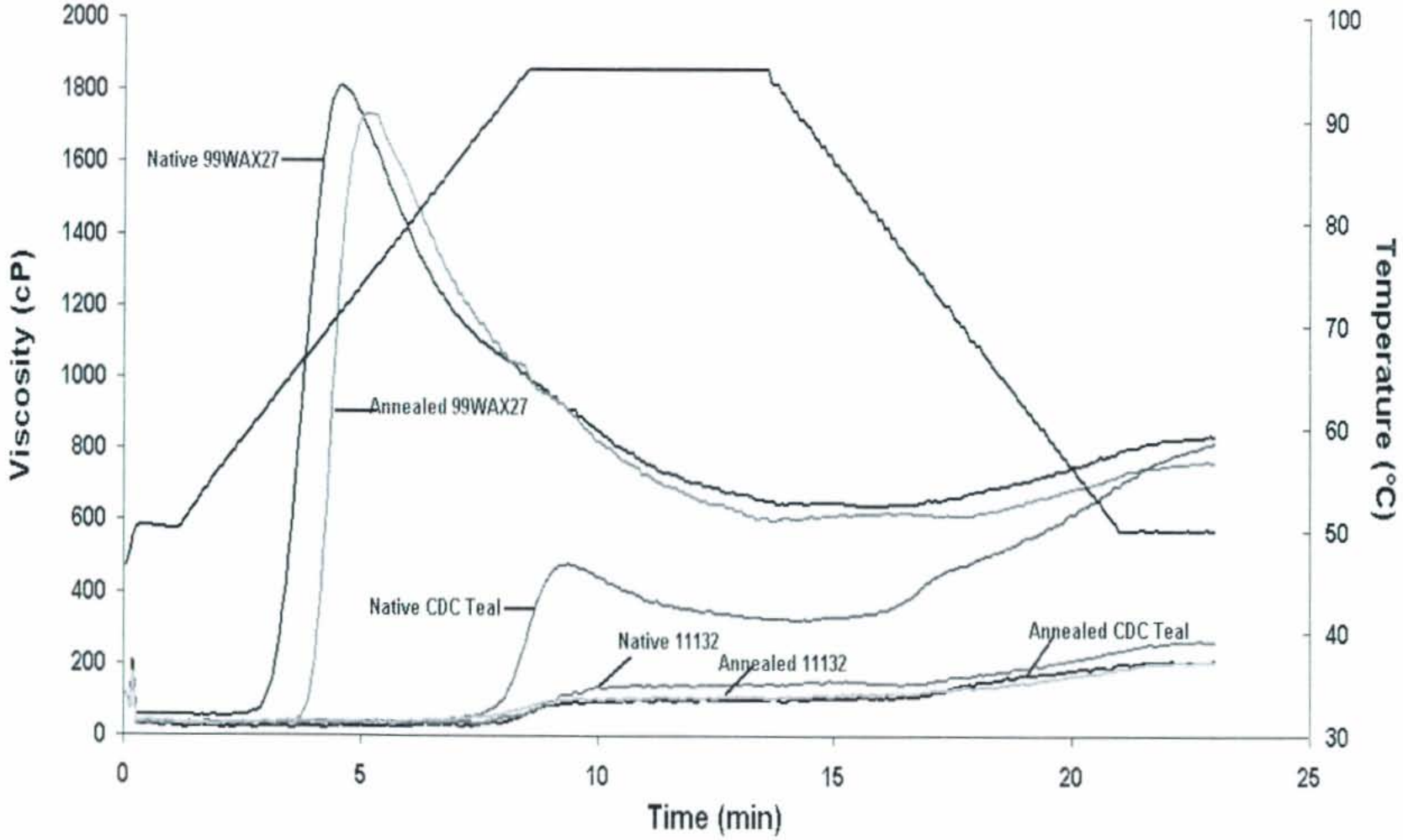
AM-AMP chains (Fig. 4.5) in the former on annealing. Reduction in SF on annealing has also been reported in barley (Waduge *et al.*, 2006), oat (Hoover & Vasanthan, 1994a) and in commercial normal wheat (Hoover & Vasanthan, 1994a) starches.

4.7 Pasting properties

A paste is defined as a viscous mass consisting of a continuous phase (a molecular dispersion) of solubilized amylose and/or amylopectin and a discontinuous phase of granule ghosts and fragments. The changes that occur during gelatinization and pasting greatly affect the rheological properties of the starch suspension. Pasting characteristics are studied by observing changes in viscosity during heating of a starch suspension.

The pasting characteristics of native and annealed wheat starches are presented in Fig. 4.6. Among the native wheat starches, peak viscosity, extent of viscosity breakdown and the final viscosity followed the order: 99WAX27 > CDC teal > 11132. Whereas, the time taken to reach peak viscosity (peak time) and the degree of set-back followed the order: 11132 > CDC teal > 99WAX27 and CDC teal > 99WAX27 > 11132, respectively. The viscosity development during heating of starch granules with water under shear has been attributed to formation of tightly packed array of swollen deformable granules, friction between swollen granules,

Figure 4.6 Pasting curves of native and annealed wheat starches


Viscosity (cP)
Temperature (°C)
Time (min)
Native 99WAX27
Annealed 99WAX27
Native CDC Teal
Native 11132
Annealed 11132
Annealed CDC Teal
2000
1800
1600
1400
1200
1000
800
600
400
200
0
100
90
80
70
60
50
40
30
0
5
10
15
20
25

amount of leached amylose and amylopectin content (Singh *et al.*, 2006, Sasaki *et al.*, 2000, Jacobs *et al.*, 1995). The viscosity rise during cooling of a heated starch suspension has been attributed to interaction between leached amylose chains, granule size and rigid swollen granules (Hoover & Sosulski, 1985, Jacobs *et al.*, 1995, Hoover & Vasanthan, 1994a). The higher peak viscosity (PV) exhibited by native 99WAX27 starch is indicative of its higher amylopectin content (Table 4.2) and higher crystallinity (Fig. 4.3). Both these factors would enable granules of 99WAX27 starch to swell rapidly without disintegration (Fig. 4.6). It is highly unlikely, that amylose leaching (AML) has any significant impact on the PV of native CDC teal and 11132 starches (CDC teal >11132), since the difference in AML between these two starches was only marginal (Fig. 4.4). The difference in PV between CDC teal and 11132 starches is more likely due to a higher crystallinity (Fig. 4.3), higher amylopectin content (Table 4.2) and to less compactly packed amylose chains in the former (Fig.4.4). The extent of viscosity breakdown during the holding cycle is more pronounced in native 99WAX27 (Fig. 4.6) starch, due to greater susceptibility of the highly swollen granules to shear. The very high resistance of 11132 native starch to shear during the holding cycle (Fig. 4.6) could be attributed to its lower extent of granule swelling (Fig. 4.5) and to a more compact arrangement of amylose chains within the amorphous domains of the granule (Fig. 4.4). Jacobs *et al.* (1995) and Hoover & Vasanthan (1994a)

