EFFECT OF ANNEALING ON THE STRUCTURE AND PROPERTIES OF BARLEY STARCHES

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NOTE TO USERS

Page 93

Effect of Annealing on the Structure and Properties of Barley Starches

By

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TABLE OF CONTENTS

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List of Figures

List of Tables

List of Abbreviations

AFM	Atomic force microscopy
AM	Amylose
AML	Amylose leaching
AMP	Amylopectin
ANOVA	Analysis of variance
BV	Blue value
CL	Chain length
CP/MAS	Cross polarization/magic angle spinning
CSLM	Confocal scanning laser microscopy
Da	Dalton
DMSO	Dimethyl sulphoxide
DP	Degree of polymerization
DP_n	Degree of polymerization by number
DP_w	Degree of polymerization by number
DSC	Differential scanning calorimetry
ΔH	Enthalpy
FFA	Free fatty acid
GL	Glycolipids
IA	Iodine affinity
LPL	Lysophospholipids
M	Molarity (Molar)
MALDI-MS	Matrix-assisted laser desorption/ionization mass spectrometry
M_w	Weight average molecular weight
NMR	Nuclear magnetic resonance
PL	Phospholipids

RC	Relative crystallinity
SAXS	Small angle X-ray scattering
SD	Standard deviation
SEM	Scanning electrone microscopy
SF	Swelling factor
Tc	Conclusion temperature
To	Onset temperature
Tp	Peak temperature
TEM	Transmission electron microscopy
TAG	Triacylglycerides
XRD	X-ray diffractometry
°C	Centigrade
λ_{max}	Maximum wave length
v/v	Volume/volume
w/v	Weight/volume
w/w	Weight/weight
db	Dry basis

Abstract

Starches from normal (CDC McGwire, SR 93102), waxy (CDC Fibar, HB 364), and high-amylose (SB 94897, SB 94893) hull-less barley cultivars were isolated and their structure, morphology, and properties studied before and after one step annealing (50°C for 72h at a moisture content of 75%). The starches from all genotypes consisted of a mixture of large (spherical, disk, lenticular) and small (irregular) granules. Pores were present on the granule surface of all starches. The total amylose content, the bound lipid content and the total phosphorous content ranged from 0.00 to 55.30%, 0.10 to 0.72%, and 0.024 to 0.060%, respectively. The amylopectin structure of all starches was nearly identical. The X-ray pattern of CDC Fibar, HB 364, and CDC McGwire starches was of the 'A'- type. Whereas, SR 93102, SB 94897, and SB 94893 starches exhibited a mixed 'A+B'- type pattern. The relative crystallinity (RC), swelling factor (SF), amylose leaching (AML), gelatinization temperature range (GTR), enthalpy of gelatinization (ΔH), amylose-lipid complex melting temperature (Tp_{CX}) and the enthalpy of melting of the amylose-lipid complex (ΔH_{CX}) ranged from 37.0 to 44.3%, 41.0 to 54.2% (at 90°C), 4.0 to 31.0% (at 90°C), 11.4 to 22.5°C, 6.0 to 13.0 J/g, 84.9 to 89.1°C and 0.4 to 1.8 J/g, respectively. The RC of CDC Fibar, HB 364, SR 93102 and CDC McGwire starches increased on annealing, whereas, it remained unchanged in SB 94897 and SB 94893 starches. The 'A'- type X-ray pattern of CDC Fibar, HB 364, and CDC McGwire starches remained unchanged on annealing. However, the 'A+B'- type X-ray pattern of

SR 93102, SB 94897 and SB 94893 starches resembled more closely the 'A'- type pattern on annealing. In all starches, the X-ray intensity of the V-amylose-lipid complex peak increased on annealing. Annealing increased the gelatinization transition temperatures and decreased the GTR in all starches. The ΔH of SB 94893 starch increased on annealing, whereas it remained unchanged in the other starches. Tp_{CX} of SR 93102 and SB 94897 remained unchanged on annealing, whereas Tp_{CX} of CDC McGwire increased slightly. ΔH_{CX} of native and annealed CDC McGwire, SR 93102 and SB 94897 were similar. Tp_{CX} and ΔH_{CX} were not detectable in annealed SB 94893 starch. In all starches, SF decreased on annealing. Annealing decreased AML in SR 93102, SB 94897 and SB 94893 starches in the temperature range of 50-90°C, but increased AML in HB 364 and CDC McGwire starches at higher temperatures. Annealing decreased acid hydrolysis in CDC Fibar starch during the early stages of hydrolysis. Thereafter, both native and annealed CDC Fibar starches were hydrolyzed to the same extent. However, all other starches showed no significant changes in acid hydrolysis on annealing.

Chapter 1. Introduction

1.1 Introduction

Barley (*Hordeum vulgare L.*) is a versatile crop and can be grown over a wide range of environmental conditions. Cultivated varieties are six-row and two-row type. The two-rowed barley is mostly used for malting/brewing and food processing, while the six-rowed type has been utilized mainly as livestock feed (Bhatty, 1993). Barley grains have traditionally been covered with hulls. However, recently hull-less barley has been developed for food and industrial applications (Bhatty, 1999). There is an increasing research interest in the utilization of barley as human foods and for industrial applications. This has been boosted by the recent recognition by the Food and Drug Administration (FDA, 1997) health claim for oat β-glucan, which can benefit human cardiovascular health. Although the claim is specifically for oat, it is known that barley and oat are the two cereal crops with high amounts of β-glucan. As an excellent source of soluble dietary fiber, β-glucan offers health benefits such as cholesterol lowering effect, cancer prevention, prevention of heart diseases, and lower risk of developing gallstones in women (The World Healthiest Foods, 2004). Furthermore, barley is a very good source of selenium, phosphorous, copper and niacin (The World's Healthiest Foods, 2004).

Canada is one of the leading producers of hull-less barley in the world. Several two- and six-rowed cultivars of hull-less barley have been registered in Canada in the last

15 years (Bhatty, 1999). Hull-less barley has been used for the preparation of food malt, production of ethanol, extraction and enrichment of β-glucan, preparation of native and modified starches, and preparation of bran and flour for use in bakery products (Bhatty, 1993, 1999). In Canada, the emphasis now is to extend the use of hull-less barley in food and non-food applications, including the malting and brewing industries (Bhatty, 1999). Currently, plant-breeding techniques have resulted in the production of waxy (<10% amylose) and high-amylose (>35% amylose) 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 requirements of the modern food industry. Therefore, to meet 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, 1999d).

Annealing is a process whereby a material is held at a temperature somewhat lower than its melting temperature, which permits modest molecular reorganization to occur and a more organized structure of lower free energy to form (Blanshard, 1987). Annealing of starches has been studied at various starch: water ratios (1:1, 1:3, 1:5, w/w) and at temperatures ranging from 40 to 75°C (Kuge & Kitamura, 1985; Yost & Hoseney, 1986; Krueger *et al.*, 1987a,b; Knutson, 1990; Larsson & Eliasson, 1991; Lopez & Lopez, 1991; Cameron & Donald, 1992; Stute, 1992; Seow & Teo, 1993; Hoover & Vasanthan, 1994a,b; Jacobs *et al.*, 1995; Muhrbeck & Svenson, 1996; Hoover & Manuel, 1996; Wang *et al.*, 1997; Jacobs *et al.*, 1998a,b,c; Tester *et al.*, 1998; Andreev *et al.*,

1999; Tester *et al.*, 2000; Atichokudomchai *et al.*, 2002; Nakazawa & Wang, 2003; Ozcan & Jackson, 2003; Gomez *et al.*, 2004; Nakazawa & Wang, 2004; Qi *et al.*, 2004; Kiseleva *et al.*, 2004; Genkina *et al.*, 2004 a,b).

The above studies have shown that changes to starch structure (increase in granular stability, starch chain interactions [within the amorphous and crystalline domains of the granule], perfection of starch crystallites, formation of double helices and compartmentalization of amylopectin-amylopectin, amylose-amylopectin and amylose-amylose helices) and properties (elevation of starch gelatinization temperatures, narrowing of the GTR, decrease in SF and AML and increase in hot and cold paste viscosities) occur on annealing. However, a discrepancy still exists with regard to the susceptibility of annealed starches towards acid and α-amylase hydrolysis (Hoover & Vasanthan, 1994a; Jacobs *et al.*, 1998a,b; Atichokudomchai et al., 2003; Nakazawa & Wang, 2003; Qi *et al.*, 2004; Nakazawa & Wang, 2004). Furthermore, there is a dearth of information with regard to the formation of V-amylose-lipid complexes on annealing. Most studies on annealing have involved starches from potato, rice, wheat, maize, sago, pea and cassava. However, relatively little work has been done to investigate the effect of annealing on starches extracted from cultivars (varying in amylose / amylopectin ratio) belonging to a particular starch source. Annealing of maize starches of varying amylose content has been studied mainly by differential scanning calorimetry (DSC), SF measurements and ^{13}C-CP/MAS-NMR (Knutson, 1990; Tester *et al.* 2000), whereas, annealing of barley starches of varying amylose content has been investigated only by DSC (Kiseleva *et al.*, 2004). Furthermore, Perez *et al.* (2001, 2003) have shown that

starches extracted from ground maize or from whole maize kernels that were steeped in water prior to starch extraction exhibit *in-vivo* annealing. The extent of *in-vivo* annealing was found to increase with steeping time. Thus, it is difficult to interpret the exact molecular mechanism of annealing in commercial maize starches (of varying amylose content), since they may have undergone some degree of *in-vivo* annealing during the steeping (45-55°C for 36 h) step of the maize wet milling process, and the effect of annealing as a function of amylose content remains to be investigated.

1.2 Objectives of the research

The objectives of this study were two fold:

1) To determine the granule morphology, composition, and molecular structure of barley starches varying in amylose content, isolated from recently released hull-less barley grains grown in the same lot and under identical field and environmental conditions (in order to eliminate the influence of environmentally driven reorganization [in-vivo annealing] of starch structure [Tester & Debon, 2000]), in Saskatoon, Canada.
2) To examine changes to barley starch structure and physicochemical properties during single-step annealing.

This study is of great significance since there is a growing interest in physically modified starches for food and non-food applications.

Chapter 2. Literature Review

2.1 Barley grain

2.1.1 Introduction

Barley, *Hordeum vulgare L.*, belongs to the tribe *Triticeae* and the genus *Hordeum* of the grass family *Poaceae* (Nilan and Ullrich, 1993). It is one of the oldest cereals in recorded history and originated in Ethiopia and Southern Asia, where it has been cultivated for more than 10,000 years (the World's Healthiest Foods, 2004). It is a versatile crop and can be grown over a wide range of environmental conditions. Cultivated varieties are six-row and two-row type, depending on the number of fertile spikelets on the rachis. The two-rowed barley is mostly used for malting/brewing and food processing, while the six-row type has been utilized mainly as livestock feed (Bhatty, 1993). Barley grains have traditionally been covered with hulls or husks, where the lemma and palea adhere to the caryopsis and do not thresh freely. However, hull-less barley, also referred to as naked barley, has recently been developed mainly for food and industrial applications (Bhatty, 1999). In hull-less barley, the hull is loosely attached and, therefore, falls off during harvesting and threshing.

2.1.2 Production and Utilization

Barley is the fourth major cereal crop in the world after wheat, rice, and maize, with annual production for 2003 of 141.5 million metric tons (FAO, 2004). By far the

leading producers are the European Union (EU), Canada, the Russian Federation, the United States of America (USA), Turkey, Australia, Ukraine, Iran (Islamic Rep of), and China. Canada is the third largest producer of barley in the world with an annual average production of 13.5 million metric tons, which accounts for 10.2% of total world production (Agriculture and Agri-Food Canada, 2003). As the second major cereal in Canada, approximately 88% of barley is produced in the prairie provinces of Alberta, Saskatchewan and Manitoba (Jadhar *et al.*, 1998).

Barley is grown for feed, malting, and food. Of the total world barley production, about 50% is used as animal feed, 30% is used for malt (to produce whiskey and beer) and 10% is for foods (World Crops and Cropping Systems, 2003). Utilization of barley in Canada is mainly in the industries of feed (75%), malting and brewing (20%) and the remaining (5-6%) for other human food uses (Alberta Agriculture, Food and Rural Development, 2004). Earlier, barley cultivars were mainly being developed for either malting/brewing or feed purposes. However, new barley varieties (normal, waxy, and high-amylose) are now being developed for food and non-food uses.

2.1.3 Composition of barley grain

The chemical composition of barley grain is presented in Table 2.1. Hull-less barley contains more starch, protein, total dietary fiber, and β-glucan compared to hulled barley (Table 2.2). The chemical composition of barley grain is influenced by both environmental conditions and genetic (varietal) factors.

Table 2.1 Chemical composition of barley grain

Component	Dry Weight (% w/w)
Carbohydrates	78-83
Starch	63-65
Sucrose	1-2
Other sugars	1
Water-soluble polysaccharides	1-1.5
Alkali-soluble polysaccharides	8-10
Cellulose	4-5
Lipids	2-3
Proteins	10-12
Albumins and globulins	3-5
Hordeins	3-4
Glutelins	3-4
Nucleic acids	0.2-0.3
Minerals	2
Others	5-6

Source: MacGregor & Fincher (1993)

2.2 Starch

2.2.1 Introduction

Starch is abundant in all major agricultural crops. Starch content (dry basis) ranges from 40 to 90% in cereals, 30 to 70% in legumes, and 65 to 85% in roots and tubers (Guilbot & Mercier, 1985) and its availability depends, mainly on geographical and climatic conditions. Over the years, maize has been the world's major source of starch followed by potato, cassava, and wheat. Starch is also produced commercially from rice, sorghum, sago, and amaranth. Barley starch has been commercialized in Finland. Although Canada is the leading producer of barley in the world, at present there is no commercial production and usage of barley starch.

2.2.2 Production and utilization

The USA and EU are the two largest producers of starch in the world. The world total production of starch in 2000 was 48.5 million metric tons (Table 2.3) (LMC International, 2002). Fifty one percent of the total world starch production comes from the USA, almost entirely depending upon maize. Over 80% of world starch production comes from maize, while wheat (>8%), potato (>5%), and cassava (>5%) mainly share the rest of the production. Starches such as rice, barley, oats, sweet potatoes, sago, etc. contribute to the world starch production in small quantities. The USA (38%) and China (20%) are the two largest maize producers. In the EU, starch is produced only from maize, wheat, and potatoes. Sixty three percent of the world's production of potato starch

Table 2.2 Chemical composition of hulled and hull-less barley grains

	Hulled (% db)		Hull-less (% db)	
Starch	52.1-63.8	49.4-63.1	23.9-64.4	49.4-66.2
Protein	8.7-10.5	9.3-15.5	11.3-18.1	10.6-2.9
Dietary fiber	18.9-23.8	18.1-27.5	13.5-34.5	13.6-20.2
β-glucan	2.8-6.9	3.8-6.3	4.6-14.9	4.7-7.9
Lipid	2.2-3.5	2.1-3.1	2.9-6.2	2.1-3.7
Ash	2.3-2.6	1.9-2.3	2.0-2.3	1.3-2.1
Cellulose	3.5-4.7	3.1-7.0	1.4-4.1	1.7-5.0
Arabinoxylans	7.5-9.0	0.4-0.7	4.8-12.2	0.7-0.9
Lignin	1.4-1.7	1.0-1.9	0.7-1.1	0.5-0.9
Uronic acids	4.4-5.2	0.5-1.1	3.4-5.7	0.5-0.7
LMWC[b]	-	0.8-1.4	-	0.9-2.2
Reference	Andersson *et al.* (1999)	Oscarsson *et al.* (1996)	Andersson *et al.* (1999)	Oscarsson *et al.* (1996)

Table 2.3 World starch production by raw material in 2000 (million tons, starch content)

Country	Maize	Potatoes	Wheat	Other	Total
EU	3.9	1.8	2.8	0.0	8.4
USA	24.6	0.0	0.3	0.0	24.9
Other countries	10.9	0.8	1.1	2.5	15.2
World	39.4	2.6	4.1	2.5	48.5

Source: LMC International (2002)

comes from Northern Europe and Russia, whereas wheat starch comes from Europe (42%) and North America (11%). Approximately 90% of world rice production comes from South and South East Asia. Outside the USA and EU, a significant amount of cassava starch is produced mainly in Southeast Asia and legume starch is produced only in Canada. Starches from rice, sorghum, sweet potato, arrowroots, mung bean and sago are also produced in Asia.

Native starches have diverse properties and have been utilized for various food and non-food applications (Table 2.4). Physical and chemical modifications have been employed to improve the properties of native starches, and therefore widen the scope of their applications. The various starch derivatives are shown in Figure 2.1. However, there has been some concern in recent years for physical modification of starches to improve their functional properties. Currently, the trend is shifting towards the use of plant breeding techniques to improve functional properties of starch from various plant sources. This has resulted in the production of waxy (<5% amylose) and high-amylose (>35% amylose) maize, rice, and barley cultivars.