have shown that the extent of setback is influenced by the amount of leached amylose, granule size, and to the presence of unfragmented rigid swollen granules embedded in the leached amylose network. The extent of set-back in the native wheat starches (CDC teal >99WAX27 >11132) (Fig. 4.6) cannot be solely attributed to AML for the following reasons: 1) native 99WAX27 starch exhibits a set-back that is higher (Fig. 4.6) than that of native 11132 starch, although it is devoid of amylose (Table 4.2), and 2) 11132 starch exhibits a lower degree of set-back than CDC teal (Fig. 4.6) although it exhibits a higher degree of AML (Fig. 4.4). This suggests that set-back in the above native starches is influenced mainly by the interplay between granule size and amylopectin chain length distribution. Among the starches, set-back is more pronounced in native CDC teal due to its higher proportion of large A-type (> 10μm granules) (Fig. 4.2). These granules (fragmented or unfragmented) if embedded within the leached amylose matrix, would increase starch viscosity , thereby enhancing the viscosity increase during the cooling cycle. The difference in set-back between native CDC teal and 11132 starches could be attributed to the small proportion of A-type granules (Fig. 4.2) and to a higher proportion of amylopectin chains with dP 6-12 (Table 4.3) in the latter. Chains with dP (6-12) have been shown to hinder association between amylopectin chains (Silverio *et al.*, 2000, Shi & Seib, 1992). The difference in set-back between native 99W AX27 and 11132 starches (99WAX27 >11132) could

only be attributed to differences in the proportion of long amylopectin chains with dP 37-50 (99WAX27 >11132) (Table 4.3), since 99WAX27 starch is devoid of amylose (Table 4.2) and its proportion of large A-type granules is smaller than in 11132 starch (Fig. 4.2). The higher set-back in 99WAX27 starch (Fig. 4.6) is indicative of the presence of a more extensively hydrogen bonded network structure formed by interaction between long amylopectin chains (dP 37-50) during the cooling cycle.

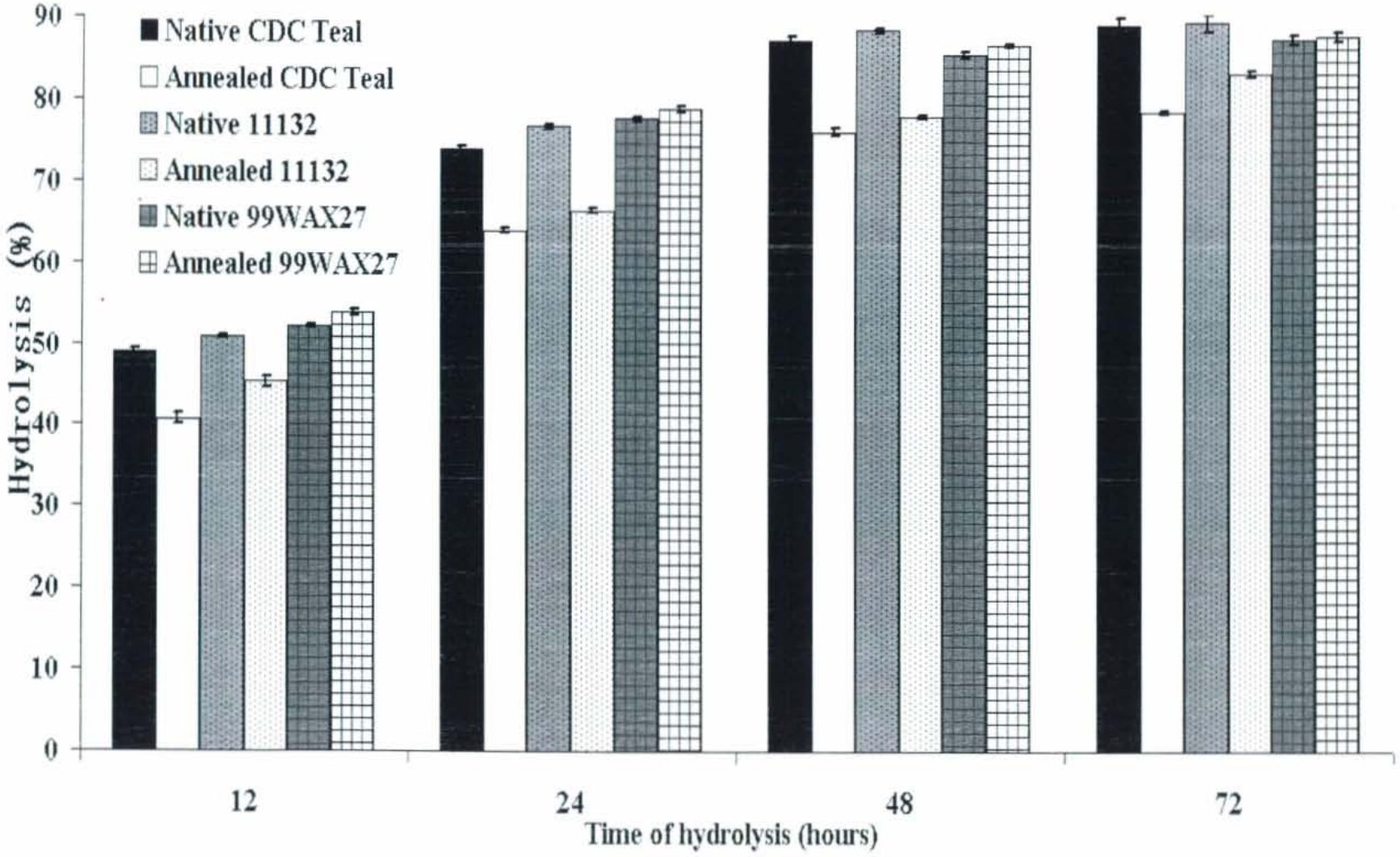
Annealing decreased PV (CDC teal >11132 >99WAX27), breakdown viscosity (CDC teal >99WAX27 >11132), final viscosity (CDC teal >99W AX27 >11132), set-back (CDC teal >99WAX27 >11132) and increased peak time (CDC teal >99WAX27 >11132). The decrease in PV on annealing can be attributed to decreased SF (Fig. 4.5) and AML (Fig. 4.4). This decrease is more pronounced in CDC teal (Fig. 4.6), due to greater reductions in AML (Fig. 4.4) and SF (Fig. 4.5). The increase in thermal stability on annealing (Fig. 4.6) is mainly due to the decrease in SF (Fig. 4.5). In CDC teal and 11132 starches, the decrease in set-back (Fig. 4.6) on annealing is influenced by the decrease in AML (Fig. 4.4) and SF (Fig. 4.5), whereas, in 99WAX27 starch, the main causative factor influencing the decrease in set-back is the decreased SF (Fig. 4.5). Jacobs et a1. (1995) and Hoover & Vasanthan (1994a) showed that in commercial normal wheat starch, peak viscosity and set-back increased on annealing. The annealing parameters

used in the above studies were 45°C/24h (Jacobs *et al.* 1995) and 50°C/72h (Hoover & Vasanthan, 1994a). The discrepancy between our results and those of the above author's may have been due to differences in cultivar, isolation procedures, annealing temperature, annealing time and the temperature and time used for drying the isolated starch.