2.2.3 Application of starch and starch derivatives

Starch is used in both the food and non-food industries (Table 2.4). Figure 2.1 provides an overview of the wide range of products obtained from starch. It is a renewable and biodegradable resource, abundant, environmentally friendly, cost competitive, and versatile. The variations in starch source, composition, structure, and the diversities in properties make starches suitable for various applications contributing to

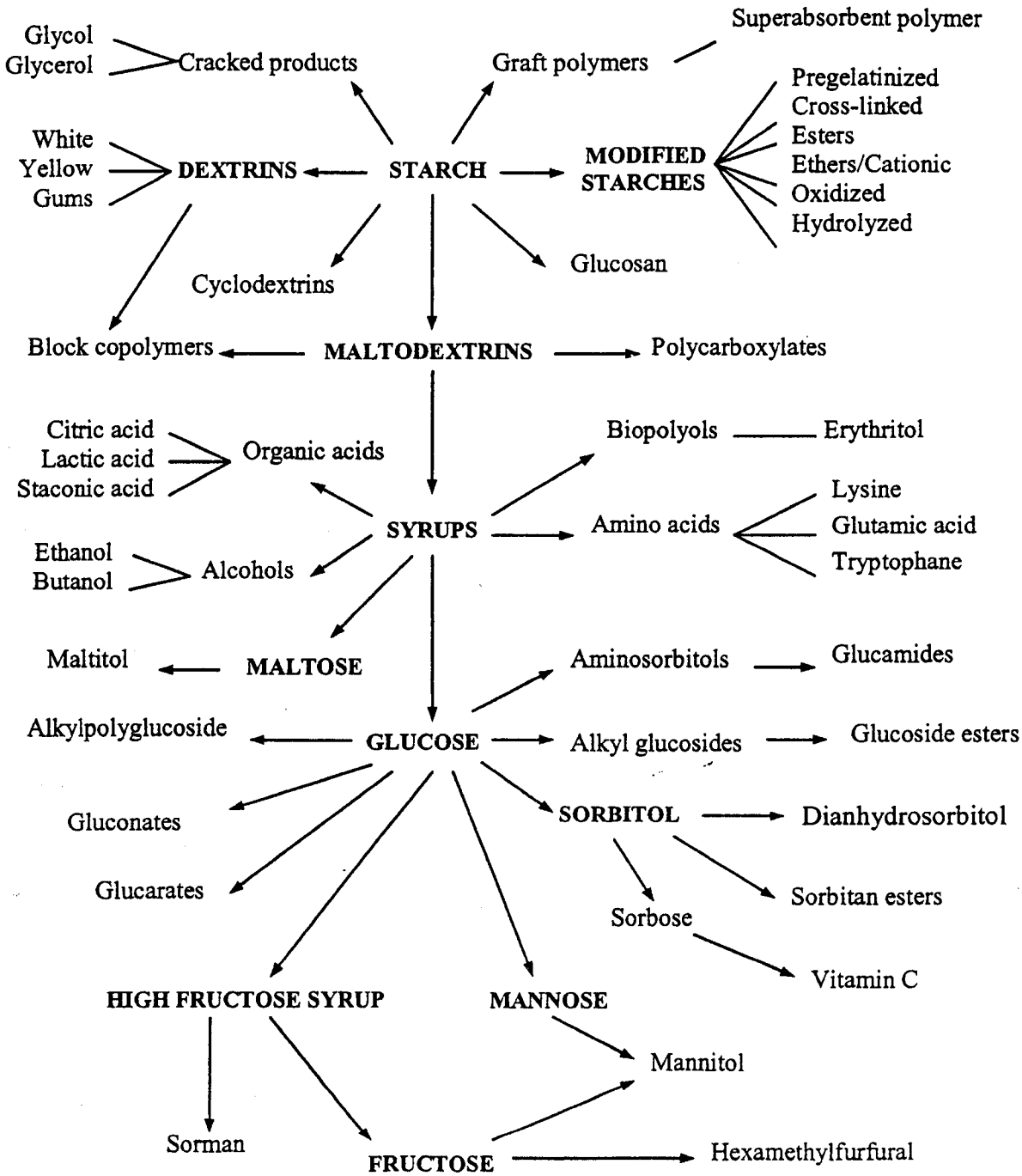
Table 2.4 Application of starch and starch derivatives in food and non-food industries

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

Source: Zhou (2003)

Figure 2.1 Products derived from starch

Adopted from Röper (2002)


Glycol
Glycerol
Cracked products
Graft polymers
Superabsorbent polymer
Pregelatinized
Cross-linked
Esters
Ethers/Cationic
Oxidized
Hydrolyzed
MODIFIED STARCHES
White
Yellow
Gums
DEXTRINS
STARCH
Cyclodextrins
Glucosan
Block copolymers
MALTODEXTRINS
Polycarboxylates
Citric acid
Lactic acid
Staconic acid
Organic acids
Biopolyols
Erythritol
SYRUPS
Amino acids
Lysine
Glutamic acid
Tryptophane
Ethanol
Butanol
Alcohols
Maltitol
MALTOSE
Aminosorbitols
Glucamides
Alkylpolyglucoside
GLUCOSE
Alkyl glucosides
Glucoside esters
Gluconates
Glucarates
SORBITOL
Dianhydrosorbitol
Sorbitan esters
Sorbose
Vitamin C
HIGH FRUCTOSE SYRUP
MANNOSE
Mannitol
Sorman
FRUCTOSE
Hexamethylfurfural

different functionalities. Physical and chemical modifications generally improve these functionalities of native starches and extend the range of starch applications in food, and non-food industries.

Barley starches with waxy (0-5%), normal (20-35%), and high-amylose (>35%) types are currently available from new barley varieties and compete with maize starches for their properties (Vasanthan & Bhatty, 1996) as well as for industrial uses (Jadhar *et al.*, 1998).

2.2.4 Starch granule morphology

The shape, size, and surface features of starch granules depend on the biological origin, with many different forms found in nature (Eliasson & Gudmundsson, 1996; Buléon *et al.*, 1998; Singh *et al.*, 2003). Despite their wide variety of morphologies, starch granules from different botanical sources show some common features specific for each species. Light microscopy (Bogracheva *et al.*, 1998; Jayakody *et al.*, 2005), transmission electron microscopy (TEM) (Li *et al.*, 2004), scanning electron microscopy (SEM) (Song & Jane, 2000; Li *et al.*, 2001a, 2004; Sing & Kaur, 2004; Suh *et al.*, 2004; Tester and Qi, 2004; Zhou *et al.*, 2004), atomic force microscopy (AFM) (Ridout *et al.*, 2003), and confocal scanning laser microscopy (CSLM) (Velde *et al.*, 2002) are some of the microscopic methods that have been employed to study the surface characteristics of the starch granule. The granule size distribution and surface area can be measured by particle size counters, image analyzers, laser light scattering, and sedimentation field flow fractionation (Li et al, 2003; Lindeboom *et al.*, 2004).

2.2.4.1 Granule shape, size and size distribution

The shape and size of starch granules from different botanical sources are presented in Table 2.5. Variously shaped granules (oval/ellipsoidal, round/spherical, polygonal, lenticular, disk/plate shaped, 'dumb bell' shaped, 'honey comb' shaped, and irregular) are found in starches with sizes ranging from 1 to 100μm in diameter (Table 2.5). Generally, granules from tuber and root starches are relatively larger in size (2-100μm). Most of the tuber and root starch granules are oval, although round, polygonal and irregularly shaped granules have also been found. Most of the legume starch granules are oval in shape, however, round and irregularly shaped granules are also common in legume starches. Among cereal starches, a wide variation in granule shape and size can be found. Generally, maize, barley, and wheat starch granules are large oval and small irregular in shape. The granule size of rice, oat, and millet starches are smaller than other cereal starches (2-15μm). Their shapes vary mainly from round to oval to polyhedral to irregular.

Native starch granules from different genotypes have been shown to exhibit unimodal, bimodal, and trimodal distribution patterns (Table 2.5). Most of the legume starches, some cultivars of barley (waxy, normal), and potato (normal) exhibit a mixed population of large, medium, and small granules (trimodal distribution), whereas some cultivars of barley (waxy, normal, high-amylose), wheat, and rye have shown a mixed population of large and small granules (bimodal distribution). However, starches from maize (waxy, normal, amylo-), rice, millet, and potato (waxy) have shown a unimodal distribution pattern.

Table 2.5 Granular shapes, sizes and size distributions of starches from various botanical sources

Starch source	Shape	Size (μm)	Distribution (μm)	References
Cereals				
Barley (waxy)	---	1.15-3.5(s), 3.5-7.0(m), 7.0-39.2(l)	Trimodal	Tang *et al.*, 2002
	Oval, irregular, 'dumb bell' shaped, compound	2-10(s), 10-30(l)	Bimodal	Li *et al.*, 2001a
Barley (normal)	---	1.51-3.5(s), 3.5-7.0(m), 7.0-44.9(l)	Trimodal	Tang *et al.*, 2002
	Oval, irregular, 'dumb bell' shaped, compound	2-10(s), 10-30(l)	Bimodal	Li *et al.*, 2001a
	Disk, spherical, irregular/polygonal, compound	1-4(s), 9-22(l)	Bimodal	Suh *et al.*, 2004
Barley (high-amylose)	Lenticular, irregular	2-26	Unimodal	You & Izydorczyk, 2002
	Lenticular, irregular,	2-10(s). 10-30(l)	Bimodal	Li *et al.*, 2001a
Maize (waxy)	Angular, spherical, 'honey comb' shaped	3(s), 10-18(m)	-	Li *et al.*, 2001a
	Spherical, polyhedral	2-30	Unimodal	Tester *et al.*, 2004
Maize (normal)	Spherical, polyhedral	2-30	Unimodal	Tester *et al.*, 2004
	Round, polyhedral	5-30	-	Jane *et al.*, 1994
Maize (high-amylose)	Highly elongated irregular filament	5-25	Unimodal	Buléon *et al.*, 1998
Wheat	Polygonal	3-26	-	Blanshard, 1987
	Lenticular, spherical	2-10(s), 15-35(l)	Bimodal	Tester *et al.*, 2004
	Round	5-45	-	Velde *et al.*, 2002
Rice	Polyhedral	3-8	Unimodal	Tester *et al.*, 2004
Rice (waxy)	Irregular, polygonal, compound	3-8	-	Jane *et al.*, 1994
Rice (normal)	Irregular, polygonal, compound	3-8	-	Jane *et al.*, 1994
Oat	Irregular, polygonal	2-15	Unimodal	Tester *et al.*, 2004
Millet	Polyhedral	4-12		
Tuber and root			Unimodal	McPherson & Jane, 1999
Potato (waxy)	Round, oval	14-44	Trimaodel	Sing & Kaur, 2004
Potato (normal)	Spherical, ellipsoidal, irregular, cuboidal	5-20(s), 25-40(m), 40-85(l)	-	Velde *et al.*, 2002
Tapioca	Truncated, round	5-30	-	Jane *et al.*, 1994
Lily	Oval, irregular	30-35	-	Hoover, 2001
Sweet potato	Round, oval, polygonal	2-42	-	Velde *et al.*, 2002
	Round, truncated	5-30	-	Jane *et al.* 1994
Canna	Oval, round	30-100		
Legumes			Trimodal	Hoover & Sosulski, 1985
Black bean	Elliptical, oval, irregular	8-34	-	Velde *et al.*, 2002
Mung bean	Oval	5-30	Trimodal	Hoover & Sosulski, 1985
Kidney bean	Elliptical, oval, irregular	20-42	-	Zhou *et al.*, 2004
Lentil	Round, oval, irregular	6.0-37.0	-	Zhou *et al.*, 2004
Wrinkled pea	Irregular, compound/rounded rosette	5.0-37.0	-	Zhou *et al.*, 2004
Smooth pea	Round, oval, irregular	8.0-50.0		

2.2.4.2 Granule surface

The outer surface of the starch granule plays an important role in many applications of starch (Galliard & Bowler, 1987) and has been extensively studied using light microscopy and scanning electron microscopy (SEM). Most starch granules (i.e., legumes, tubers and roots) are relatively smooth with no evidence of pores, cracks or fissures (Jane *et al.*, 1994; Hoover & Sosulski, 1985; Gunaratne & Hoover, 2002; Hoover, 2001; Zhou *et al.*, 2004; Singh & Kaur, 2004).

Jane *et al.* (1994) have observed indentations/cuts in many legume starches (lima bean, chick pea, lentil, mung bean, green pea); sharp edges in rice (wild type and waxy), and maize (normal) starches; pocks in wheat, oat, millet, and triticale starches; and rough surfaces in waxy maize starches. These surface features are partly due to close packing of granules or packing with protein bodies within the plant cells (Hood & Liboff, 1983). Equatorial grooves or furrows are present in larger granules of barley, maize (Li *et al.*, 2001a, 2004), pigeon pea (Hoover *et al.*, 1993), and wheat (Thomas & Atwell, 1999a) starches. Recently, studies on high resolution imaging of granule surface using atomic force microscopy revealed that potato and wheat starches possess a rough granule appearance with smaller (10-50nm), and larger (200-500nm) protrusions on the granule surface, respectively (Baldwin *et al.,* 1998).

Surface pores have been observed in starches of maize (Fanon *et al.*, 1992; BeMiller, 1997; Li *et al.*, 2001a; Jayakody & Hoover, 2002; Velde *et al.*, 2002), rice (Jayakody & Hoover, 2002), sorghum (Fannon *et al.*, 1992; BeMiller, 1997), barley (Fannon *et al.*, 1992; Li *et al.*, 2001a), Innala (Jayakody *et al.*, 2005), millet, large

granules of wheat and rye (Fannon *et al.*, 1992). Pores have also been observed along the equatorial groove of large granules of wheat, rye, and barley starches (Fannon *et al.*, 1992). These pores are exterior openings to internal channels that penetrate into the granule interior, perhaps even into the hilum (Fannon *et al.*, 1992; BeMiller, 1997). They, together with internal channels, are true architectural features of starch granules, potentially increasing the granule surface area available for chemical and enzymatic reactions (Huber & BeMiller, 2000). Recently however, Juszczak *et al.* (2003) studied the granule surfaces of commercial starches of barley, oat, maize (waxy, normal), and wheat using non-contact atomic force microscopy and observed depressions in all the above starches. They interpreted these depressions as typical surface pores or ends of channels penetrating the whole granule. They further observed that the shapes and sizes of these depressions depend on the starch origin. In the case of barley starch, observed depressions had a slit-like shape with the larger axis greater than 100nm. The slit-like pores were found also on maize and rye starch granules, whereas the surface of oat and rice starch had more round or oval depressions. The diameters were less than 100nm and 40 nm in rice and oat starch, repsectively.

2.2.5 Composition and structure

2.2.5.1 Composition

2.2.5.1.1 Major components

The major portion (98-99%) of isolated starch is composed of amylose (AM) and amylopectin (AMP), and the ratio of amylose and amylopectin depends on the botanical origin of the starch (Tester *et al.*, 2004). The 'waxy' starches contain less than 15% amylose, while 'normal' starches 20-35% and 'high-' (amylo-) amylose starches greater than about 40% (Topping *et al.*, 2003; Tester *et al.*, 2004). There are two types of amylose in lipid containing starches, namely lipid free amylose and lipid-complexed amylose (Morrison *et al.*, 1993b).

2.2.5.1.2 Minor components

Apart from amylose and amylopectin, starch contains small quantities of surface and integral proteins and lipids, as well as a trace amount of minerals (Tester, 1997a; Tester *et al.*, 2004). The starch bound proteins and lipids have been reported to influence starch digestibility, swelling, solubilization, retrogradation, and granule integrity (Appelqvist & Debet, 1997; Han & Hamaker, 2002), but the most dramatic effect of these components is on the flavor profile of the starch (Thomas & Atwell, 1999b). Compared with most cereal starches, tapioca and potato starches are considered to be very bland in flavor because of the small amounts of lipid and protein present. Surface components could be removed readily by appropriate treatment without granule damage/disruption. Integral components, which can only be extracted near or above the starch gelatinization temperature, are deeply embedded and possibly covalently bound to the starch matrix (Galliard & Bowler, 1987; Baldwin, 2001). The amount of these components depends on

the botanical origin and the starch isolation procedure (Morrison & Laignelet, 1983; Morrison & Karkalas, 1990).

2.2.5.1.2.1 Lipids

Starch lipids are found both on the surface of and inside granules (Morrison, 1985, 1988, 1995). It is likely that surface lipids are distributed unevenly at the granule surface and would be present in multimolecular droplet or micellar forms (Galliard & Bowler, 1987). The surface lipids are mainly triacylglycerides (TAGs), followed by free fatty acids (FFAs), glycolipids (GLs) and phospholipids (PLs) (Morrison, 1985,1988). The internal lipids of cereal starches are predominantly monoacyl lipids, with the major components being lysophospholipids (LPLs) and FFAs (Morrison, 1985,1988,1995). It is likely that both surface and internal lipids may be present in the free state as well as bound to starch components, or linked via ionic or hydrogen bonding to hydroxyl groups of the starch components (Vasanthan & Hoover, 1992). Thus, solvent extractable lipids at ambient temperature mainly represent free surface lipids while those lipids extracted at elevated temperatures represent surface bound lipids, and internal free and bound lipids (Vasanthan & Hoover, 1992).

In general, cereal starches contain 0.5-1.8% lipids (Table 2.6). The non-waxy cereal starches are unusual among food starches because they contain significant amounts of monoacyl lipids (Morrison, 1988, 1995; Vasanthan & Hoover, 1992). In starches of wheat, rye, barley and triticale, the lipids are almost exclusively LPLs, while in oat, rice,

Table 2.6 Proximate composition of starches from different botanical origin.

Starch source	Amylose content (%)		Lipid content (%)			Phosphorous content (%)	Nitrogen content (%)	Reference
	Apparent	Total	Surface	Bound	Total			
Barley (waxy)	0-4.42	0-6.44	0.14-0.22	0.34-0.75	-	0.01-0.04	0.25-0.38	Tester & Qi, 2004, Li *et al.*, 2001a
Barley (normal)	22.5-24.6	23.6-29.0	0.11-0.2	0.8-1.32	-	-	0.30-0.43	Li *et al.*, 2001a
Barley (high-amy)	33.9-38.6	41.7-44.5	0.12-0.17	1.22-1.69	-	-	0.41-0.43	Li *et al.*, 2001a
Rice (waxy)	-	0.2-2.1				3.3-4.4	0.12-0.48	Qi *et al.*, 2003
Wheat	21.1	27.3	0.04	0.64	0.70		0.04	Hoover & Vasanthan, 1994a
Oat	16.7	19.4	0.07	1.05	1.13		0.05	Hoover & Vasanthan, 1994a
Potato	25.2	28.1	0.08	0.12	0.20	0.1	0.09	Gunaratne & Hoover, 2002
Cassava	19.8	22.4	0.06	0.08	0.12	0.01	0.02	Gunaratne & Hoover, 2002
Lentil	27.35-28.78	30.05-32.29	0.01	0.72-0.81	0.71-0.83	-	0.04-0.05	Zhou *et al.*, 2004
Smooth pea	27.35-31.04	30.51-35.09	0.01-0.02	0.47-0.72	0.48-0.71	-	0.05-0.08	Zhou *et al.*, 2004
Wrinkled pea	68.84	78.42	0.05	0.80	0.84	-	0.03	Zhou *et al.*, 2004
Black bean	33.07-35.21	37.17-39.32	0.08-0.10	0.26-0.43	0.35-0.52	-	0.03-0.05	Zhou *et al.*, 2004

maize, millet and sorghum, they consist of FFAs and LPLs in proportions characteristic of each species (Morrison, 1988). Legume, root, and tuber starches generally contain less (<0.12) than do cereal starches (Vasanthan & Hoover, 1992). Lipids in cereal starches are proportional to the amylose content and are fully complexed with a portion of amylose, but the relationship between starch lipids and amylose is quite different among starches (Morrison, 1988,1995).

2.2.5.1.2.2 Protein

Nitrogen present in the starch is generally considered to be present as protein, but it may also be a part of starch lipids. Protein content in a purified starch is a good indicator of starch purity. Purified starches contain less than 0.6% protein (Tester *et al.*, 2004; Table 2.6). In common with starch lipids, proteins occur on the surface and, regardless of origin, are embedded within the matrix of granules (Tester *et al.*, 2004). Collectively, the proteins are referred to as starch granule associated proteins and may be associated with lipids on granule surfaces (Baldwin, 2001). Starch granule bound proteins are mainly located in the granule interior as integral proteins (Li *et al.*, 2003), and may influence starch physicochemical properties such as digestibility, swelling, solubilization, retrogradation, and granular integrity (Appelqvist & Debet, 1997).

2.2.5.1.2.3 Minerals

Starches also contain relatively small quantities (<0.4%) of minerals such as calcium, sodium, magnesium, potassium, and phosphorous, which are, with the exception

of phosphorous, of little functional significance (Tester *et al.*, 2004). Phosphorous is found in three major forms: phosphate monoesters, phospholipids, and inorganic phosphates (Lim *et al.*, 1994; Kasemsuwan & Jane, 1996; Blennow *et al.*, 2002). Phosphorous in normal and waxy cereal starches is mainly in the form of LPL and phosphate monoesters, respectively (Lim *et al.*, 1994; Kasemsuwan & Jane, 1996), however, in amylomaize, all three forms are present. Tuber, root and legume starches are LPL-free and the phosphorous is present mainly in the form of phosphate monoesters (Lim *et al.*, 1994; Kasemsuwan & Jane, 1996). The degree of phosphorylation depends on the cultivar, growth conditions, temperature, fertilizer, and storage (Hizukuri *et al.*, 1970; Nielsen *et al.*, 1994, Blennow *et al.*, 2002). Amylopectins from most plant sources contain small amounts of glucose moieties (0.1-1%) with phosphate groups (Blennow *et al.*, 1998). Generally, amylopectins from tuber and root starches have the highest degree of phosphorylation (Lim *et al.*, 1994). Potato amylopectin contains 200-1000ppm of the esterified phosphorous, and those from other roots contain 40-150ppm; cereals contain less than 20ppm of the esterified phosphorous, with the exception of amylomaize, which contains 110-260ppm (Hizukuri, 1996). Waxy varities of amaranth, rice, and maize appeared to bind inorganic phosphate (10-50ppm) tightly and could not be extracted with water (Hizukuri, 1996). The high phosphate monoester content of potato starch confers enhanced paste clarity, high peak consistency, significant shear thinning and slow rate and extent of retrogradation (Galliard & Bowler, 1987; Jane *et al.*, 1996).

Most of the phosphate groups in tuberous starch are covalently bound to amylopectin but not to amylose (Blennow *et al.*, 2002). These phosphate monoesters are selectively bound to specific regions (i.e., C-2, C-3, and C-6) within the amylopectin molecule (Hizukuri, 1996; Kasemsuwan & Jane, 1996; Blennow *et al.*, 1998,2002). Lim *et al.* (1994) have shown that larger amounts of starch phosphates are on C-6 than on C-3. Both the crystalline and amorphous regions of amylopectin contain esterified phosphates (Blennow *et al.*, 2000, 2002). However, the major part of the monoesterified starch phosphate is located in the amorphous regions of the starch granule (Blennow *et al.*, 1998,2000b).

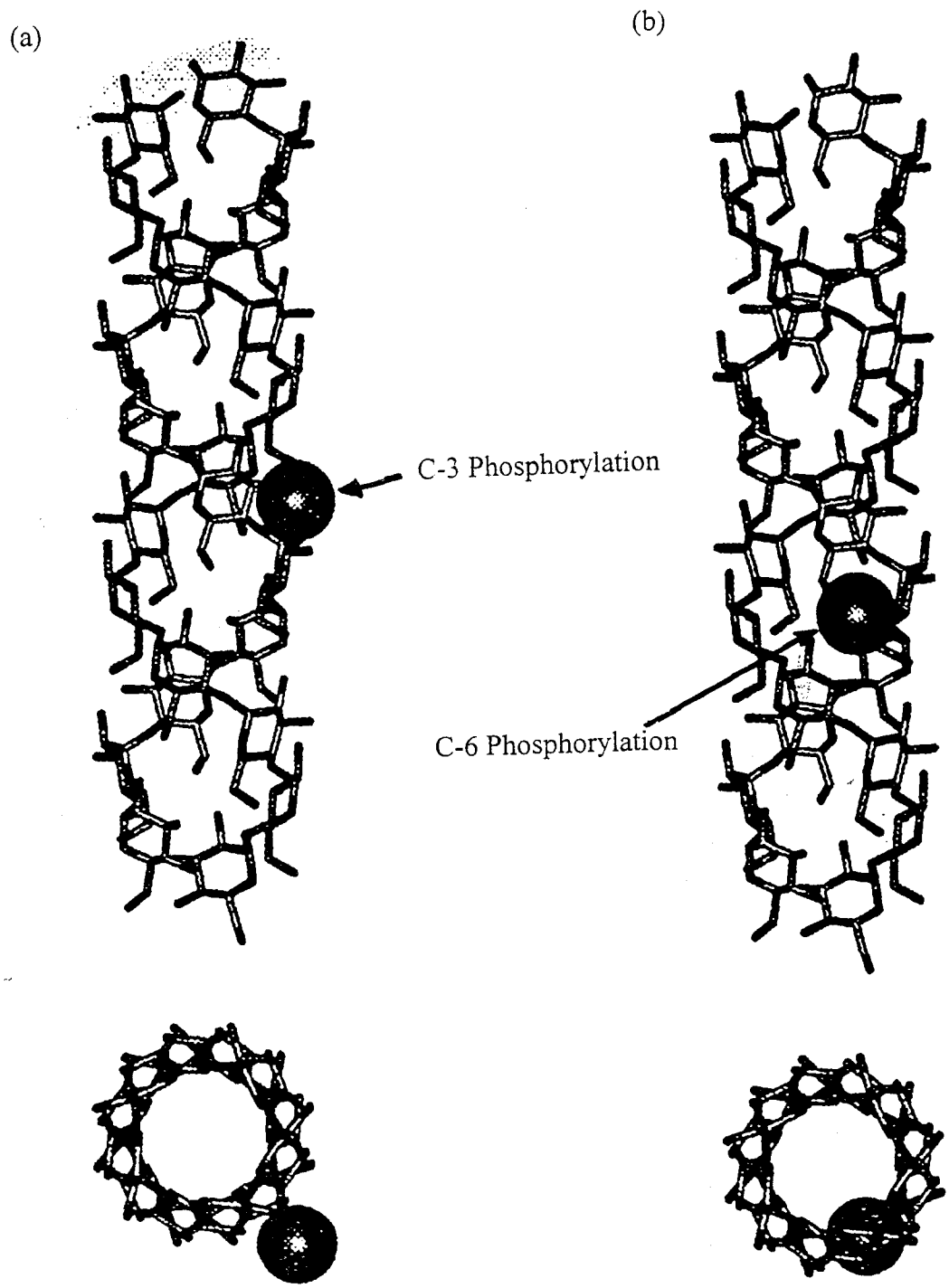
In the amylopectin double helix, the free C-3 and C-6 hydroxyl groups are located at the hydrophilic surface of the double helix. Therefore, phosphate groups, which are attached to C-6 or C-3 positions, will align with or protrude from the helix surface, which might affect the solubility of the helices or the side-by-side packing of the helices, and hence, the crystallinity of the starch (Blennow *et al.*, 2002; Figure 2.2). According to these molecular models, C-3 phosphorylation has more effect on starch granule crystallinity than C-6 phosphorylation.

2.2.5.1.3 Intermediate components

Some starches contain a third polysaccharide fraction, usually referred to as an intermediate fraction, which has more or less branched materials (Banks & Greenwood, 1975). However, the average chain length and the number of chains per molecule differ from those of amylose and amylopectin. Therefore, this intermediate fraction cannot be

Figure 2.2. A molecular model of phosphorylated starch. The helices are phosphorylated on the same glucose residue, at the C-3 (a) and C-6 (b) positions.

Source: Adopted from Blennow *et al.* (2002)


(a)
(b)
C-3 Phosphorylation
C-6 Phosphorylation

categorized either as amylose or amylopectin (Colonna & Mercier, 1984; Hizukuri, 1996). However, Wang and White (1994) found that the intermediate fraction of oat starches is close to amylopectin in structure, but with longer branch chain lengths. This intermediate fraction has been observed in normal (barley, oat, rye, and wheat) and high- amylose (maize, barley rice, and pea) starches (Banks & Greenwood, 1975; Asaoka *et al.*, 1986; Inouchi *et al.*, 1987).

2.2.5.2 Ultra structure

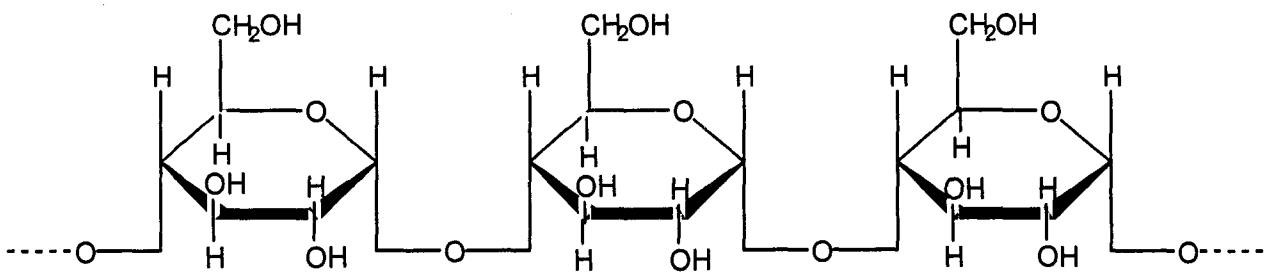
2.2.5.2.1 Macromolecules

2.2.5.2.1.1 Amylose

Amylose was initially found to consist of relatively long linear chains of α-(1→4) linked D-glucopyranosyl units (Figure 2.3a). However, it is now recognized that some amylose molecules have several branches (Manners, 1985; Hizukuri, 1996; Buléon *et al.*, 1998). The extent of branching depends on the botanical origin of amylose and it increases with the molecular size (Takeda *et al.*, 1987; Buléon *et al.*, 1998; Yoshimoto *et al.*, 2000; Hoover, 2001; Tester *et al.*, 2004). A summary of the general characteristics of amylose is given in Table 2.7. Amylose has a molecular weight of ~10^5–10^6 Da (Buléon *et al.*, 1998; Biliaderis, 1998; Gidley, 2001). The degree of polymerization (DP) of amylose by number (DPn) is 324-4920 and it contains around 9-20 branch points

Figure 2.3. Structure of amylose (a) and amylopectin (b).

(a)



(b)

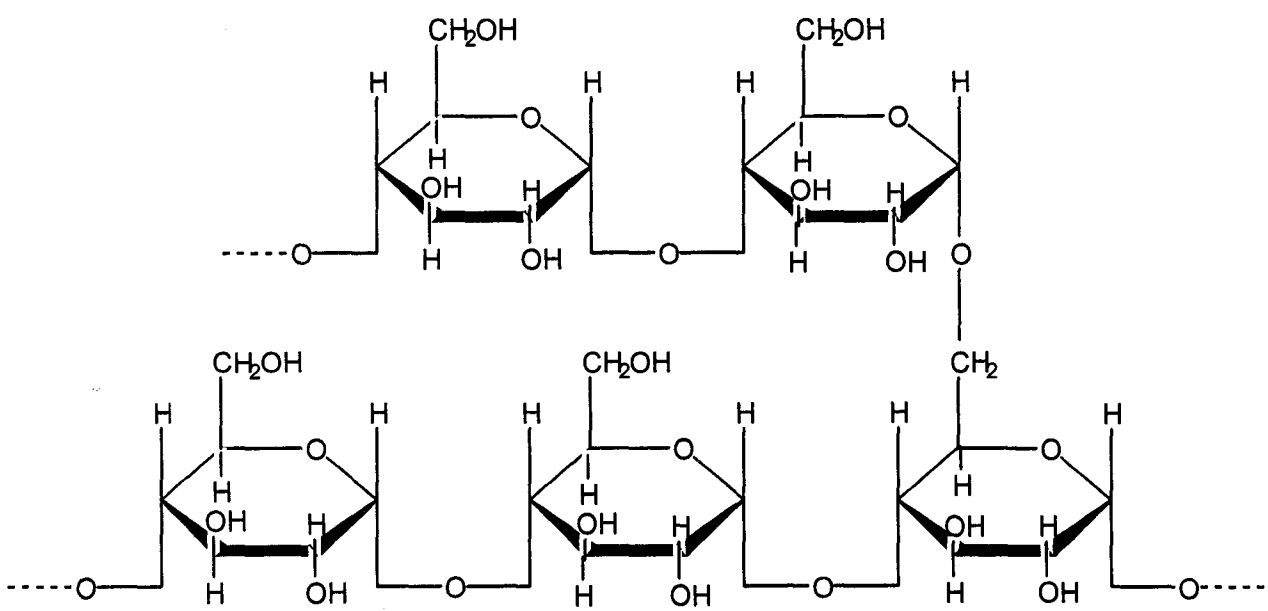


Table 2.7 General characteristics of amylose and amylopectin

Property	Amylose	Amylopectin
Molecular structure	Essentially linear, α-(1→4)-glucosidic linkage	Branched, α-(1→4) and α-(1→6)-glucosidic linkage
Degree of branching (%)	0.2-0.7	4.0-5.5
Degree of polymerization (DP)	700-5000	10^4-10^5
Molecular weight (Da)	10^5-10^6	10^7-10^9
Average chain length	100-550	18-25
Structural conformation	Partly complexed with lipid, amorphous	Double helix, partly crystalline
Iodine complex		
Iodine affinity (IA, g/100g)	19-20.5	0-1.2
λ_{max} (nm)	640-660	530-570
Blue value (BV)	1.2-1.6	0-0.2
Color	Blue	Purple
β-amylosis limit (%)	70-95	55-60
Stability of dilute aqueous solutions	Unstable (retrogradates)	Stable
Gel texture	Stiff, thermally irreversible	Soft, thermally reversible (<100°C)
Film properties	Strong, coherent	Brittle

Reference : Biliaderis, 1991; Hizukuri, 1996

Table 2.8 Molecular characteristics of amylose from various cereal starches

Property	Maize		Rice	Wheat	Barley			
	Normal	Amylomaize			Waxy	Normal	High-amylose	Oat
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	20.0-21.1	19.3-20.2	19.5-19.8	20.0-20.1	1.36-1.43	18.4-18.9
Blue value	-	1.32-1.39	-	1.36-1.48	1.35-1.42	1.33-1.63	18.8-20.0	1.32-1.44
β-amylosis limit	84	75-78	73-84	76-82	77-82	76-87	70-73	-
λ_{max}	-	645-650	-	645-657	643-655	643-664	643-646	659-662
DPw (range)	390-13100	210-8940	210-12900	-	-	180-17200	130-19000	392-2920
DPw (mean)	2550	1810-1990	2750-3320	-	-	3440-5580	4080-4920	939-1208
DPn (mean)	960	690-740	920-1110	1020-1380	1560-1680	940-1570	950-1080	-
DPw/DPn	2.66	2.45-2.88	2.64-3.39	-	-	3.56-4.1	4.3-4.6	-
Chain length	305	215-255	250-370	270-380	460-510	210-530	350-450	-
Chain number	3.1	2.9-3.2	2.5-4.3	4.8	3.3-3.4	1.8-5.3	2.4-2.7	-
Unbranched amylose (mol%)	52	53-58	69	63-77	55-74	65-83	80-85	-
Reference:	Morrison & Karkalas, 1990	Takeda *et al.*, 1988	Morrison & karkalas, 1990	Morrison & karkalas, 1990; Yoshimoto *et al.*, 2004	Yoshimoto *et al.*, 2002	Takeda *et al.*, 1999; Yoshimoto *et al.*, 2000, 2004;	Yoshimoto *et al.*, 2000	Wang & White, 1994

equivalent to 3-11 chains per molecule (Takeda *et al.*, 1987; Morrison & Karkalas, 1990; Wang & White, 1994; Yoshimoto *et al.*, 2000). Each chain contains ~200-700 glucose residues (Morrison & Karkalas, 1990) equivalent to a molecular weight of 32,400-113,400 Da (Morrison & Karkalas, 1990). Cereal starches have much smaller average molecular size compared to root and tuber starches (Takeda *et al.*, 1986). The physicochemical characteristics of some cereal amyloses are given in Table 2.8.

The conformation of amylose in aqueous solution has generated much controversy and many models have been proposed. Hollo *et al.* (1961) proposed a tightly wound helix. They suggested that the helical segments are interspersed by regions of random coils. Banks and Greenwoods (1975) postulated that amylose, in water and neutral aqueous potassium chloride solutions, exists in the form of random coil with no helical segments; in formamide and dimethyl sulfoxide, in the form of expanded coil; and in the presence of complexing agent or in alkaline solutions, in helical form (Figure 2.4).

2.2.5.2.1.1.1 Amylose inclusion complexes

Amylose, the essentially linear polymer of starch, has the unique ability to form helical inclusion complexes (Figure 2.5a,b) with several organic and inorganic complexing agents such as lipids, iodine, dimethylsulfoxide, flavor compounds and aliphatic alcohols. These complexing agents induce the formation of single left-handed amylose helices with a pitch of 0.805nm, also known as V-amylose (Veregin *et al.*, 1987; Morrison *et al.*, 1993a, b; Buléon *et al.*, 1998; Takahashi *et al.*, 2004). In the V-form, a single chain of amylase forms a helix with a relatively large cavity, in which

Figure 2.4 Models proposed for the amylose molecule in aqueous solution:

a) Random coil

b) Interrupted helix

c) Deformed helix.