4.8 α-amylase hydrolysis

The susceptibility of native and annealed wheat starches towards porcine pancreatic α-amylase is presented in Fig. 4.7. The difference in the extent of hydrolysis among the native starches was marginal. During the early stages (<24h), hydrolysis followed the order: 99WAX27 >11132 >CDC teal, whereas, beyond 24h, the order of hydrolysis was: 11132 >CDC teal >99WAX27 (Fig. 4.7). Small differences in hydrolysis between normal and waxy starches have also been observed in maize starches (Hoover & Manuel, 1996a, Zhang *et al.* 2006b). The above results on native maize and wheat starches (Fig. 4.7), suggests that amylose content does not play a significant role in influencing α-amylase hydrolysis. It is likely that the interplay of the following factors may have influenced the difference in susceptibility of normal, waxy and high amylose wheat starches towards α-amylase: 1) granule size (CDC teal >11132 >99WAX27) [larger granules will be less susceptible than smaller granules to α-amylase hydrolysis, due to their smaller surface area (Kong *et al.*, 2003)], 2) crystallinity (99WAX27

Figure 4.7 α-amylase hydrolysis (%) of native and annealed wheat starches


Native CDC Teal
Annealed CDC Teal
Native 11132
Annealed 11132
Native 99WAX27
Annealed 99WAX27
Hydrolysis (%)
0
10
20
30
40
50
60
70
80
90
12
24
48
72
Time of hydrolysis (hours)

>CDC teal >11132) [glucosidic bonds buried within starch crystallites will not be readily accessible to hydrolysis by α-amylase (Hoover & Sosulski, 1985)], 3) Extent of disruption of amylopectin clusters (11132 > CDC teal) [the presence of more amylose tie-chains in 11132 starch would disrupt the crystallites, enabling α-amylase easy access to the glucosidic bonds], and 4) lipid complexed amylose chains (11132 >CDC teal >99WAX27) [glycosidic bonds of lipid complexed amylose chains will be less accessible to α-amylase hydrolysis than those of free amylose chains, since the ability to undergo the chair to half chair conformational change will be difficult, if amylose chains are rendered immobile by the presence of lipid molecules inside their hydrophobic core].

Annealing decreased the extent of α-amylase hydrolysis in CDC teal and 11132 starches (CDC teal > 11132). However, in 99WAX27 starch, susceptibility towards α-amylase increased slightly on annealing. Decreased α-amylase hydrolysis an annealing has also been reported by Hoover & Vasanthan (1994a) on commercial normal wheat starch. The decreased hydrolysis on annealing in CDC teal and 11132 (CDC teal > 11132) starches could be explained as being due to increased crystalline perfection (CDC teal > 11132 (Table 4.4) and to an increase in the proportion of larger A-type granules (CDC teal > 11132 [Fig. 4.2]). SEM micrographs showed the presence of pores on the granule surface of CDC teal (Fig. 4.1b) on annealing. Theoretically, formation of pores on granule surfaces should

facilitate entry of α-amylase into the granule interior, thereby enhancing hydrolysis. Therefore, the decreased susceptibility of CDC teal starch towards α-amylase on annealing, suggests that the increase in granule size (Fig. 4.2) and crystalline perfection (Table 4.4) may have negated the effect of pore formation on hydrolysis. In 99WAX27 starch, the extent of increase in crystalline perfection (Table 4.4) and granule size (Fig. 4.2) was much lower than in the other two starches. However, more pores were formed on the granule surface of 99WAX27 starch on annealing (Fig. 4.1f). This suggests, that the marginal increase in hydrolysis in 99WAX27 starch on annealing (Fig. 4.7) is due to pore formation negating the effect of increased crystalline perfection and granule size on the extent of hydrolysis. It is difficult to find a consensus of the action pattern of α-amylase on annealed wheat starches (Jacobs *et al.*, 1998b, Wang *et al.*, 1997, Hoover & Vasanthan, 1994a) due to differences in α-amylase source and cultivar differences. Furthermore, the above studies have not included waxy and high amylose wheat starches.

4.9 Acid hydrolysis

The solubilization patterns of native and annealed wheat starches are presented in Table 4.5. The extent of hydrolysis among the native starches followed the order: 99WAX27 >11132 >CDC teal. A relatively slow rate was observed during the first three days, followed by a faster rate between day 4 and day 20. The highest rate of hydrolysis for all three starches occurred between the 12th and 20th day (Table 4.5).

Table 4.5 Acid hydrolysis (%) of native and annealed wheat starches[1]

Starch source	Acid hydrolysis (%)										
	Day 1	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 12	Day 15	Day 20
CDC Teal											
Native	0.6±0.2^{a}	2.6±0.1^{a}	11.7±0.2^{a}	19.2±0.3^{a}	24.7±0.3^{a}	28.0±0.5^{a}	31.7±0.3^{a}	39.5±0.3^{a}	46.4±0.4^{a}	56.6±0.3^{a}	73.7±0.4^{a}
Annealed	0.9±0.1^{a}	2.8±0.1^{a}	12.1±0.2^{a}	20.0±0.5^{a}	22.6±0.2^{b}	24.7±0.1^{b}	27.6±0.3^{b}	33.9±0.1^{b}	39.6±0.4^{b}	48.0±0.2^{b}	59.7±0.7^{b}
11132											
Native	0.9±0.2^{a}	3.1±0.1^{a}	15.5±0.3^{a}	23.1±0.2^{a}	31.5±0.2^{a}	33.8±0.2^{a}	38.3±0.2^{a}	42.9±0.5^{a}	49.8±0.8^{a}	59.8±0.3^{a}	78.4±0.4^{a}
Annealed	1.2±0.1^{a}	3.3±0.1^{a}	16.0±0.2^{a}	23.5±0.1^{a}	27.7±0.1^{b}	30.6±0.1^{b}	34.5±0.1^{b}	38.6±0.6^{b}	43.5±0.4^{b}	54.4±0.3^{b}	71.3±0.8^{b}
99WAX27											
Native	1.0±0.2^{a}	3.6±0.1^{a}	19.2±0.2^{a}	26.8±0.1^{a}	33.2±0.2^{a}	36.9±0.2^{a}	42.1±0.4^{a}	47.4±0.6^{a}	53.1±0.7^{a}	64.4±0.3^{a}	83.9±0.4^{a}
Annealed	1.3±0.1^{a}	3.8±0.1^{b}	19.6±0.2^{a}	27.1±0.3^{a}	30.0±0.5^{b}	33.7±0.4^{b}	38.5±0.5^{b}	44.8±0.4^{b}	48.9±0.8^{b}	60.9±0.2^{b}	83.0±0.8^{a}

[1] All data reported on dry basis and represent the mean + SD of three determinations. Means within each column with different superscripts for native starch and its annealed counterpart are significantly different ($P<0.05$) by Tukey's HSD test.

Acid hydrolysis has been shown to exhibit two phases in barley (Waduge *et al.*, 2006), maize (Nakazawa & Wang, 2003, Jayakody & Hoover, 2002) and wheat (Jacobs *et al.*, 1998c, Tester *et al.*, 1998, Hoover & Vasanthan, 1994a) starches. The first phase (between day 1 and day 8) has been attributed to the relatively fast hydrolysis within the amorphous lamellae, and the second slow phase (between day 9 and day 15) to hydrolysis of the crystalline lamella.