Source: Banks & Greenwood (1975), reproduced with permission from Edinburgh University press.

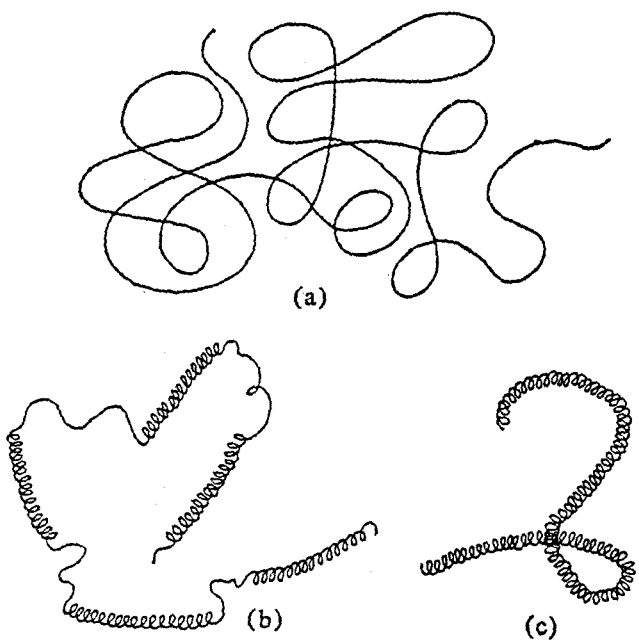

(a)
(b)
(c)

Figure 2.5 a) Schematic representation of V-amylose-lipid complex

Source: Adopted from Carlson *et al.* (1979)

b) Molecular modeling representation of V-amylose-lipid complexes

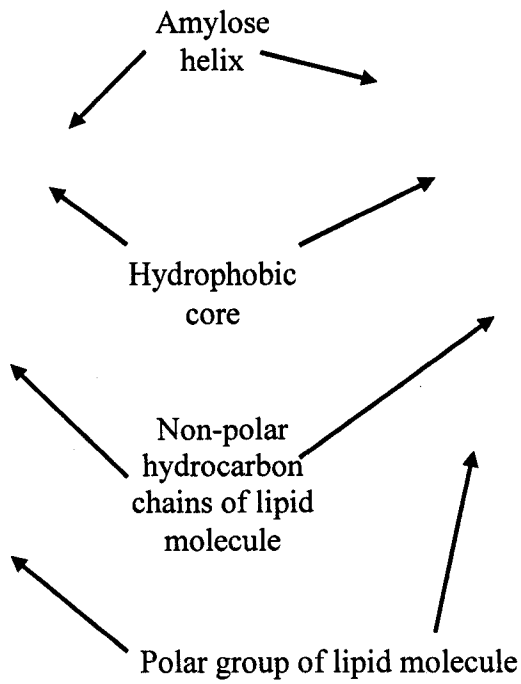
Source: Adopted from Buléon *et al.* (1998)

c) Proposed model of V-amylose-lipid complex to demonstrate the pseudo-cross link feature.

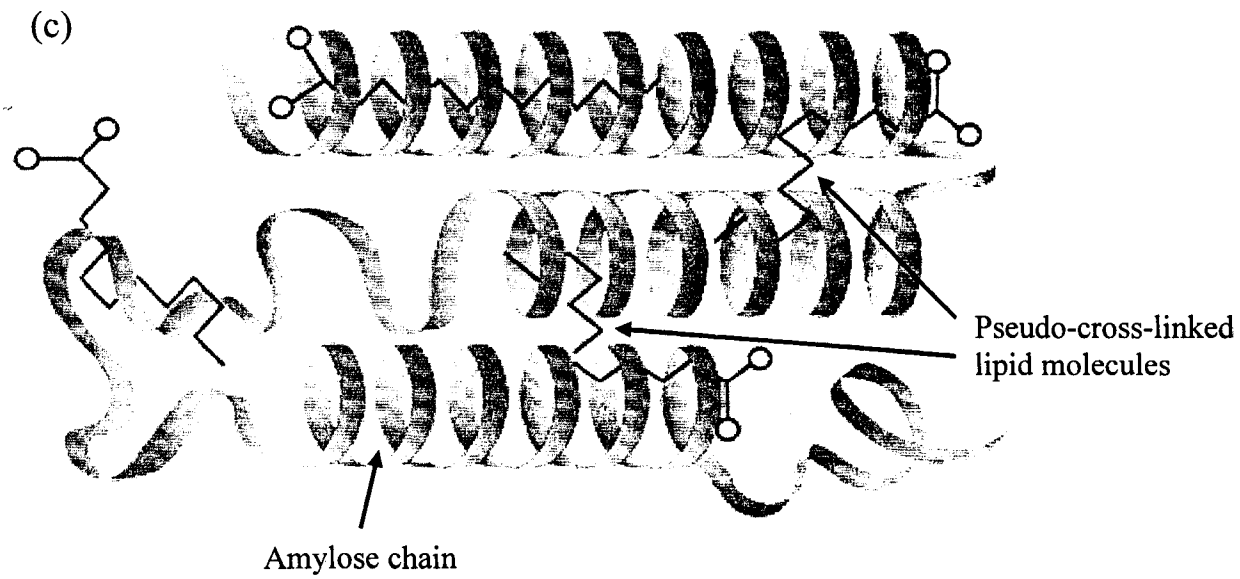
Source: Adopted from Kawada *et al.* (2004)

(a)

(b)


Amylose
helix
Hydrophobic
core
Non-polar
hydrocarbon
chains of lipid
molecule
Polar group of lipid molecule

(c)


Pseudo-cross-linked
lipid molecules
Amylose chain

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). These helices are stabilized by hydrogen bonds between hydroxyl groups of adjacent glucosyl residues located on the outer surface of the helix (Nimz *et al.*, 2004). The helix cavity is effectively a hydrophobic tube. The hydrocarbon chain of the fatty acid or lipid lies within the amylose helix (Godet *et al.*, 1993; Snape *et al.*, 1998; Nimz *et al.*, 2004) and is stabilized by Van der Waals contacts with adjacent hydrogens of amylose (Godet *et al.*, 1993; Nimz *et al.*, 2004) but the polar ends are on the outside of the helix cavity (Godet *et al.*, 1993; Snape *et al.*, 1998). However, Kawada and Marchessault (2004) recently found that complexing agents are not present regularly in the respective crystals; rather, the complexing agents are trapped in a matrix, as shown in Figure 2.5c.

Kawada and Marchessault (2004) also found that the amylose chain mobility in complexes is inversely proportional to the length of the alkyl chain of the complexing agent. Hahn and Hood (1987) reported that increased chain length of saturated fatty acids increased the number of moles of lipids bound per mole of amylose; increased unsaturation of the fatty acid decreased the formation of V-amylose-lipid complex. They further observed that the complex formation depends on the temperature, pH, and ionic strength of the system. Amylopectin also posseses some lipid binding ability (Hahn & Hood, 1987).

V-amylose-lipid complexes can be formed naturally as well as artificially, during starch thermal processing (Morrison *et al.*, 1993a). The presence of V-amylose-lipid complexes in native barley, wheat, maize, rice, oat (Morrison *et al.*, 1993a,b), and

amylomaize starches has been observed using the technique of ^{13}C CP/MAS-NMR. The formation of V-crystalline structures have been observed *in situ* by synchrotron X-ray diffraction studies, during heating of native maize starch at intermediate and high moisture contents (Le Bail *et al.*, 1999). Lipid-complexed amylose is amorphous (type I) in native starches but can be annealed into a crystalline form (type-II), which exhibits a strong V-type X-ray pattern (Biliaderis, 1991; Morrison, 1995). Type I complexes are probably the form in most cereal starches and generally dissociate on heating in water at 94-100°C. Type II complexes, which are found in starches after gelatinization, dissociate at 100-125°C (Morrison, 1995).

2.2.5.2.1.1.2 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 (1987) and Zobel (1992) postulated that amylose is located in bundles between amylopectin clusters. Later, it was shown (by cross linking) that amylose is randomly interspersed as individual molecules in both the amorphous and crystalline regions of the granule (cross linking was found only between amylopectin molecules and between amylose and amylopectin molecules, but not between amylose molecules) (Jane *et al.*, 1992; Kasemsuwan & Jane, 1994). A number of studies have indicated that the distribution of amylose is uneven within the granule. Studies on maize (Inouchi *et al.*, 1987), wheat (Morrison & Gadan, 1987), barley (McDonald *et al.*, 1991), rice (Asaoka *et al.*, 1985), and pea (Biliaderis, 1982) starches have shown that more amylose is present in

the peripheral regions of these granules. However, more recently 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.

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, suggesting that an increase in amylose content causes more separation between amylose and amylopectin molecules.

2.2.5.2.1.2 Amylopectin

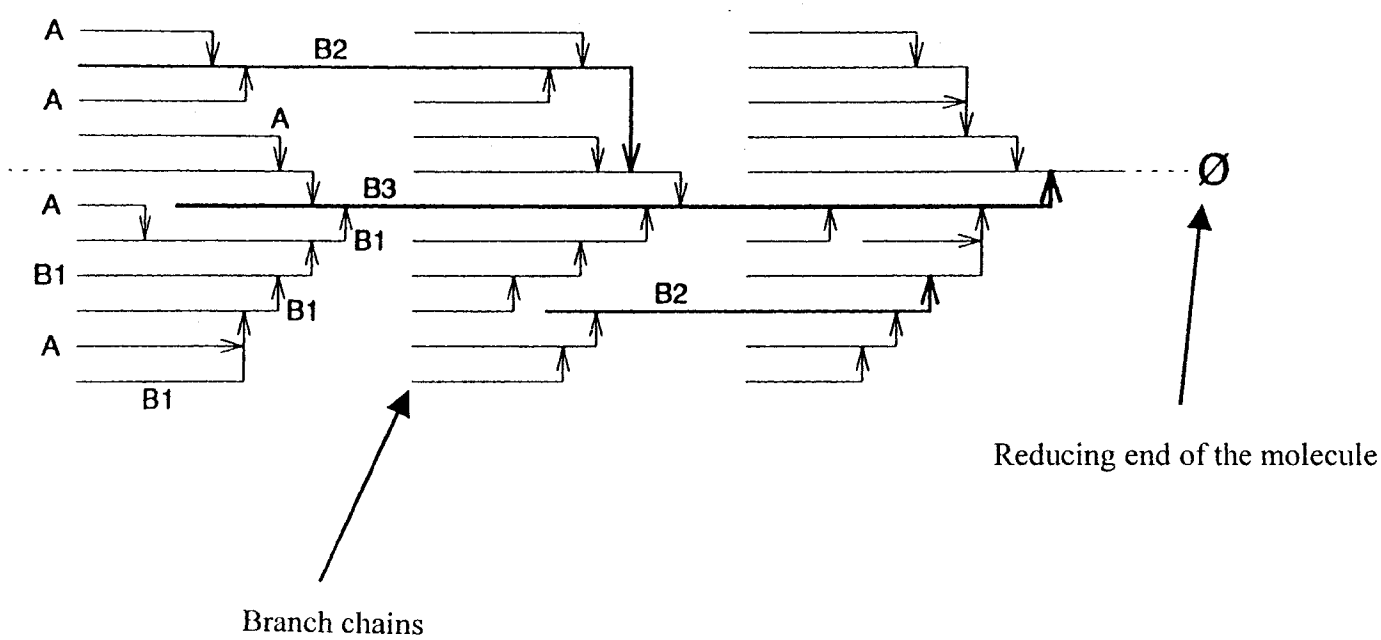
Amylopectin is the major component of most starches. It is composed of α-(1→4) linked glucopyranosyl units interconnected through α-(1→6) linkages leading to a highly branched, compact structure (Figure 2.3b) (Buléon *et al.*, 1998). Amylopectin is one of the largest polymers in nature with an average molecular weight of about 10^7-10^9 Da and a hydrodynamic radius of 21-75nm (Morrison & Karkalas, 1990; Buléon *et al.*, 1998; Biliaderis, 1998). The DPn is typically within the range of 9600-15,900, but comprises three major species, with DPn of 13,400-26,500, 4400-8400 and 700-2100 (Takeda *et al.*, 2003). Amylopectin molecular characteristics from different starch sources are summarized in Table 2.7. In common with amylose, the molecular size, shape, structure, and polydispersity of the molecule vary with botanical origin (Tester *et*

al., 2004). Amylopectin unit chains are relatively short compared to amylose molecules with a broad distribution profile. They are typically ~18-25 units long on average (Hizukuri, 1985; Morrison & Karkalas, 1990; Wang & White, 1994; Takeda *et al.*, 2003). However, Jane *et al.* (1999) have shown that this range is ~19-31, if high-amylose starches are also taken into account.

The individual chains can be specifically classified in terms of length, and consequently their position within the starch granules (Hizukuri, 1985,1986). Three types of unit chains are present, referred to as 'A'-, 'B'-, and 'C'-chains (Figure 2.6). Depending on the chain length and, correspondingly, the number (radial) of clusters traversed within the native granule, 'B'-chains are referred to as 'B_1'-'B_4' (one to four clusters). The 'A'- and 'B_1'- chains are the exterior, and form double helices within the native granules. Their chain length is typically ~12-24 depending on the botanical origin of the starch (Hizukuri, 1985; Li *et al.*, 2001a; Franco *et al.*, 2002). Starches with 'A'-type crystallinity (most cereals) have shorter chain lengths on average than 'B'-type starches (e.g. potato). Amylopectin molecules from high-amylose starches contain relatively high proportions of very long chains (Yoshimoto *et al.*, 2000). With respect to the structure of amylopectin, the 'A'- and/or exterior 'B'-chains of amylopectin are (1→6)-α- linked to interior 'B'-chains, which in turn can be linked to another interior 'B'-chain or to the 'backbone' of the amylopectin molecule, the single 'C'-chain. The 'C'-chain is the only chain with a reducing group at the end. Typical chain lengths for 'A', 'B_1-B_4' chains for different starches (after debranching with isoamylase) are in the range of 12-16, 20-24, 42-48, 69-75, and 101-119, respectively (Hizukuri, 1986; Wang &

Figure 2.6 Proposed cluster model of amylopectin. The different types of unit chains are designated as A, B_1, B_2, B_3 and C. A: outer branches, B_1-B_3: inner branches, and C: the chain that carries the reducing group (ϕ).

Source: Adopted from Hizukuri (1986)


A
B2
A
A
B3
A
B1
B1
B1
B2
A
B1
Ø
Reducing end of the molecule
Branch chains

White, 1994; Bello-Pérez *et al.*, 1996). The ratio of 'A'- to 'B'-chains is an important parameter and depends on the starch source. 'A'-chains and exterior 'B'-chains form crystalline lamellae and their α-(1→6) branch points are located in the amorphous lamellae. Some 'B'-chains are long enough to traverse through both the semicrystalline growth ring and the inter-crystalline amorphous growth ring.

2.2.5.2.1.2.1 Chain length distribution of amylopectin

The distribution of the amylopectin unit chains generally appears to be genetically controlled, and is characteristic of a species. Extensive research has been done to study the chain length profile of amylopectin from different cereal starches, such as barley (Czuchajowska *et al.*, 1998; Jane *et al.*, 1999; Song & Jane, 2000; Yoshimoto *et al.*, 2000, 2002; Li *et al.*, 2001a; Tang *et al.*, 2001; You & Izydorczyk, 2002; Tester & Qi, 2004), wheat (Hizukuri & Maehara, 1990; Jane *et al.*, 1999; Franco *et al.*, 2002; Yoshimoto *et al.*, 2004), rice (Takeda *et al.*, 1987; Jane *et al.*, 1999), oat (Wang & White, 1994; Tester & Karkalas, 1996), rye (Fredriksson *et al.*, 1998), and maize (Takeda *et al.*, 1988; Shi *et al.*, 1998; Jane *et al.*, 1999; Vermeylen *et al.*, 2004; Tziotiz *et al.*, 2004); root and tuber starches, such as tapioca (Jane *et al.*, 1999; Vermeylen *et al.*, 2004), potato (Frederiksson *et al.*, 1998; Vermeylen *et al.*, 2004), and sweet potato (McPherson & Jane, 1999); and legumes (Biliaderis *et al.*, 1981; Biliaderis, 1982; Frederiksson *et al.*, 1998; Ratnayake *et al.*, 2001; Yoshimoto *et al.*, 2001; Vermeylen *et al.*, 2004).

Isoamylase debranched amylopectin usually exhibits a bimodal (Shi *et al.*, 1998), trimodal (Wang & White, 1994; Czuchajowska *et al.*, 1998; You & Izydorczyk, 2002;

Yoshimoto *et al.*, 2004) or polymodal (Fredriksson *et al.*, 1998; Yoshimoto *et al.*, 2000,2002) distribution pattern, representing different chain groups of the amylopectin molecule. Generally, cereal starches (wheat, rye, barley [normal, waxy and high-amylose], and waxy maize) show a polymodal distribution with local peak maxima or shoulders at DP 11-12, 18-19 and 46-48 (Fredriksson *et al.*, 1998). Jane *et al.* (1999) have studied the amylopectin distribution profile of 'A'-,'B'-, and 'C'-type starches, and observed that 'A'-type starches had peaks at shorter chain lengths (DP 12-14 and 41-51) than 'B'-type starches (DP 14-16 and 48-53). 'A'-type starches also had larger proportions of short chains (DP 6-12) and smaller proportions of long chains (DP>37) than 'B'-type starches. The 'C'-type starches had substantial amounts of both short and long-branch chains. Wang and White (1994) found that amylopectins from oat starch contain short-branch chains. Amylopectins from buckwheat starches have shown a higher amount of long chains than other cereal amylopectins (Yoshimoto *et al.*, 2004). While amylopectins from amylomaize starches have relatively longer average chain lengths, and higher proportions of long chains (DP>37) compared to those from waxy, and normal maize starches (Shi *et al.*, 1998; Jane *et al.*, 1999). The amount of short chains in rice amylopectin has been shown to increase with decrease in growth temperature (Inouchi *et al.*, 2000). Branch chain length distribution of amylopectin has been shown to influence starch physicochemical properties such as gelatinization temperature, pasting properties, retrogradation and acid hydrolysis (Shi & Seib, 1992; Shi *et al.*, 1998; Jane *et al.*, 1999; Franco *et al.*, 2002).

Generally barley amylopectins have short branch chain lengths (Song & Jane, 2000). Debranched amylopectins of waxy, normal and high-amylose barley starches exhibit nearly similar chain length distributions, with the highest peak at DP 12 (Song & Jane, 2000; Yoshimoto *et al.*, 2000, 2002; Li *et al.*, 2001a; Tester & Qi, 2004). However, Czushajowska *et al.* (1998) and Yoshimoto *et al.* (2000, 2002) have observed a slight difference in chain length distribution between high-amylose barley cultivars and those of waxy and normal barley cultivars. The lengths of some linear chains in amylopectin from high-amylose barley starches have been found to be significantly longer than in normal and waxy starches (You & Izydorczyk, 2002). Amylopectins from different barley cultivars are similar in molecular structure irrespective of their amylose content (Yoshimoto *et al.*, 2000, 2002; Tester & Qi, 2004). Average branch chain length has been correlated with small granule size ($r = 0.81$, $p<0.01$), the proportion of small granules by number ($r = 0.71$, $p<0.05$), the proportion of small granules by weight ($r = 0.78$, $p<0.01$), the number of short chains ($r = -0.92$, $p<0.01$), and the number of long chains ($r = 0.99$, $p<0.01$), in 10 barley cultivars of varying amylose content (Li *et al.*, 2001a).

2.2.5.2.2 Semicrystalline structure

2.2.5.2.2.1 Growth rings

Starch granules from higher plants contain alternative zones of semicrystalline and amorphous material known as growth rings (Jenkins *et al.*, 1994; Figure 2.7a) which represent the periodic growth of starch granules (French, 1984). These growth rings have

been observed by light microscopy (Ridout *et al.*, 2003), atomic force microscopy (AFM) (Baker *et al.*, 2001; Ridout *et al.*, 2002,2003), confocal scanning laser microscopy (CSLM) (Velde *et al.*, 2002), and after treatment with acid or degradative enzymes, by scanning and transmission electron microscopy (SEM & TEM) (Pilling & Smith, 2003; Li *et al.*, 2003). Ridout *et al.* (2003) have observed that growth rings are almost continuously distributed throughout the granule of pea starch. However, two distinct regions (an area of densely packed granule growth rings, and a loose filamentous network located in the central region of the granule), have been observed in the granules of barley starches of varying amylose contents (Li *et al.*, 2003). These growth rings become closer together towards the outer edges of the granule (Baker *et al.*, 2001; Ridout *et al.*, 2003; Li *et al.*, 2003; Lemke *et al.*, 2004). The number and size of growth rings have been shown to be influenced by the genotype of the starch granule (Li *et al.*, 2003). The width of granule growth rings was found to decrease with increases in the amylose content of barley starches (Li *et al.*, 2003).

According to Cameron and Donald (1992), a semicrystalline domain is built up by ~16 repeats of alternating crystalline (5-6nm) and amorphous (2-5nm) lamellae (Figure 2.7b) with a thickness between 120 and 400nm (French, 1984). The amorphous growth ring is largely amorphous, contains more water, and is at least as thick as the semicrystalline growth ring (Cameron & Donald, 1992). The crystalline lamellae are made of amylopectin double helices, which are packed in a parallel fashion, whereas the amylopectin branch points are in amorphous zones (Figure 2.7c). This granule structure model proposed by Jenkins *et al.* (1994) agrees with the current cluster model structure

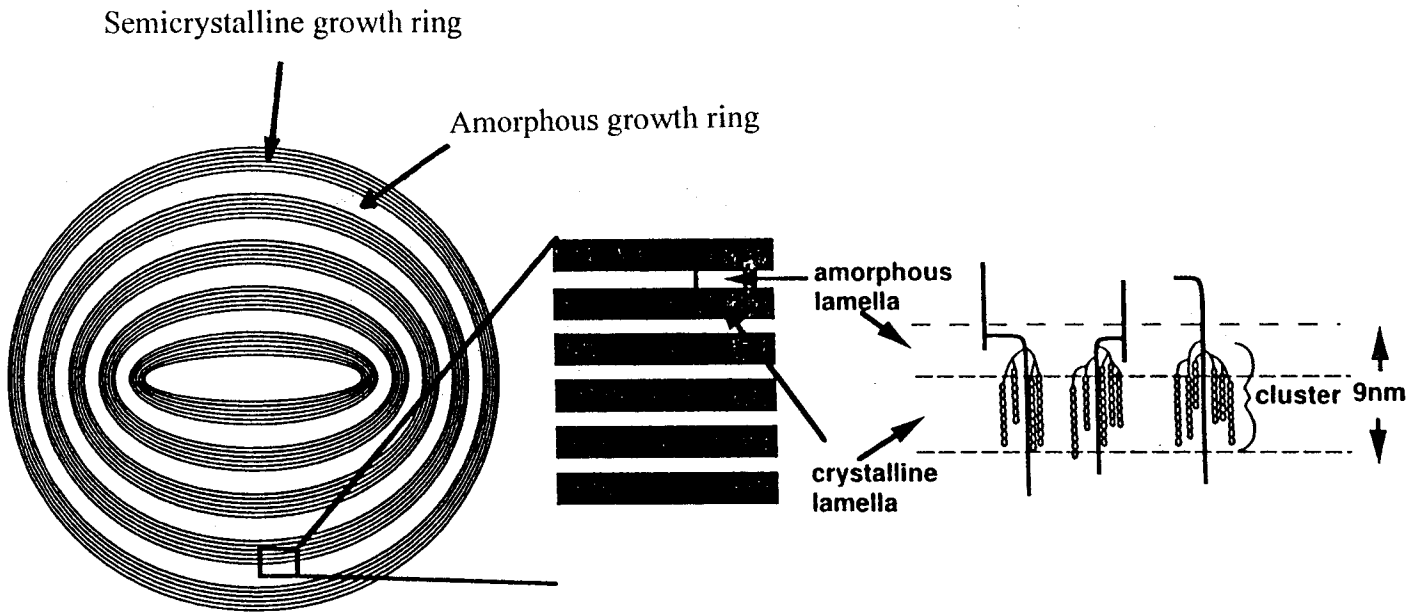
Figure 2.7 Internal structure of a starch granule showing alternating amorphous and semicrystalline growth rings.

a) Stacks of semicrystalline lamellae are separated by amorphous growth rings.

b) A magnified view of one such stack, showing that it is made up of alternating crystalline and amorphous lamellae.

c) The crystalline lamellae comprise regions of lined up double helices formed from amylopectin branches. The amorphous lamellae are where the amylopectin branch points sit.

Source: Adopted from Donald *et al.* (1997)


Semicrystalline growth ring
Amorphous growth ring
amorphous lamella
crystalline lamella
cluster
9nm

a) b) c)

for amylopectin. Recently however, Gallant *et al.* (1997) have proposed another granule structure model (Figure 2.8), in which the growth rings appear to be composed of spherical structures, namely blocklets, stacked on top of each other with sizes ranging between 20-500nm in diameter, depending on the starch source and location in the granule. This was supported by AFM studies carried out by Baker *et al.* (2001) and Ridout *et al.* (2002, 2003). These AFM images imply that the blocklet structure is continuous throughout the granule and the amorphous growth rings in the native granule are composed of a collection of localized defects in blocklet structure, distributed around the surface of shells within the granule.

2.2.5.2.2.2 Channels and central cavity

Channels and central cavities are also visible using SEM, TEM, CSLM and fluorescence microscopy. These channels have been observed in maize (Huber & BeMiller, 1997), and sorghum (Fannon *et al.*, 1993; Huber & BeMiller, 1997) starches. Velde *et al.* (2002) observed central cavities in maize and mung bean starches. In sorghum starch granules, the diameter of channels was in the range of 0.07-0.1μm (Fannon *et al.*, 1993). These channels have been shown to penetrate from the external surface inward toward a cavity at the hilum with various depths of penetration. No direct relationships have been found among surface pinholes, granule size, cavity size, and internal channels. For instance, potato starch granules have no visible holes and no channels, but still have central cavities. Huber and BeMiller (1997, 2000) have suggested that channels and cavities are more likely voids, which are formed by the crystallization

Figure 2.8 Blocklet model of starch granule structure.

a) The granule is composed of alternating crystalline (hard) and semicrystalline (soft) shells (dark and light color, respectively). The shells are thinner towards the granule exterior (due to increasing surface area to be added to by constant growth rate) and the helium is shown off center.

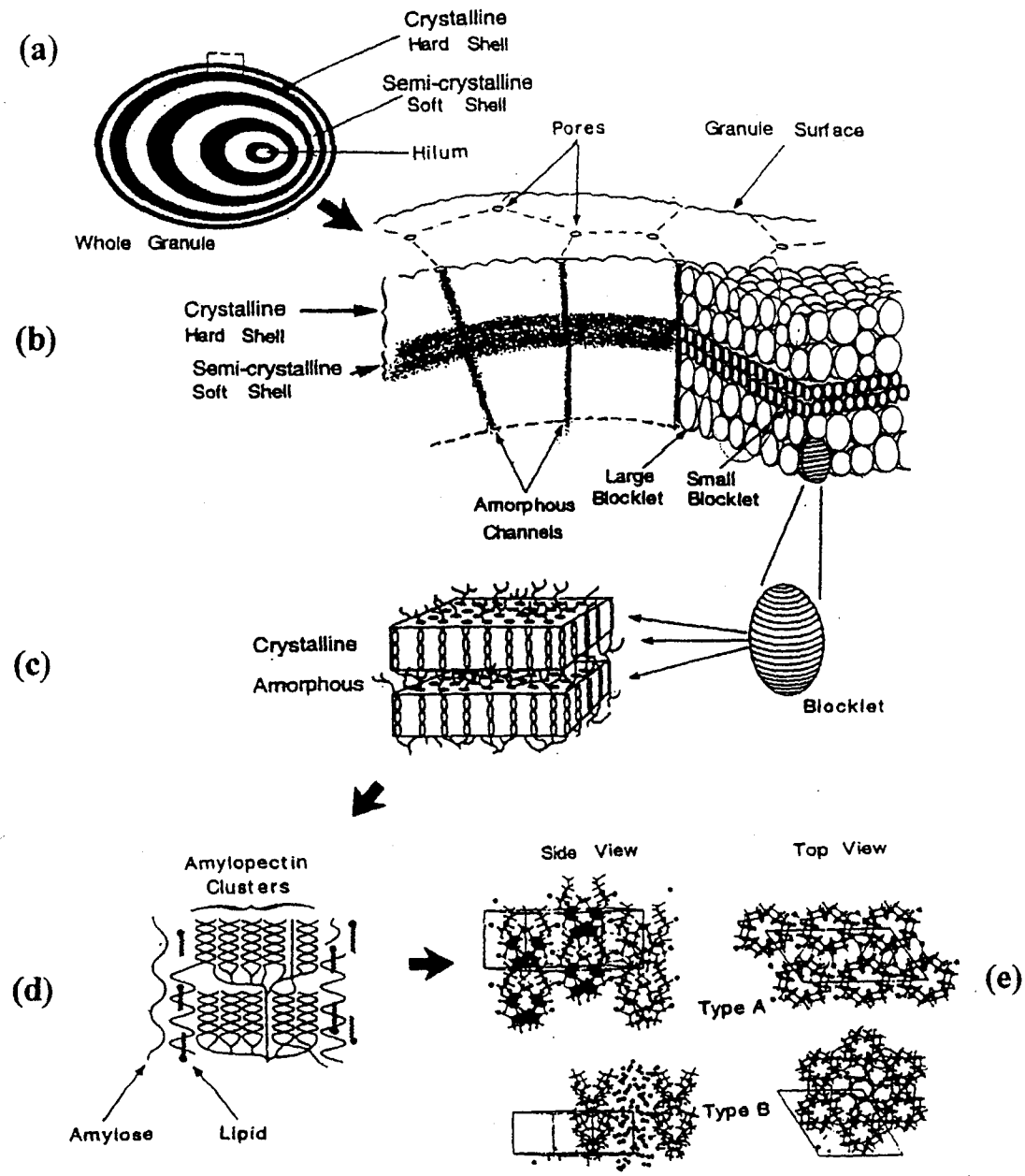
b) Blocklet structure is shown, in association with amorphous radial channels. Blocklet size is smaller in the semicrystalline shells than in the crystalline shells.

c) One blocklet is shown containing several amorphous and crystalline lamellae. The next diagram shows the magnified picture of amorphous and crystalline lamellae of amylopectin.

d) Amylose-lipid (and protein) complexes feature in the organization of the amylopectin chains.

e) The crystal structures of A and B type crystalline.

Source: Gallant *et al.* (1997), reproduced with permission from Elsevier Science.


(a)
Crystalline
Hard Shell
Semi-crystalline
Soft Shell
Hilum
Whole Granule
Pores
Granule Surface
(b)
Crystalline
Hard Shell
Semi-crystalline
Soft Shell
Amorphous
Channels
Large
Blocklet
Small
Blocklet
(c)
Crystalline
Amorphous
Blocklet
(d)
Amylopectin
Clusters
Amylose
Lipid
Side View
Top View
Type A
Type B
(e)

of amylopectin molecules and concurrent shrinkage of the matrix as the granule grows and develops. They further postulated that these channels and cavities could influence reactions within granules.

2.2.5.2.2.3 Structure of the amorphous region

The major part of starch granules is believed to be amorphous or gel phase, where several materials such as lipid-free amylose, lipid-complexed amylose, and some branch points of amylopectin are mixed together (French, 1984; Hizukuri, 1996). However, Morgan *et al.* (1995) have suggested that, in wheat starch the V-amylose-lipid inclusion complexes occur in distinct regions of the starch granule and not in the amorphous region. According to them, there are three distinct regions in the starch granule: 1) highly crystalline regions formed from double-helical starch chains, 2) solid-like regions formed from V-amylose-lipid inclusions of starch, and 3) completely amorphous regions associated with the branching regions of amylopectin and lipid-free amylose. Recent studies of starch granule structure using small angle neutron scattering revealed that amorphous lamellae and amorphous growth rings of potato ('B'-type) starch have very similar compositions, whereas the amorphous lamellae of cereal starches ('A'-type) are significantly less dense and contain more water than the amorphous growth rings (Donald *et al.*, 2001). They interpreted these observations as a result of growth conditions of the starches (i.e., permanent darkness for tubers and diurnal fluctuations of growth conditions for cereal starches).

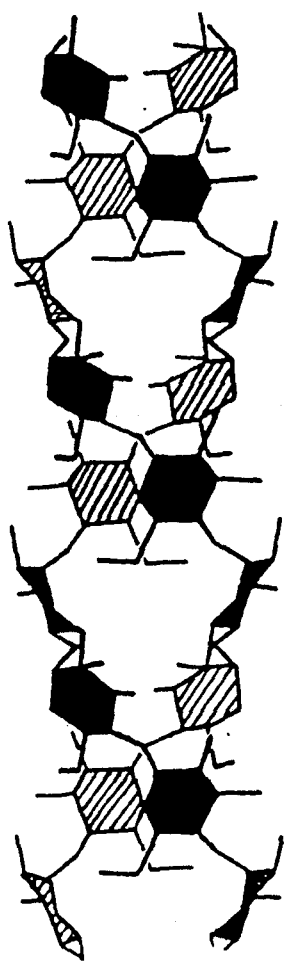
2.2.5.2.2.4 Double helices, polymorphic pattern and crystallinity

Both amylose chains and exterior chains of amylopectin can form double helices, which may in turn associate to form crystalline domains (Tester *et al.*, 2004). In most starches, these are confined to the amylopectin component. Two neighboring short chains fit together compactly with the hydrophobic parts of the opposed glucose units in close contact at the inside of the structure and the hydroxyl groups at the outside of the double helix (Figure 2.9). Because of the highly hydrophobic and compact nature of the helical core, there is no room for water or any other molecule to reside in it. The stability of the helix is attained by direct or indirect (through a bridge of water molecules) inter-chain hydrogen bonding between hydroxyl groups of two neighboring double helices. This close packing of neighboring double helices forms granule crystallites or polymorphs (Imberty & Pérez, 1988; Imberty *et al.*, 1988 a,b,1991; Wang *et al.*, 1998). One early model proposed by Wu & Sarko (1978a,b), for the packing arrangement of double helices, is illustrated in Figure 2.10.

Wu and Sarko (1978a,b) proposed two types of unit cells called 'A' and 'B', and assumed that these were in the form of right-handed, double-stranded helices which packed in an antiparallel fashion. Later, Imberty *et al.* (1987,1988a,b) reported that the molecules assumed the left-handed, double-stranded helices, and packed in a parallel fashion. However, these two types of polymorphs differ in the geometry of their single cell units, the packing density of their double helices and in the amount of bound water within the crystal structure, 'A'-type polymorph being more dense and binding less water than 'B'-type (Wu & Sarko, 1978a,b; Imberty & Pérez, 1988; Imberty *et al.*, 1988 a,b;

Figure 2.9 Double helix model of starch chain

Source: French & Murphy (1977), reproduced with permission from American Association of Cereal Chemists.



Wang *et al.*, 1998; Figure 2.10). The unit cell of 'B'-type amylose contains 36 water molecules loosely associated in a channel formed by the hexagonal packing of the helices, whereas in the orthogonal unit cell of the 'A'-type amylose, the channel was occupied by another double helix and 8 water molecules (Pfannemüller, 1987; Appelqvist & Debet, 1997).