In this study, none of the wheat starches exhibited a slow rate of hydrolysis between day 9 and day 15 (Table 4.5). This is indicative, that the crystalline regions of the above starches were not hydrolyzed during the time course of hydrolysis. Differences in the extent and rate of hydrolysis among starches has been attributed to the interplay of many factors. Studies by Vasanthan and Bhatty (1996) found that small granules of barley are hydrolyzed faster than larger granules. Morrison *et al.* (1993) reported by studies on barley starches that lipid complexed segments of single amylose helices are resistant to acid hydrolysis. Hoover and Manuel (1996a) showed by studies on native maize starches of varying amylose content, that susceptibility towards acid hydrolysis is influenced by the extent of interaction between starch chains within the amorphous domains of the granule. Srichwong *et al.* (2005a,b,c) have shown by studies on cereal and tuber starches, that although the characteristics of the amorphous lamella are a critical factor influencing hydrolysis rates, the amylopectin chain length

distribution also plays a significant role in influencing the extent of hydrolysis. These authors' showed that very short chains (DP 6-8) of amylopectin are readily hydrolyzed together with amorphous material by acid, since they are not long enough to form double helices. Jayakody & Hoover (2002) have postulated that pores on the granular surface could influence the extent of hydrolysis. An increase in amylose content in maize starches has been shown to decrease hydrolysis (Hoover & Manuel, 1996a). Our results indicate that the difference in the extent of hydrolysis among the native wheat starches is largely influenced by differences in their proportion of A-type (large) granules (CDC teal >11132 >99WAX27) (Fig. 4.2). Granules of 99WAX27 starch are hydrolyzed faster, since the surface area accessible to acid is much larger (due to a lower proportion of large A-type granules). It is likely, that differences in granule size may have negated the effect of lipid complexed amylose chains (11132 >CDC teal >99WAX27), crystallite disruption (11132 >CDC teal) amylose content (11132 >CDC teal >99WAX27) and proportion of dP 6-12 chains (11132 > CDC teal >99WAX27) on the rate and extent of acid hydrolysis in the native starches.

In all annealed starches, the extent of acid hydrolysis between day 1 and day 5 was marginally higher than that of their native counterparts (Table 4.5). Between day 6 and day 20, all annealed starches were hydrolyzed to a lesser extent than their native counterparts (Table 4.5). The extent of this decrease between day 6 and day

8 was marginal and nearly similar for all starches (Table 4.5). However, beyond day 9, differences in hydrolysis between native and annealed starches were more pronounced and followed the order: CDC teal >11132 > 99WAX27. The effect of annealing on the acid hydrolysis profile of normal wheat starches from other cultivars has been shown to be different from that reported in this study. For instance, Tester *et al.* (1998) showed that during the rapid phase of hydrolysis (2M HCl at 35°C/10 days), annealed normal wheat starch was more extensively degraded than its native counterpart, while during the slow phase of hydrolysis, there was no difference. Hoover & Vasanthan (1994a) reported a slight reduction (~5%) in hydrolysis on annealing (2.2M HCl, 35°C/20 days) normal wheat starch during both phases. Jacobs *et al.* (1998c) reported no difference in hydrolysis (2.2M HCl, 35°C,20 days) between native and annealed normal wheat starch. In barley starches (normal, waxy and high amylose) the extent of decrease in annealing throughout the time course of hydrolysis has been shown to be less than 5% (Waduge *et al.*, 2006).

The extent of decrease in acid hydrolysis on annealing has been attributed to: 1) perfection of starch crystallites, 2) increased resistance of α (1→6) branch points, 3) formation of V-amylose lipid complexes and 4) formation of amylose double helices (Hoover & Vasanthan, 1994a, Jacobs & Delcour, 1998a). Jacobs & Delcour (1998a) have postulated, that perfection of starch crystallites during

annealing, causes some of the α (1→6) branch points in the amorphous regions to become more embedded in the crystalline structure and, as a result, less susceptible to acid hydrolysis. The results (Table 4.5) suggest, that the extent of decrease in acid hydrolysis on annealing is a reflection on: 1) the amount of α (1→6) branch points that may have become embedded within the starch crystallites (greater in CDC teal, due to larger differences in crystalline perfection between the native and annealed states [Table 4.4]), and 2) the extent of interaction between AM-AM chains (greater in CDC teal [Fig. 4.5]). It is highly unlikely, that acid hydrolysis is influenced by the amount of lipid complexed amylose chains (LCAC), since LCAC decreased on annealing (Table 4.2).

4.10 Retrogradation

Retrogradation occurs when the starch components in gelatinized starch reassociate in an ordered structure. In its initial phase, two or more glucan chains may form a simple juncture point, which may then develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears. In this study, retrogradation of the wheat starches was determined by turbidity and DSC measurements.

4.10.1 Transmission measurements

The percentage light transmission (%T) of native and annealed starch pastes stored at 40°C for periods ranging from 1 to 17 days is presented in Table 4.6. The %T of

Table 4.6 Transmittance[1] of native and annealed wheat starches as a function of days of storage[2]

Starch source	Transmittance (%) at 640nm Time of storage (days)								
	1	3	5	7	9	11	13	15	17
CDC Teal									
Native	37.9±0.2^{a}	36.2±0.0^{a}	35.1±0.1^{a}	34.3±0.1^{a}	33.7±0.1^{a}	33.2±0.1^{a}	32.7±0.1^{a}	32.4±0.1^{a}	32.1±0.1^{a}
Annealed	12.8±0.1^{b}	11.6±0.0^{b}	10.5±0.0^{b}	10.0±0.0^{b}	9.8±0^{b}	9.5±0^{b}	9.3±0^{b}	9.1±0^{b}	8.9±0^{b}
11132									
Native	11.2±0.0^{a}	7.6±0.1^{a}	7.1±0.1^{a}	6.7±0.1^{a}	6.4±0.1^{a}	6.1±0.1^{a}	5.8±0.1^{a}	5.6±0.1^{a}	5.3±0.1^{a}
Annealed	6.4±0.1^{b}	4.9±0.1^{b}	4.8±0.1^{b}	4.8±0.1^{b}	4.7±0^{b}	4.6±0^{b}	4.5±0^{b}	4.4±0^{b}	4.3±0^{b}
99WAX27									
Native	68.5±0.3^{a}	66.3±0.2^{a}	64.8±0.2^{a}	63.1±0.3^{a}	61.8±0.2^{a}	60.8±0.1^{a}	59.8±0.1^{a}	59.0±0.1^{a}	58.3±0.1^{a}
Annealed	62.9±0.1^{b}	60.4±0.1^{b}	58.2±0.2^{b}	55.9±0.2^{b}	54.0±0.1^{b}	52.4±0.1^{b}	51.0±0.1^{b}	49.7±0.1^{b}	48.6±0.1^{b}

[1] All data reported on dry basis and represent the mean + SD of three determinations. Means within each column with different superscripts for native starch and its annealed counterpart are significantly different ($P<0.05$) by Tukey's HSD test.