In addition to the differences in their crystallite arrangements and water content (Figure 2.10), the amylopectin chain length (Pfannemüller, 1987; Gidley & Cooke, 1991; Hizukuri, 1996), and branching pattern (Jane *et al.*, 1997) also differ from each other in 'A'- and 'B'-type polymorphs. The crystallization of 'A'-type polymorphs over 'B'-type has been shown to be influenced by shorter amylopectin chain lengths, higher temperatures, high concentrations and the presence of salt, water soluble alcohols, and water content (Gidley, 1987; Pfannemüller, 1987; Imberty *et al.*, 1991; Genkina *et al.*, 2004a,b). The difference in the average chain length between 'A'- and 'B'-types can be as little as one glucose unit (Hizukuri, 1996). In 'A'-type amylopectin, the α-(1→6)-branch linkages are more scattered and mainly located within the crystalline region/ lamellae (Figure 2.11). In 'B'-type amylopectin, most of the α-(1→6)- branch linkages are clustered in the amorphous region (Figure 2.11).

Starch is classified according to the packing arrangement of the amylopectin double stranded helices in the granule, namely 'A'-, 'B'-, and 'C'-type (Figure 2.12), as determined by differences in the X-ray diffraction pattern (Imberty *et al.*, 1991). The 'A'- and 'B'-types are believed to be independent, while the 'C'-type is a mixture of 'A'- and 'B'- type crystallites in varying proportions (Wu & Sarko, 1978 a,b; Garnat *et al.*,

Figure 2.10 Packing arrangement of double helices of 'A'- and 'B'-type crystallite unit cells.

Source: Wu & Sarko (1978a,b), reproduced with permission from Elsevier Science.

A-type

B-type

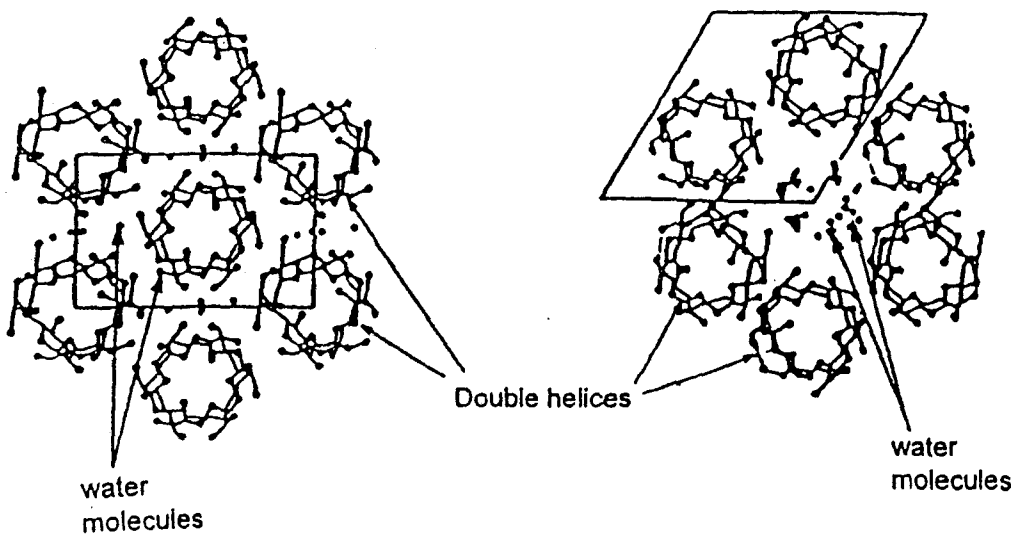


Figure 2.11 Proposed models for branching patterns of 'A'-type starch and 'B'-type starch. A and C stand for the amorphous and crystalline regions, respectively.

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

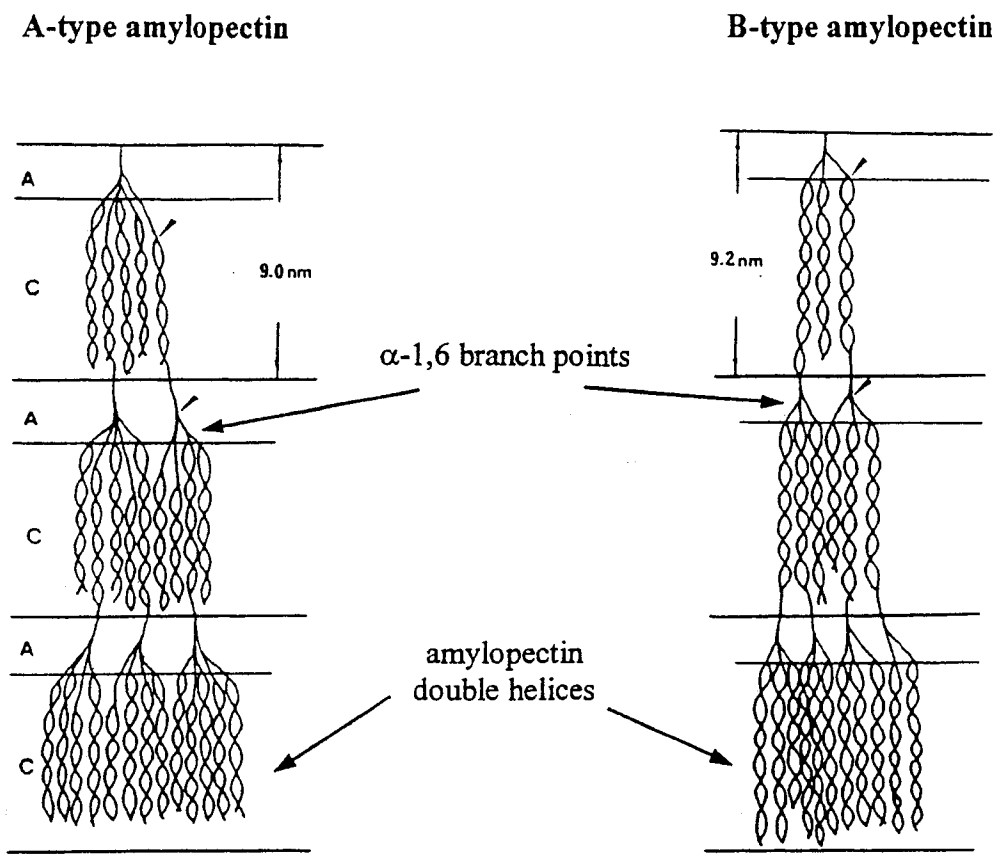

A-type amylopectin
B-type amylopectin
A
C
9.0 nm
A
C
A
C
9.2 nm
α-1,6 branch points
amylopectin
double helices

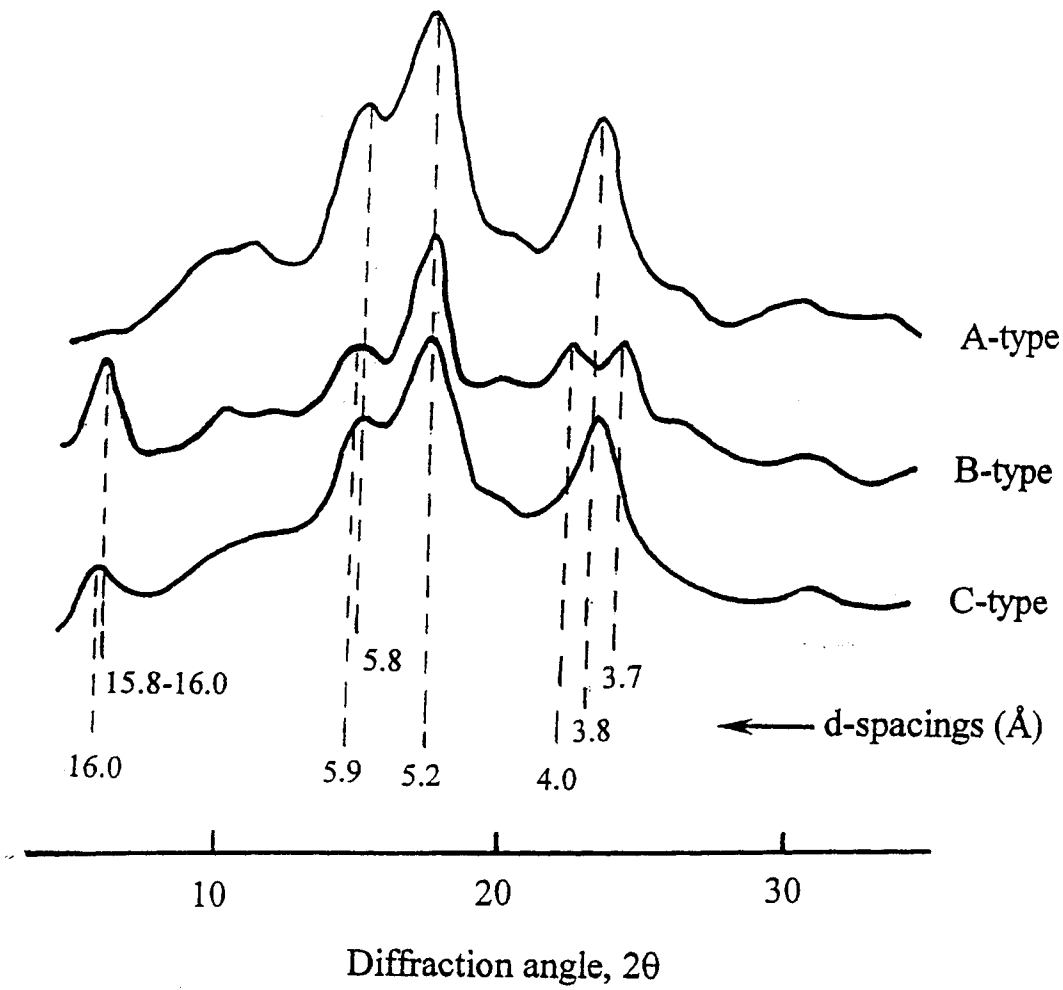
Figure 2.12 X-ray diffraction patterns of 'A'-, 'B'-, and 'C'-type starches with their characteristic *d*-spacing.

'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 at 17.05 2θ (*d*=5.2 Å) and 18.1 (*d*=4.9 Å). The *d*-spacing at 4.4 Å is characteristic to amylose-lipid complex (Vasanthan & Bhatty, 1996).

'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: Adopted from Zobel (1988)


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

1990; Hizukuri, 1996). Further classification of the 'C'-type as 'Ca', 'Cb', and 'Cc' is on the basis of their resemblance to the 'A'-type, 'B'-type, and a combination of two types, respectively (Hizukuri, 1996). In addition to these three types of diffraction patterns, another pattern called 'V'-type was also reported, which is mainly characterized by crystalline amylose helical inclusion complexes (Blanshard, 1987; Eliasson & Gudmundessson, 1996).

The 'A'-type X-ray diffraction pattern is common in most cereal starches (Cheetham & Tao, 1998a; Matveev *et al.*, 2001; Tang *et al.*, 2002; Yoshimoto *et al.*, 2002; Qi *et al.*, 2003,2004), and in some root and tuber starches (Cheetham & Tao, 1998a; Hoover, 2001; Gunaratne & Hoover, 2002). Tuber and root starches (Hoover, 2001; Gunaratne & Hoover, 2002), and some high-amylose barley, maize, and rice (Hizukuri, 1996; Cheetham & Tao, 1998a; Matveev *et al.*, 2001) and wrinkled pea (Zhou *et al.*, 2004) starches have been shown to exhibit the 'B'-type X-ray diffraction pattern. However, there are some reports that certain cultivars of barley containing amylose content in the range 33.4-48.0% exhibit an 'A'-type X-ray pattern similar to waxy and normal barley starches (Vasanthan & Bhatty, 1996; Song & Jane, 2000; Yoshimoto *et al.*, 2000). The 'C'-type pattern commonly appears in legume starches (Hoover & Sosulski, 1985; Zhou *et al.*, 2004).

'C'-type is believed to be a mixture of 'A'- and 'B'-type crystallites. The way 'C'-types crystallites are structured is still not fully understood. Gernat *et al.* (1990) have observed that legume starches consist of starch granules of pure 'A'- and pure 'B'-type in varying proportions. However, Gérard *et al.* (2001) have shown that heterogeneous

distributions of 'A'- and 'B'-type crystallites were present within the granule of mutant maize starches with various 'A'- and 'B'-polymorphic ratios. In 'C'-type pea starches, Bogracheva *et al.* (1998) found that both 'A'- and 'B'-type polymorphs are present in the same granule and 'B'-type polymorphs are present in the granule interior, while 'A'-types are located in the periphery of the granule.

Double helical content and the degree of crystallinity of starches determined by ^{13}C NMR and X-ray diffraction, respectively, are presented in Table 2.9. The degree of crystallinity ranges from 12 to 45% by weight depending on the starch origin and hydration. Cheetham and Tao (1998a,b) observed a strong correlation between the relative crystallinity with amylose content; the relative crystallinity being inversely proportional to the amylose content. Amylopectin is the main crystalline component of the starch granule and amylose acts as a diluent in normal and waxy starches (Banks & Greenwood, 1975; Blanshard, 1987; Zobel, 1988; Hoover, 2001). However, there is a controversy regarding the contribution of amylose to the crystallinity of high-amylose starches. Banks and Greenwood (1975) suggested that amylose contributes significantly to increasing the crystallinity of high- amylose starches, whereas Jenkins (1994) suggested that amylose disrupts the packing of amylopectin double helices within crystalline lamellae (Figure 2.13).

Table 2.9 Degree of crystallinity, polymorphic pattern, and double helical content of starches from different botanical sources

Starch source	Amylose content (%)	Crystalline pattern	Degree of crystallinity (%)	Double helical content (%)
Maize (waxy)	0	A	41.8	36-53
Maize (normal)	28	A	30.3	38-43
Maize (amylo)	40	C	21.8	38
	56-84	B	17.2-19.5	38
Barley (waxy)	-	A	36.4-44.8	-
Barley (normal)	-	A	22-27.4	-
Rice	-	-	-	49-63
Wheat	-	-	-	32-46
Potato	28.1	B	30.0	40-64
Tapioca	22.4	A	37.0	44
Smooth pea	30-43	C	26-32	-
Wrinkled pea	78.42	B	17.7	-
Lentil	30.51-32.29	C	31.7-32.3	-

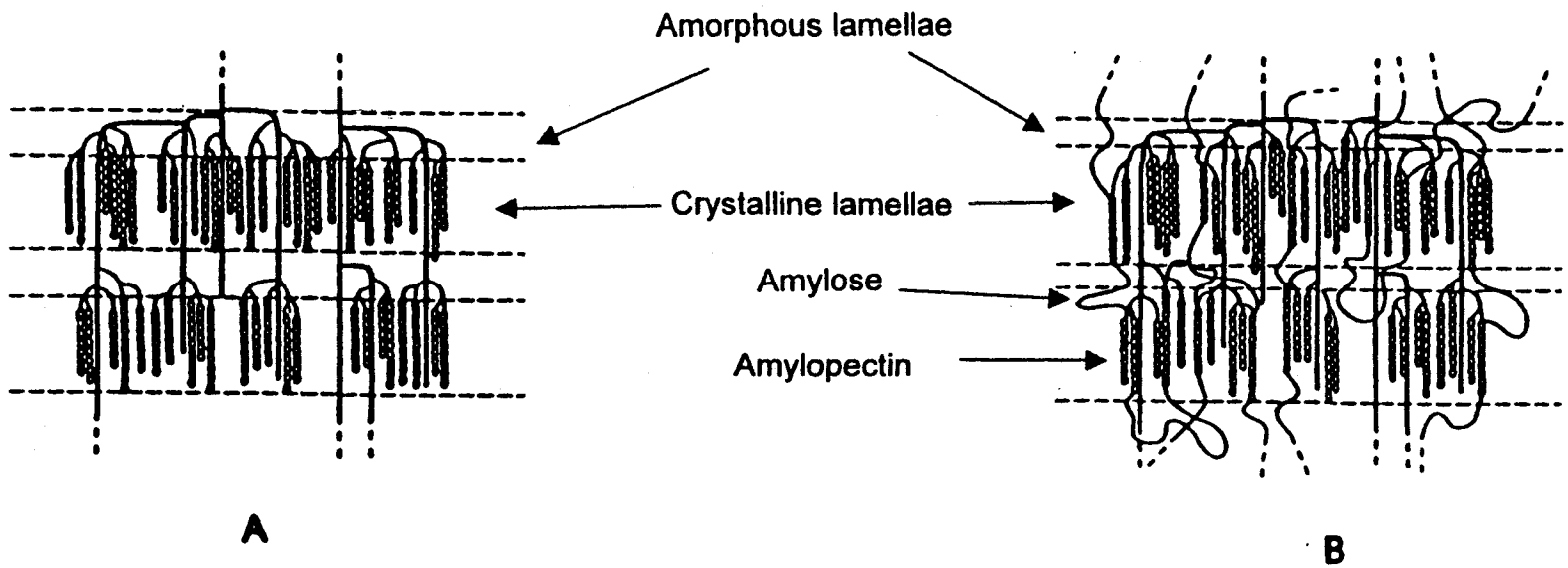
Reference: Cheetham & Tao, 1998a,b; Davydova, *et al.*, 1995; Tester *et al.*, 2004; Gunaratne & Hoover, 2002; Zhou *et al.*, 2004; Qi *et al.*, 2004;

Figure 2.13 A possible mechanism to explain the disruption of amylopectin double helical packing by amylose.

A) Amylopectin structure in the absence of amylose showing small crystalline lamellae size

B) Cocrystallinity between amylose and amylopectin pulls a number of amylopectin chains out of register resulting an increased lamellae size.

Source: Jenkins & Donald (1995), reproduced with permission from Elsevier Science.


Amorphous lamellae
Crystalline lamellae
Amylose
Amylopectin
A
B

2.2.6 Physicochemical properties

2.2.6.1 Granular swelling and amylose leaching

When dry starch granules are placed in water, a small amount of water is absorbed. If the temperature is increased, the amount of absorbed water increases and results in swollen granules. 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). This swelling is often measured as an increase in gel volume. The irreversible swelling starts at a temperature corresponding to onset temperature (To) in DSC measurements (Tester & Morrison, 1990a,b). The swelling is rapid during the first 5-10 min at a certain temperature, and continues during further heating (Tester & Morrison, 1990a). The swelling is not affected by presoaking before heating, but increases with the water/starch ratio at ratios up to 25 mL/g of starch (Tester & Morrison, 1990a).

Granular swelling is primarily a property of intact amylopectin, and amylose acts as a diluent (Tester & Morrison, 1990a; Morrison, 1995). It has been shown to be influenced by: 1) amylose content (Tester & Morrison, 1990a; Sing & Kaur, 2004; Zhou *et al.*, 2004), 2) the amount of lipid-complexed amylose (Tester & Morrison, 1990a; Morrison *et al.*, 1993b; Morrison, 1995; Gunaratne & Hoover, 2002), 3) extent of interaction between starch chains within the amorphous and crystalline domains of the starch granule (Hoover & Sosulski, 1986; Zhou *et al.*, 2004; Singh & Kaur, 2004), 4) amylopectin molecular structure (Shi & Seib, 1992; Tester *et al.*, 1993; Qi *et al.*, 2003), 5) phosphorous content (Swinkels, 1985; Galliard & Bowler, 1987; Gunaratne & Hoover,

2002), 6) temperature (Colonna & Mercier, 1985; Hoover & Vasanthan, 1994a; Davydova *et al.*, 1995; Qi *et al.*, 2003), 7) starch damage (Karkalas *et al.*, 1992), and 8) granule size (Vasanthan & Bhatty, 1996; Tang *et al.*, 2002, 2004; Singh & Kaur, 2004).

Jenkins *et al.* (1994) showed that the initial absorption of water and the location of swelling occur primarily within the amorphous growth ring rather than the amorphous lamellae. In general, starches from legumes, roots and tubers exhibit a single-stage swelling (Hoover & Sosulski, 1986; Hoover, 2001). Of these, potato starch has the highest swelling power due to a high phosphate ester content of amylopectin (Galliard & Bowler, 1987). In contrast to legume and root and tuber starches, normal cereal starches show a two-stage swelling (Leach *et al.*, 1959; Doublier *et al.*, 1987; Langton & Hermansson, 1989). Recently, however, Li *et al.* (2001b) and Vasanthan and Bhatty (1996) have observed a two-stage swelling in waxy barley starches and a single-stage swelling in normal and high-amylose starches. A rapid increase of swelling power at lower temperatures, has been observed for waxy maize and zero-amylose barley starches (Li *et al.*, 2001b).

During heating, at the same time as the absorption of water, material is leached out from the starch granule. This material is mainly amylose, although amylopectin might be leached out, depending on the type of starch and conditions (Tester & Morrison, 1990a). The extent of AML has been shown to be influenced by: 1) 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), 2) the amount of lipid-complexed amylose chains (Morrison *et al.*, 1993b; Ratnayake *et al.*,

2001; Gunaratne & Hoover, 2002; Nakazawa & Wang, 2004), 3) phosphate content (Gunaratne & Hoover, 2002), 4) granular size (smaller granules leach more amylose than larger granules) (Lindeboom *et al.*, 2004), and 5) heating temperature (Hoover & Vasanthan, 1994b; Vasanthan & Bhatty, 1996; Gunaratne & Hoover, 2002). Materials leached out at higher temperatures are composed of molecules with a high molecular weight and are more branched (Hizukuri, 1996). It has been found that not all amylose leaches out during heating (Eliasson & Budmundsson, 1996). 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).

2.2.6.2 Gelatinization

Starch, when heated in the presence of excess water, undergoes an order-disorder phase transition called geltinization over a temperature range characteristic of the starch source. The above 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, loss of optical birefringence, uptake of heat, loss of crystalline order, uncoiling and dissociation of double helices (in the crystalline regions), and amylose leaching (French, 1984; Tester, 1997a; Hoover, 2001). Of the various methods, which have been used for the characterization of starch gelatinization, such as polarized light microscopy, XRD, DSC, NMR spectroscopy, enzymatic digestibility, viscoamylogarphy and small-angle light scattering, DSC has been more widely used for evaluating gelatinization parameters (onset temperature [To], peak temperature [Tp],

conclusion temperature [Tc], and enthalpy [ΔH]) (Zobel, 1984; Tester & Debon, 2000; Appendix A.3). According to Jenkins (1994), gelatinization in excess water is primarily a process driven by swelling. The swelling within the amorphous region acts to destabilize the amylopectin crystallites within the crystalline lamellae, which are ripped apart. Smaller crystallites are destroyed first. This process occurs rapidly for an individual crystallite, but over a limited temperature range for a single granule (1-2 °C) and a wider range (10-15 °C) for a whole population of granules (French, 1984; Eliasson & Gudmundsson, 1996).

Gelatinization is primarily a property of amylopectin in which gelatinization temperature reflects crystalline perfection (double helical length) and gelatinization enthalpy is a measure of the overall crystallinity (quality and quantity of crystallites) (Tester & Morrison, 1990a,b). Cooke and Gidley (1992) suggested that ΔH primarily reflects the loss of double helical order rather than loss of X-ray crystallinity. Noda *et al.* (1998) demonstrated that gelatinization temperatures are influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains (DP 6-11), and not by the proportion of the crystalline region, which corresponds to the amylose/amylopectin ratio. Qi *et al.* (2004) suggested that the crystalline lamellae region controls the gelatinization parameters of starch, and although the crystallite length contributes to the origin of the gelatinization temperatures (but not enthalpy), it is the overall optimization of registration of these double helices that control the gelatinization process (i.e., more or less restricted hydration of starch granules when heated in water).

Gelatinization transition temperatures and enthalpy have been shown to be controlled by: 1) molecular structure of amylopectin (unit chain length, extent of branching, molecular weight, polydispersity) (Tester, 1997a,b; Jane *et al.*, 1999; Kohyama *et al.*, 2004), 2) amylose content (Russel, 1987; Hoover & Manuel, 1996; Tester, 1997a,b; Fredriksson *et al.*, 1998; Matveev *et al.*, 2001; Yoshimoto *et al.*, 2002; MacGregor *et al.*, 2002), 3) lipid-complexed amylose (Morrison et al, 1993b, 1995; Tester, 1997a,b), 4) phosphorous content (Tester, 1997a,b; Blennow *et al.*, 2000), 5) granule architecture (crystalline/amorphous ratio) (Tester & Morrison, 1993; Tester, 1997a,b; Noda *et al.*, 1998; Qi *et al.*, 2004), 6) size of the granule (Vasanthan & Bhatty, 1996; Li *et al.*, 2001b; Tang *et al.*, 2001, 2002; Sing & Kaur, 2004), and 7) growth temperature (Myllärinen *et al.*, 1998; Kiseleva *et al.*, 2003; Kohyama *et al.*, 2004). The addition of sugars, polyhydric alcohols, and salts (Evans & Haisman, 1982; Bogracheva *et al.*, 1998) during heating, (Donovan, 1979), and starch modification (Biliaderis, 1991; Jacobs & Delcour, 1998c) have also been shown to influence gelatinization parameters.

2.2.6.3 Retrogradation

Starch retrogradation is a process, which occurs when starch chains begin to reassociate in an ordered structure. In its initial phases, two or more starch chains may form a simple juncture point which then may develop into more extensively ordered regions. Ultimately, under favorable conditions, a crystalline order appears (Atwell *et al.*, 1988). Retrogradation is especially evident when a gelatinized starch is cooled. Although, amylopectin can retrogradate upon cooling, linear amylose molecules have a

greater tendency to reassociate and form hydrogen bonds than the larger amylopectin molecules (Thomas & Atwell, 1999b). 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).

The interactions that occur during retrogradation, are found to be time and temperature dependent (Hoover, 2001). The retrogradation tendency of starches from different origins varies greatly. The rate and extent of retrogradation of starches are mainly influenced by starch composition and structure, starch concentration, storage conditions (i.e., moisture, temperature), the presence of other substances (i.e., lipids, surfactants), and starch modifications (Shi & Seib, 1992; Eliasson & Gudmundson, 1996; Fredriksson *et al.*, 1998; Lai *et al.*, 2000).

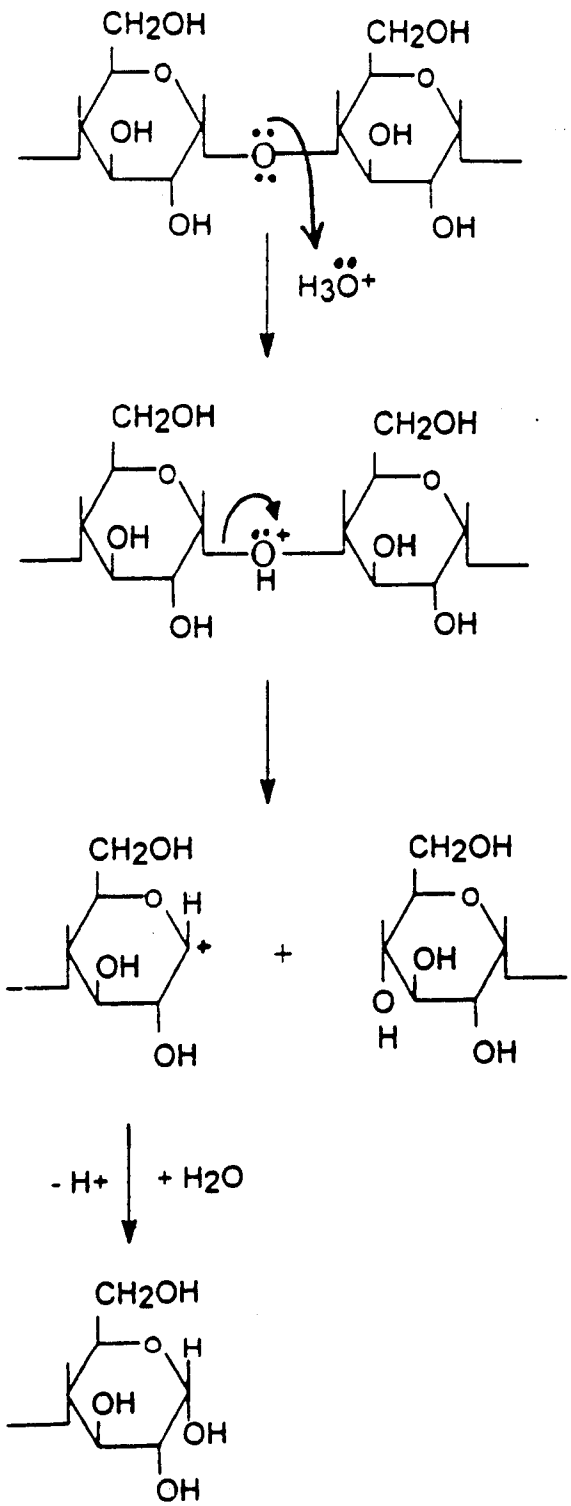
2.2.6.4 Acid hydrolysis

Acid causes scissions of the glycosidic linkages (Figure 2.14), thereby altering the structure and properties of the native starch. Starch treated with sulfuric acid (15% v/v) is referred to as Nägeli amylodextrins, while starch treated with hydrochloric acid (7.5% v/v) 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).

The differences in the rate and extent of acid hydrolysis between starches have been attributed to differences in granular size, extent of starch chain interactions within

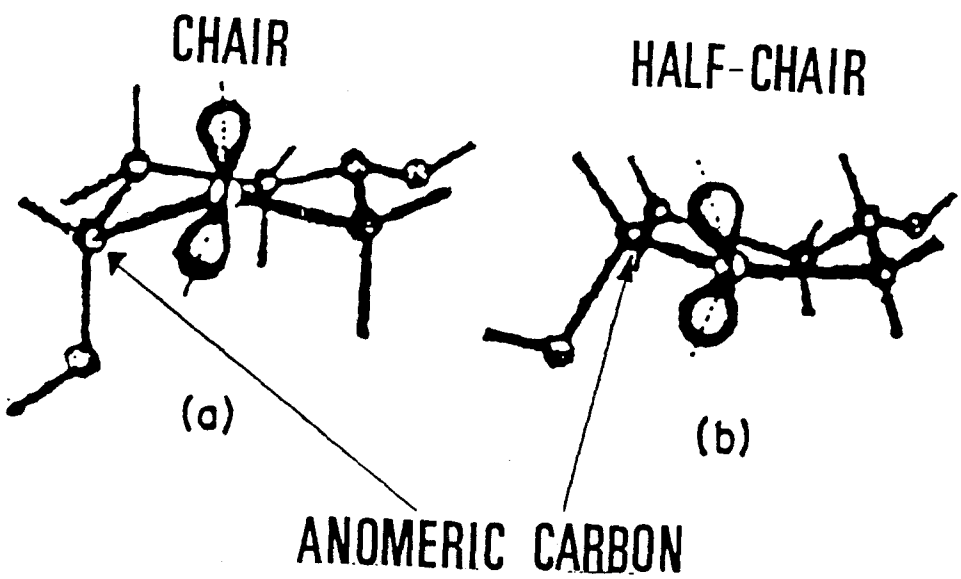
Figure 2.14 Mechanism of acid hydrolysis of starch

Source: Hoover (2000), reproduced with permission from Marcel Dekker, Inc.


CH2OH
CH2OH
OH
OH
OH
OH
H3O+
CH2OH
CH2OH
OH
OH
H
OH
OH
CH2OH
CH2OH
H
OH
OH
OH
H
OH
- H+
+ H2O
CH2OH
H
OH
OH
OH

the amorphous and crystalline regions of the granule, and starch composition (amylose content, extent of phosphorylation, and lipid-complexed amylose) (Vasanthan & Bhatty, 1996; Jane *et al.*, 1997; Shi *et al.*, 1998; Song & Jane, 2000; Hoover, 2001; Li *et al.*, 2001b; Gérard *et al.*, 2002; Jayakody & Hoover, 2002; Hoover *et al.*, 2003). 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; Jacobs *et al.*, 1998a; Li *et al.*, 2001b; 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). Differences in the extent and rate of hydrolysis among the starches during the first stage of hydrolysis have been mainly attributed to: 1) the amount of lipid-complexed amylose (lipid-complexed amylose chains resist degradation by H_3O^+) (Morrison *et al.*, 1993a,b; Jacobs *et al.*, 1998a; Hoover *et al.*, 2003), 2) the extent of interaction between starch chains (retrograded amylose) within the amorphous regions of the granule (presence of double helices and close packing of non-helical amylose chains within the amorphous regions will hinder the conformational transformation [chair→half chair; Figure 2.15] required for protonation of glycosidic oxygens) (Hoover, 2000; Gunaratne & Hoover, 2002; Hoover *et al.*, 2003), 3) granule size (small granules are hydrolyzed faster and to a greater extent than large granules) (Vasanthan & Bhatty, 1996), and 4) the amount of very short amylopectin branch chains (DP 2-8, which exist as dangling chains on the

Figure 2.15 Chair→half chair conformation of glucose molecule


CHAIR
HALF-CHAIR
(a)
(b)
ANOMERIC CARBON

surface of crystallites or at weak points of the crystallites) (Biliaderis *et al.*, 1981; Jane *et al.*, 1997; Gérard *et al.*, 2002). Lipids within the amylose helix may decrease the extent and rate of hydrolysis by hindering the conformational transformation (chair→half chair) required for protonation of the glycosidic oxygen (Hoover, 2000). However, Gérard *et al.* (2002) have shown that the V-type amylose-lipid complex is preferentially degraded by acid in mutant maize starches. Differences in hydrolysis at the second stage have been attributed to: 1) the degree of packing of the double helices that form the crystalline lamellae (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).

The influence of acid hydrolysis on X-ray pattern has been shown to vary with starch source. Maize (waxy, normal, amylo-) (Jayakody & Hoover, 2002), rice (Jayakody & Hoover, 2002), some cultivars of barley (Morrison *et al.*, 1993c), oat (Jayakody & Hoover, 2002), potato, and legume (Hoover, 2000) starches exhibit unchanged X-ray patterns on acid hydrolysis. However, the acid hydrolysis has been shown to change the 'A'-type X-ray pattern in some barley starches (Morrison *et al.*, 1993c) and the 'B'-type pattern in cassava starch (Garcia *et al.*, 1996) to a 'C'-type pattern. The relative crystallinity of acid treated starches of wheat (Muhr *et al.*, 1984), maize (normal, amylo-) (Komiya *et al.*, 1987; Raja, 1994; Jayakody & Hoover, 2002), mutant maize (Gérard *et al.*, 2002), rice (Jayakody & Hoover, 2002), oat (Jayakody & Hoover, 2002), potato (Muhr *et al.*, 1984), and cassava (Raja, 1994; Atichokudomchai *et al.*, 2002) have been shown to increase with hydrolysis time. However, the relative

crystallinity of waxy maize starch has been shown to decrease with the time of hydrolysis (Jayakody & Hoover, 2002). Acid treated non-waxy barley starches have been shown to exhibit a higher double helical content and degree of crystallinity compared to its native counterpart, whereas a significant change was not observed for acid treated waxy barley starches (Morrison *et al.*, 1993c).

Acid hydrolysis has been shown to increase gelatinization transition temperatures (To, Tp, Tc) and the gelatinization temperature range (Tc-To) in cassava (Garcia *et al.*, 1996; Atichokudomchai *et al.*, 2002), potato (Komiya & Nara, 1986; Jenkins & Donald, 1997; Jacobs *et al.*, 1998a), barley (Shi & Seib, 1992; Morrison *et al.*, 1993c), rice (Chun *et al.*, 1997; Jayakody & Hoover, 2002), maize (Shi *et al.*, 1998; Jayakody & Hoover, 2002), oat (Jayakody & Hoover, 2002), and pea (Jacobs *et al.*, 1998a) starches. However, the influence of acid hydrolysis on gelatinization enthalpy has been reported to vary with the starch source and hydrolysis time (Muhr *et al.*, 1984; Komiya & Nara, 1986; Hoover & Vasanthan, 1994a; Garcia *et al.*, 1996; Jenkins & Donald, 1997; Jacobs *et al.*, 1998a; Atichokudomchai *et al.*, 2002; Jayakody & Hoover, 2002).

2.2.7 Starch annealing

2.2.7.1 Introduction

Starch annealing, a hydrothermal treatment that modifies the physicochemical properties of starch without destroying the granule structure, is described as the perfection of the amorphous and crystalline lamellae of the starch granule. Annealing is defined as a physical treatment that involves incubation of starch granules in excess water

or at intermediate water content, i.e., at or above 40% water (w/w), for a certain period of time, at a temperature above the glass transition, but below the gelatinization of the native starch (Jacobs & Delcour, 1998; Tester & Debon, 2000), which implies that amorphous glassy starch molecules become mobile and reorganize to form an improved crystalline structure during annealing (Figure 2.16).