[2] Samples stored at 4°C for 24h to initiate nucleation and then at 40°C for the time periods indicated.

the native starches followed the order: 99WAX27 >CDC teal >11132 throughout the storage period. In the native starches, %T of CDC teal and 99WAX27 starches decreased gradually during storage. However, 11132 starch exhibited a steep decrease (Table 4.6) in %T at the end of the third day of storage followed by a gradual decrease thereafter. At the end of the storage period (17 days), %T had decreased by 15.3%, 52.6% and 14.8% in native CDC teal, 11132 and 99WAX27 starches, respectively. This suggests that the extent of retrogradation is most pronounced in native 11132 starch.

In all starches, %T decreased on annealing (CDC teal >11132 >99WAX27). In both annealed CDC teal and 11132 starches, the change in %T was most pronounced at the end of the third day of storage. At the end of the 17th day, %T had decreased by 43.6%, 32.8% and 22.7% in annealed CDC teal, 11132 and 99WAX27 starches, respectively. This suggests that, among the annealed starches, retrogradation is most pronounced in CDC teal starch.

The extent of %T has its origin in refractive index fluctuation over a distance scale comparable to the wavelength of observation. In a polymer solvent system this is caused by density fluctuation over the same distance scale and has been attributed to polymer-polymer aggregation (Gidley and Bulpin, 1989). Gidley and Bulpin (1989) have shown, on the basis of their studies on amylose aggregation in aqueous systems, that even at the onset of detectable transmission, highly

aggregated polymer structures are present. Craig *et al.* (1989) have classified starch pastes into three categories depending on their behavior in light: 1) high clarity and almost no whiteness [due to little or no refraction of light resulting from lack of swollen granule remnants and little reflection of light (due to limited association of starch chains), 2) moderate clarity and high whiteness [due to little refraction (few granular remnants) and high reflection of light (due to interaction between polymer chains)], and 3) low clarity and low whiteness (due to high refraction of light by swollen granular remnants but little reflection by collapsed or associated starch granules). SF measurements (Fig. 4.5) showed the highest granular swelling for native 99WAX27 starch. This suggests that granule remnants may not have been present during transmission measurements (since the granules of 99WAX27 starch would have been very fragile and thus prone to extensive disintegration). This would then partly explain the high %T of native 99WAX27 starch during the storage period. In addition, weak interaction between amylopectin chains of 99WAX27 starch during storage may have also accounted for its high %T. The difference in %T between native CDC teal and 11132 starches (CDC teal > 11132) (Table 4.6) could be attributed to the greater extent of amylose leaching (Fig. 4.4) and to the presence of more intact granule remnants in the latter [since the SF of 11132 starch was less than that of CDC teal starch (Fig. 4.6)]. It is also likely, that interactions between amylose chains may have been more

pronounced in 11132 starch (due to a greater extent of amylose leaching) than in CDC teal starch (Fig. 4.4). This would then also explain the difference in %T between these two starches. The steeper reduction in %T during the first three days of storage in CDC teal and 11132 starches, is indicative of rapid amylose-amylose interactions and/or binding of granule remnants into assemblies by leached amylose and amylose aggregates (Jacobson *et al.*, 1997). The plateaued %T after 5 days storage in CDC teal and 11132 starches, and after 9 days storage in 99WAX27 starch, corresponds to slow aggregation of amylose-amylose and amylopectin-amylopectin chains (Table 4.6).

The reduction in %T on annealing (Table 4.6) reflects reduction in SF (Fig. 4.5). A reduction in SF would make the granules less fragile, and thus increase the amount of intact granule remnants The effect of reduction in % T on annealing is more pronounced in CDC teal starch, since the extent of reduction in SF on annealing followed the order: CDC teal >11132 >99WAX27 (Fig. 4.5). The results showed that the rate of retrogradation (determined by noting the extent of decrease in %T at the various time intervals of storage) of annealed CDC teal and 11132 starches (Table 4.6) were lower (due to a decrease in amylose leaching) than that of their native counterparts (Table 4.6), whereas, in 99WAX27 starch, annealing increased the rate of retrogradation (Table 4.6). The effect of annealing on the rate of retrogradation of waxy (99WAX27) and amylose containing starches (CDC teal,

11132) can be explained by considering the changes that occur within the crystalline lamella during gelatinization. Waigh *et al.* (2000b) have postulated that during gelatinization in excess water, the double helices forming the crystalline lamella undergo slow side by side dissociation, followed by a fast helix to coil transition. DSC results (Table 4.4) showed that crystalline perfection occurs on annealing. This suggests that the extent of dissociation and unraveling of the double helices during gelatinization would be slower in annealed starches. Consequently, during gel storage, the formation and lateral association of the unraveled double helices would be faster in annealed than in native starches. This would then explain the difference in the rate of retrogradation between native and annealed (annealed >native) 99WAX27 starches. The difference in the rate of retrogradation between native and annealed (native >annealed) CDC teal and 11132 starches, suggests that in the annealed starches, reduced amylose leaching (Fig. 4.4) negates the faster rate of recrystallization of amylopectin double helices.

4.10.2 DSC measurements

The DSC parameters of native and annealed starches stored at 4 °C for 24h and then at 40°C for two and seven days are presented in Table 4.7. The enthalpy (ΔH_R) of retrogradation (mainly representing the dissociation, unraveling and melting of the double helices formed by associations between the outer A-chains of amylopectin during storage) of the native starches at the 2nd and 7th day of

Table 4.7 DSC analysis of native and annealed retrograded wheat starches[1]

Starch source	Time of storage (days) at 40°C							
	Day 2				Day 7			
	To (°C)[2]	Tp (°C)[2]	Tc (°C)[2]	ΔH_R (J/g)[3]	To (°C)[2]	Tp (°C)[2]	Tc (°C)[2]	ΔH_R (J/g)[3]
CDC Teal								
Native	59.3±0.1[a4]	65.6±0.1[a4]	71.8±0.4[a4]	3.97±0.12[a4]	61.9±0.1[a4]	67.8±0.5[a4]	73.0±0.1[a4]	4.48±0.09[a4]
Annealed	58.5±0.1[b]	65.6±0.1[a]	72.4±0.1[a]	4.17±0.20[a]	60.8±0.1[b]	67.2±0.1[a]	74.0±0.5[b]	4.60±0.20[a]
11132								
Native	59.1±0.1[a]	65.1±0.1[a]	70.8±0.1[a]	2.07±0.11[a]	61.3±0.3[a]	67.0±0.2[a]	72.1±0.1[a]	2.37±0.12[a]
Annealed	58.7±0.2[a]	65.1±0.1[a]	71.2±0.6[a]	2.09±0.13[a]	60.2±0.1[b]	66.7±0.1[a]	72.4±0.1[a]	2.64±0.11[a]
99WAX27								
Native	60.1±0.4[a]	66.3±0.1[a]	72.3±0.1[a]	4.32±0.19[a]	62.0±0.1[a]	67.9±0.1[a]	72.0±0.4[a]	5.15±0.15[a]
Annealed	59.4±0.1[b]	66.2±0.1[a]	72.7±0.3[a]	4.80±0.23[a]	61.4±0.2[b]	67.9±0.1[a]	74.0±0.1[a]	5.35±0.20[a]

[1] Starches were initially stored at 4°C for 24h to increase nucleation and then at 40°C for 2 and 7days.

[2] To, Tp, and Tc represent the onset, peak, and final gelatinization enthalpy of the retrograded starches.

[3] Enthalpy of retrogradation (J/g of dry matter).

[4] Means within each column with different superscripts for native starch and its annealed counterpart are significantly different ($P<0.05$).

Summary and Conclusions

The main thrust of the thesis was to gain a deep insight into the impact of annealing on the molecular structure and physicochemical properties of wheat starches varying in amylose content.

The first part of the thesis involved a detailed study of the surface characteristics, granule size distribution, composition, molecular structure and properties of starch from normal (CDC teal), high amylose (line 11132) and waxy (99 WAX 27) bread wheat (*Triticum aestivum* L.) cultivars grown under the same environmental conditions in Saskatoon. All isolated starches were pure and undamaged. The granule diameters ranged from 2 to 38μm in 99WAX27 and 11132 starches, and from 2 to 36μm in CDC teal starch. The shapes of native wheat starches were oval to elliptical to round in shape. The granule surfaces of all native starches appeared smooth with no evidence of pores, cracks or indentations. The total phosphorus, total amylose, and lipid complexed amylose chains ranged from 0.007-0.058% (CDC teal >11132 >99WAX27), 26.9-32.3% (11132 > CDC teal), and 13.4-18.7% (11132 >CDC teal >99WAX27), respectively. The amylopectin branch chain length distribution and the average chain length ($\overline{CL}$) ranged from 35.53-39.57% (degree of polymerization [DP] 6-12), 45.33-48.89% (DP 13-24), 11.65-12.54% (DP 25-36), 2.57-3.08% (DP 37-50) and 16.49-17.00, respectively. Among these three native wheat starches, 11132 exhibited an 'A'-type X-ray

pattern. However, CDC teal and 99WAX27 starches exhibited 'A+B'- type crystallites. Crystallinities ranged from 28.6-42.8% (99WAX27 > CDC teal > 11132) and a V-lipid amylose complex peak was also visible in the X-ray pattern of all starches. The gelatinization transition temperatures (T_o [onset], T_p [peak], T_c [conclusion]), gelatinization temperature range (T_c-T_o) and gelatinization enthalpy (ΔH), ranged from 52.6-59.6°C, 57.2-65.5°C, 65.4-73.9°C, 12.7-14.3°C, and 11.3-13.3 J/g, respectively. Among the native wheat starches, T_o, T_p, T_c, T_c-T_o and ΔH followed the order: 99WAX27 >CDC teal >11132. Swelling factor (at 90°C), and amylose leaching (at 90°C) ranged from 27.6-72.1 (99WAX27 >CDC teal >11132) and 22.2-26.2% (11132 >CDC teal), respectively. Peak viscosity, thermal stability, set-back and susceptibility towards acid hydrolysis followed the order: 99WAX27 >CDC teal >11132, 11132 >CDC teal >99WAX27, CDC teal > 99WAX27 >11132, and 99WAX27 >11132 >CDC teal, respectively. Susceptibility towards α-amylase hydrolysis followed the order: 99WAX 27 > 11132 > CDC teal (<24h) and 11132 >CDC teal >99WAX27 (>24h). The extent of retrogradation measured by spectroscopy and differential scanning calorimetry followed the order: 11132 > CDC teal > 99WAX27 and 99WAX27 >CDC teal > 11132, respectively.

The second part of thesis was to determine the impact of one step annealing on the composition, morphology, structure and physicochemical properties of wheat starches. The shape of all wheat starch granules remained unchanged on annealing.

However, except for 11132, whose granule surface remained unchanged on annealing, the surfaces of many granules of annealed CDC teal and 99WAX27 starches appeared rough and were covered with pores and indentations. The extent of these changes were more pronounced in the latter. Annealing decreased the proportion of granules having diameters in the range 2 to 8 μm (CDC teal, 1132) and 2 to 10μm (99W AX27). In all starches, concentration of amylose (apparent & total), lipid complexed amylose chains, gelatinization temperature range, swelling factor, amylose leaching, peak viscosity, breakdown viscosity, final viscosity, set-back, light transmission, susceptibility towards α-amylase and acid hydrolysis decreased on annealing. The extent of decrease in above parameters followed the order: CDC teal~11132, 11132> CDC teal, CDC teal > 99WAX27 >11132, CDC teal > 11132 >99WAX27, CDC teal>11132, CDC teal > 11132 >99WAX27, CDC teal > 99WAX27 >11132, CDC teal > 99WAX27 >11132, CDC teal > 99WAX27 >11132, CDC teal >11132 >99WAX27, CDC teal >11132, and CDC teal >11132 >99WAX27, respectively. However, The gelatinization transition temperatures (T_o, T_p, T_c) (CDC teal >11132 >99WAX27), thermal stability (CDC teal > 99WAX27 >11132) and crystallinity (99WAX27 >11132 >CDC teal) increased on annealing. The 'A'-type X-ray pattern of 11132 remained unchanged, while the 'A+B'-type X-ray pattern of CDC teal and 99WAX27 starches resembled more closely the 'A'-type pattern on annealing. The enthalpies of gelatinization and

retrogradation and the amylopectin chain length distribution remained unchanged in all starches.

The results showed that changes to physicochemical properties of normal, waxy and high amylose wheat starches on annealing, are influenced by changes in amylose conformation (helix to coil), increase in crystalline perfection, enhanced interaction between starch chains, decrease in the proportion of small B-type granules, and by native starch structure (crystalline defects, amylopectin chain length distribution, amylose concentration, and degree of association between starch chains within the amorphous and crystalline domains of the granule).

Recommendation for future research

This study has shown that structural changes occur within the amorphous and crystalline domains of wheat starch granules. The extent of these changes is influenced by the composition and structure of the native starches. It would be interesting to investigate, whether the structural changes on annealing could influence the reactivity of wheat starches towards reagents used for chemical modification. Furthermore, a study on the effect of heat-moisture treatment (100°C, moisture content 25%-27%, 16h) followed by annealing, and annealing followed by heat-moisture treatment on the structure and properties of starches varying in amylose content, may provide further insights into the role played by moisture and heat during hydrothermal treatment.

Publications

A) Research papers & articles:

1. **H. Lan,** R. Hoover, Q. Liu, E. Donner, M. Baga, E. K. Asare, P. Hucl, and R. N. Chibbar (2008). Impact of annealing on the molecular structure and physicochemical properties of normal, waxy and high amylose bread wheat starches. **Food Chemistry** (In Press, accepted 15 April 2008).
2. L. Jayakody, **H. Lan**, R. Hoover, P. Chang, Q. Liu, and E. Weber (2007). Composition, molecular structure and physicochemical properties of starches from grass pea (*Lathyrus sativus* L.) cultivars grown in Canada. **Food Chemistry**, 105, 116-125.

B) Abstracts in conference proceedings/presentations:

1. R. Hoover, **H. Lan,** L. Jayakody, Q. Liu, E. Donner, M. Baga, A. Erick, P. Hucl, and R. N. Chibbar (2008). Impact of annealing on the molecular structure and physicochemical properties of wheat starches. 236th American Chemical Society National Meeting (ACS). Philadelphia, PA, USA. August.
2. L. Jayakody, R. Hoover, P. Chang, **H. Lan**, Q. Liu, and E. Donner (2006). Composition, molecular structure and physicochemical properties of starches from two grass pea (*Lathyrus sativus* L.) cultivars grown in Canada. 6th Canadian pulse research workshop (Pulse innovation project symposium), Hilton Garden Inn, Saskatchewan, Canada. P. 26.
3. L. Jayakody, R. Hoover, P. Chang, **H. Lan**, Q. Liu, and E. Donner (2006). Molecular structure and physicochemical properties of grass pea (Lathyrus sativus L.) starches. Canadian Institute of Food Science & Technology (CIFST), Montreal, Canada, p.211

Scholarships and Awards

2005-2007 Graduate Fellowship, School of Graduate Studies, Memorial Universtity, Canada

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Appendices

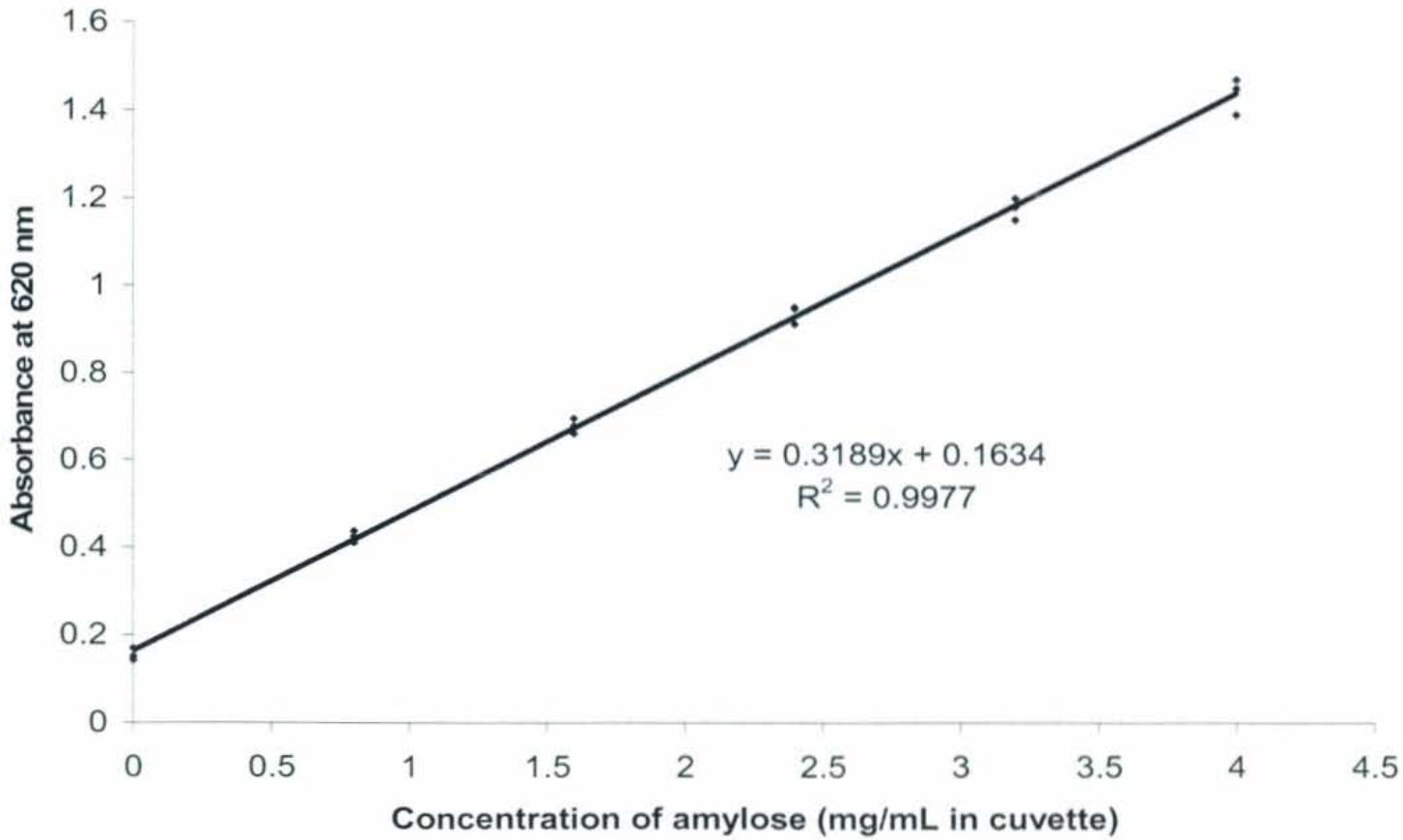


Figure A.1 Standard curve for amylose determination at 620 nm

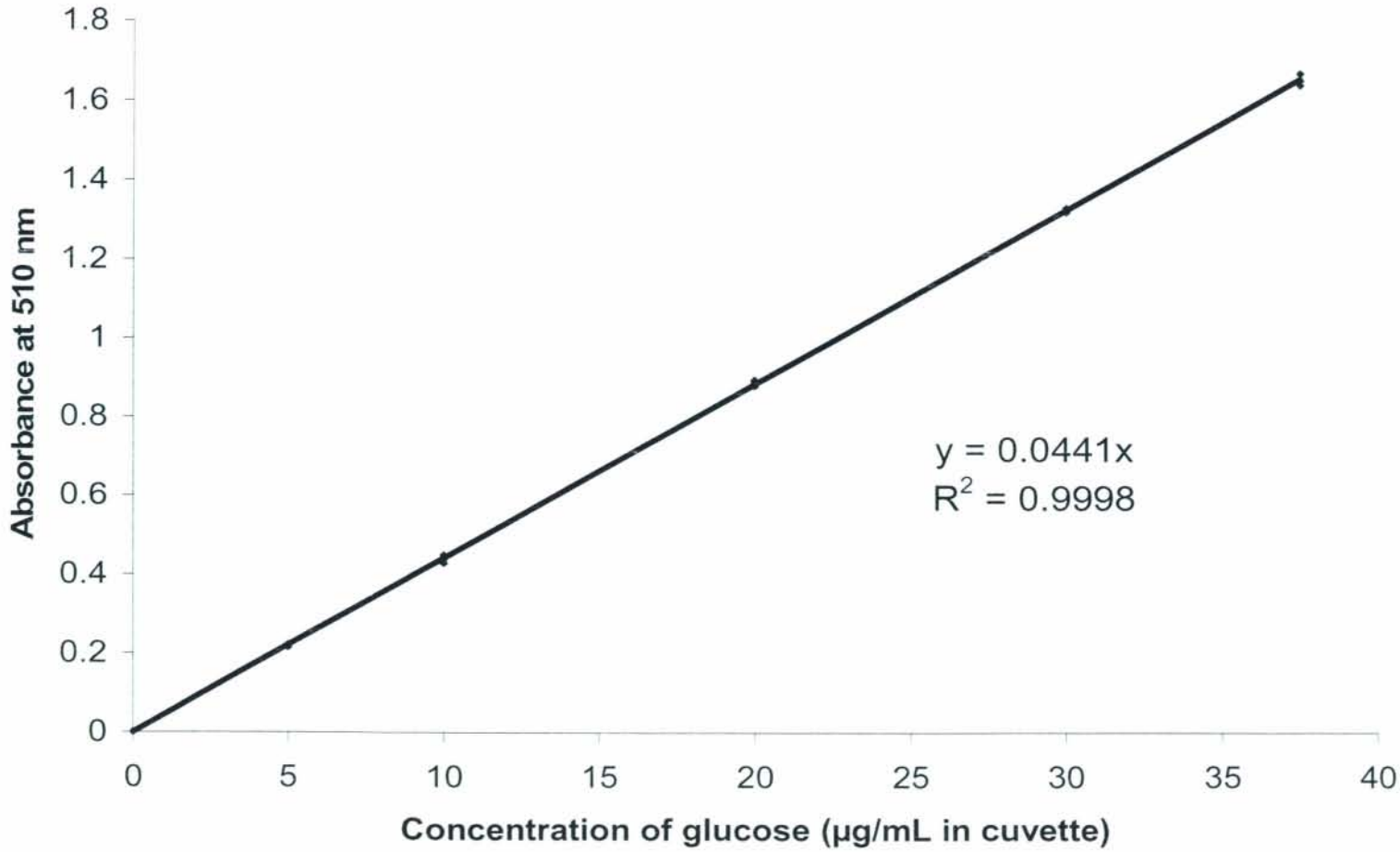


Figure A.2 Standard curve for determination of total carbohydrate as glucose 510 nm

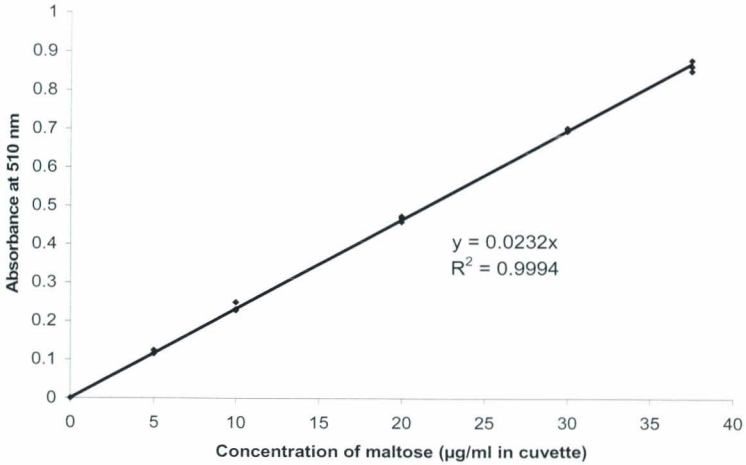
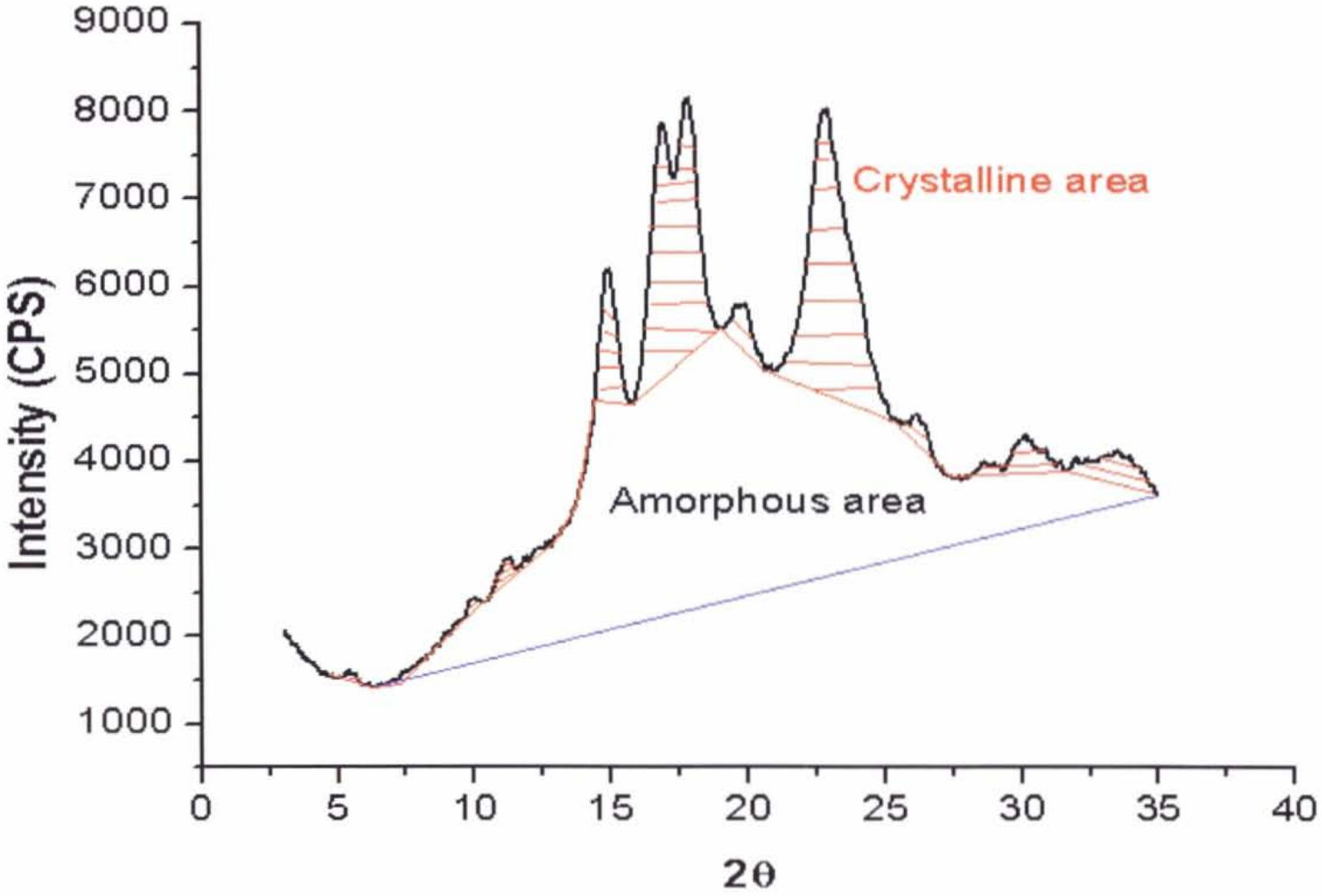


Figure A.3 Standard curve for determination of total carbohydrate as maltose 510 nm

Figure A.4 Crystallinity calculation by drawing a base line and connecting the base of the major peaks


Intensity (CPS)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
5
10
15
20
25
30
35
40
2θ
Crystalline area
Amorphous area