Annealing of starch has been studied at various starch:water ratios (1:1, 1:3, 1:5, w/w) and at temperatures ranging from 50°C to 75°C (Hoover & Vasanthan, 1994a). The annealing process has been studied as a single event (single-step) (Jacobs *et al.*, 1998a,b,c; Yamamoto & Shirakawa, 1999; Tester *et al.*, 2000; Adebowale & Lawal, 2002; Gomes *et al.*, 2004), two starch-water/temperature/time events (double-step) (Jacobs *et al.*, 1998a,b,c; Tester *et al.*, 2000), or even many individual steps (multi-step) (Knutson, 1990; Nakazawa & Wang, 2003). This double- or multi-step approach is often used to promote annealing without gelatinization, and the double/multi-step process potentially produces higher gelatinization temperatures than the single-step process (Jacobs & Delcour, 1998).

2.2.7.2 Impact of annealing on starch structure

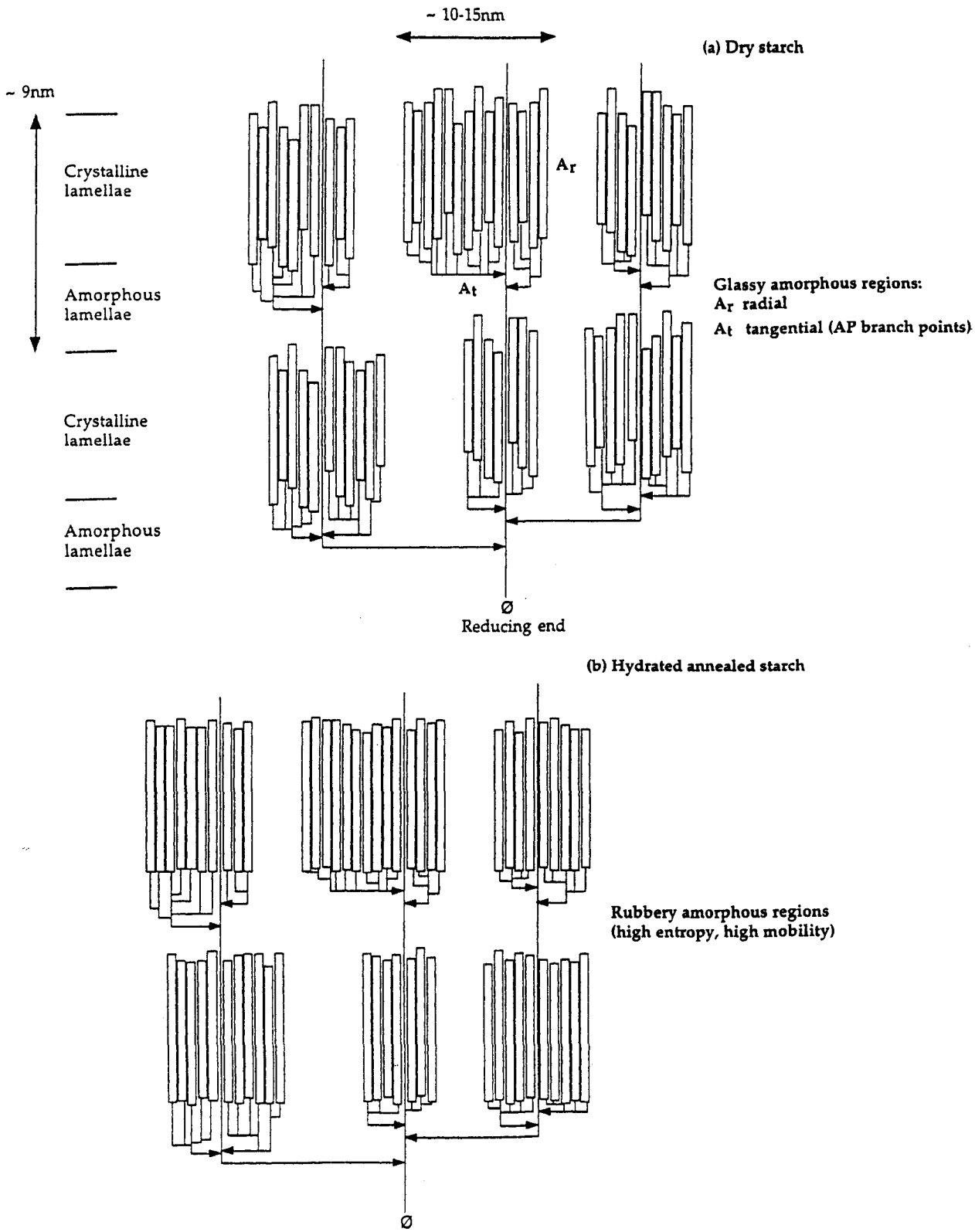
Annealing causes no effect on starch granule size or shape (Stute, 1992; Hoover & Vasanthan, 1994a; Adebowale & Lawal, 2002). Jacobs *et al.* (1998b) found no significant difference in ^{13}C CP/MAS NMR spectra of native and annealed wheat, potato and pea starches, indicating no changes in double helical content. However, with respect to the effect of annealing on the double helix content of starches, the situation in

Figure 2.16 Pictorial representation of the effect of hydration, and subsequent annealing on the semicrystalline 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 *et al.* (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

amylomaize is far more complex than for waxy or normal starches. In amylomaize starch, there is evidence from ^{13}C CP/MAS NMR that amylose also forms some double helices and that, upon annealing, there is a partitioning of amylopectin and amylose helical structures (Shi *et al.*, 1998; Tester *et al.*, 1999, 2000). Recently, Gomes *et al.* (2004) confirmed this observation and postulated that the increase in helical order is not induced by the formation of V-amylose-lipid complexes but by interactions between AM-AMP, AM-AM and/or AMP-AMP chains.

Annealing of wheat (Gough & Pybus, 1971; Jacobs *et al.*, 1998a), pea (Jacobs *et al.*, 1998a), and potato (Stute, 1992; Jacobs *et al.*, 1998a; Genkina *et al.*, 2004b) starches have shown no effect on their polymorphic pattern. However, annealing has been shown to decrease the 'B'-polymorphic content of cassava (Gomes *et al.*, 2004) and sweet potato (Genkina *et al.*, 2004a) starches. X-ray peak intensities of rice (Yamamoto & Shirakawa, 1999), wheat, lentil, and oat (Hoover & Vasanthan, 1994a) starches, have been shown to increase on annealing, whereas a decrease was reported for potato starch (Hoover & Vasanthan, 1994a). The degree of crystallinity of potato, wheat, and pea starches has been shown to remain unchanged on annealing (Jacobs *et al.*, 1998c). Hoover and Vasanthan (1994b) reported that annealing had no effect on X-ray *d*-spacing of potato, wheat, lentil and oat starches. However, cassava starch has been shown to exhibit a reduced *d*-spacing during annealing (Gomes *et al.*, 2004).

Small angle X-ray scattering (SAXS) quantifies differences (periodicity) at the level of amorphous-crystalline lamellae. SAXS data of wheat and potato starches has shown that annealing does not change the repeat distances of amorphous and crystalline

lamellae (Jacobs *et al.*, 1998c). They further observed a higher electron density contrast between amorphous and crystalline regions. They attributed this to either a higher electron density in the crystalline zone (due to a closer packing of the double helices), or a lower electron density in the amorphous zone. However, they further postulated that annealing may affect the individual lamella sizes, without affecting the overall repeat distance. This is in agreement with more recent studies carried out by Genkina *et al.* (2004a,b), on potato and sweet potato starches grown at different soil temperatures. They observed an increase in crystalline lamellae thickness during annealing, in the above starches, and the extent of the increase was more pronounced for starches grown at low soil temperatures. They further observed a similar crystalline lamellae thickness for starches, which were grown under various soil temperatures, after long term annealing.

Native cereal starch granules contain V-amylose-lipid complexes as shown by NMR (Cheetham & Tao, 1998b; Blennow *et al.*, 2000; Tester & Debon, 2000). The effect of annealing on V-amylose-lipid complexes is, predictably, very unlikely, because the annealing temperature (e.g. 35-50°C) is much lower than the peak transition temperature of these complexes (e.g. 95-115°C). However, Lorenz and Kulp (1984) have observed the development of a 'V'-type pattern (attributed to crystalline amylose-lipid complexes) after annealing of normal and high amylose barley starches. Evidences of crystalline V-amylose-lipid complexes have not been found after the annealing of other lipid containing starches. For wheat starch, the amount of amylose-lipid complex was found to be unchanged during annealing (Larsson & Eliasson, 1991; Morrison *et al.*, 1993a,b; Jacobs *et al.*, 1998b).

2.2.7.3 Impact of annealing on gelatinization parameters

The effect of annealing on gelatinization characteristics is well established, particularly using DSC, where there tends to be an increase in To, Tp, and Tc, a decrease in the gelatinization temperature range (Tc-To) and either no change or an increase in gelatinization enthalpy (Gough & Pybus, 1971; Krueger *et al.*, 1987a,b; Tester & Morrison, 1990a,b; Stute, 1992; Hoover & Vasanthan, 1994a; Jacobs *et al.*, 1998a; Tester *et al.*, 1998, 2000; Nakazawa & Wang, 2003; Genkina *et al.*, 2004a; Gomes *et al.*, 2004). These changes have been shown to be influenced by heating temperature, time, moisture content, annealing procedure (i.e., single-, double-, or multi-step), growth temperature, and starch composition.

Annealing occurs most rapidly, and to the largest extent, just below the temperature at which gelatinization starts (Lorenz & Kulp, 1984; Krueger *et al.*, 1987b; Knutson, 1990; Larsson & Eliasson, 1991). Nevertheless, the phenomenon has been reported to occur at temperatures down to 25°C with increased holding time (Lorenz & Kulp, 1984). Multi-step annealing allows higher annealing temperatures than can be obtained by one-step annealing (Knutson, 1990; Stute, 1992; Jacobs *et al.*, 1998a). For instance, annealing temperatures were 48°C (first step) and 53°C (second step) for wheat starch, 50°C (first step) and 55°C (second step) for potato starch, and 50°C (first step) and 56°C (second step) for pea starch (Jacobs *et al.*, 1998a).

Increasing annealing time has been shown to increase the To, Tp, Tc and ΔH and decrease (Tc-To) in lentil, oat, wheat (Hoover & Vasanthan, 1994a), maize (Krueger *et*

al., 1987b), potato (Hoover & Vasanthan 1994a; Genkina *et al.*, 2004b), and cassava (Gomes *et al.*, 2004) starches. Krueger *et al.* (1987a) and Larsson and Eliasson (1991) reported that the largest changes in gelatinization temperatures for maize and wheat starches occur during the first 2-6h (at 50°C). Increases in enthalpy as a result of annealing at 50°C were evident after only 48, 6, 2 and 1h for wheat, oat, potato and lentil starches, respectively (Hoover & Vasanthan, 1994a). Genkina *et al.* (2004a) have shown that the increased holding time during annealing of potato starches grown at different soil temperatures results in similar gelatinization temperatures and enthalpy.

With regard to the moisture content during annealing, Krueger *et al.* (1987a) observed a slightly larger and at the same time significant change in gelatinization temperature of maize starch with increasing moisture content up to 67% (w/w), but no effect at higher water contents. Increasing moisture content has not been shown to influence enthalpy. However, Hoover and Vasanthan (1994a) have observed that the magnitude of changes in gelatinization temperatures and enthalpies of wheat, oat, potato and lentil starches increased with increasing moisture content (up to 80%, w/w).

Several researchers (Krueger *et al.*, 1987b; Knutson, 1990; Jacobs & Delcour, 1998) have shown that high-amylose maize starches exhibit pronounced changes in DSC parameters on annealing. When potato starches with varying degrees of phosphorylation were annealed, the highest increase in gelatinization temperature occurred in samples with the lowest degree of phosphorylation. However, the largest increase in gelatinization enthalpy was observed for the highly phosphorylated starches (Muhrbeck & Svensson, 1996). Annealing has been shown to influence the formation of new V-

amylose-lipid complexes in normal maize, normal and high-amylose barley, wheat, and rye starches (Andreev *et al.*, 1999). Genkina *et al.* (2004b) studied the effect of annealing on DSC parameters of sweet potato starches grown at different soil temperatures. They showed that soil temperature influences the changes in DSC parameters on annealing. The changes in gelatinization temperatures (increase) and the gelatinization temperature range (decrease) was observed to be more pronounced for sweet potato starches grown at low soil temperatures. This was attributed to the crystalline structure being poorly ordered in granules of sweet potato starches grown at low soil temperatures.

Different authors have given different explanations for the effect of annealing on gelatinization parameters. Tester *et al.* (1999) suggested that increasing incubation temperature enhanced the order of the amorphous lamellae and subsequently the order of double helices of amylopectin to form more perfect helix aggregation. The helix aggregation caused an increase in the length of double helices without increasing hydrogen bonding. The increased double helix length might contribute to improved ordered structure and crystallinity, which consequently increased the gelatinization temperature of annealed starch. Tester *et al.* (2000) explained their observation of increased enthalpy on the basis of increased double helical content during annealing. However, Hoover and Vasanthan (1994a) suggested that an increase in ΔH was due to the interaction between amylose and amylopectin.

2.2.7.4 Impact of annealing on swelling, and amylose leaching

Annealing has been shown to decrease the swelling power, and AML in wheat, potato, oat, lentil (Hoover & Vasanthan, 1994a), bambarra groundnut (Adebowale & Lawal, 2002), and cassava (Gomes *et al.*, 2004) starches, in the temperature range from 50 to 95°C. The extent of these reductions for wheat, oat, potato and lentil starches have been shown to be influenced by amylose content (Hoover & Vasanthan, 1994a). Increasing annealing time increased the extent of reduction of swelling power and AML of cassava starch (Gomes *et al.*, 2004). However, the multi-step annealing of potato, cassava, and maize starches (Nakazawa & Wang, 2004) decreased AML (temperature range 50-100°C), but did not influence granular swelling (temperature range 60-100°C). They further observed that the extent of decrease in AML was greater with increase in heating temperature.

2.2.7.5 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, multi-step), annealing temperature, type of the acid, and starch source (Hoover & Vasanthan, 1994a; Jacobs *et al.*, 1998a; Nakazawa & Wang, 2003). Annealing (single-step) has been reported to decrease the acid susceptibility of wheat, potato and lentil starches, but increase the acid susceptibility of oat starch (Hoover & Vasanthan, 1994a). Jacobs *et al.* (1998a) showed that acid susceptibility of potato starch decreased (during the second phase of hydrolysis)

on annealing (single- and double-step), but there were no difference during the first phase of hydrolysis. However, no differences were observed between native and annealed wheat and pea starches, throughout the time course of hydrolysis. Tester *et al.* (1998) reported that during the first phase of acid hydrolysis, annealed (single-step) wheat starch was more extensively degraded than its native counterpart, while uring the second phase, there was no difference in the extent of hydrolysis for native and annealed wheat starches. Nakazawa and Wang (2003) showed that multi-step annealing of wheat, tapioca, potato, mung bean, normal maize, waxy maize and amylo-maize starches increased the acid susceptibility during both phases of acid hydrolysis, with potato starch showing the greatest and high amylose starches showing the least changes.

Hoover and Vasanthan (1994a) postulated that the slight difference in hydrolysis during the first phase, between native and annealed starches is due to the formation of double helices (which do not form into a crystalline array) within the amorphous regions of annealed starches. The greater difference in hydrolysis between native and annealed starches during the second phase was attributed to the increase in crystalline order that occurs on annealing. Jacobs *et al.* (1998a) studied the acid hydrolyzed residues of native and annealed starches, by DSC and high-performance anion exchange chromatography (HPAEC). The DSC studies suggested that the amorphous regions of the granule influence the structural changes that occur during annealing, whereas the results obtained by HPAEC suggested that the branch points of amylopectin become more resistant to acid hydrolysis as a result of annealing (by perfection of crystalline structure, some branch points may become more embedded in this structure and as a result, less

susceptible to hydronium ions). The explanation put forward by Tester *et al.* (1998) for the enhanced hydrolysis of amorphous regions after annealing was that the amorphous regions become more concentrated due to the enhanced glassy structure (enhanced order and decrease in free volume). On the other hand, the small difference or decrease in hydrolysis patterns during the crystalline hydrolysis phase (>7 days) was attributed to enhanced registration of double helices (improved perfection).

The results obtained by Nakazawa and Wang (2003) for potato starch was attributed to its 'B'-type polymorphic structure (which is loosely organized), and high phosphorous content. The differences in hydrolysis exhibited by the maize starches were attributed to the variations in amylose content. Based on their observations, the above authors postulated that: 1) a starch with a more open loose structure would rearrange to a greater degree and create void areas that were more susceptible to acid hydrolysis after annealing, 2) reorganization of starch molecules occur mainly within the crystalline lamellae during annealing (consequently, void spaces are created that allows penetration of H_3O^+ into the granule interior), and 3) branch linkages at the imperfect double helices become more perfected by the improved crystalline structure that occurs on annealing.

Chapter 3. Materials and Methods

3.1 Materials

Hull-less barley cultivars (CDC Fibar, HB 364, CDC McGwire, SR 93102, SB 94897, SB 94893), grown and harvested in Saskatoon in 2000, were obtained from the Crop Development Center, University of Saskatchewan, Saskatoon, Canada. The barley grains were ground in a UDY cyclone sample mill equipped with a 0.5 mm screen. Isoamylase (EC 3.2.1.68) and maltoheptaose (DP 7, internal standard) were obtained from Sigma Chemical Co (St. Louis, MO, USA), Sepharose C18 cartridges from Waters Corp (Milford MA, USA), Macro-sep centrifuge concentrators (30 K) from Filtron Tech Corp (Northborough, MA, USA) and Sephadex G-10 (desalting columns) from Amersham Pharmacia Biotec AB, (Uppsala, Sweden). All chemicals and solvents were of ACS certified grade.

3.2 Methods

3.2.1 Starch isolation

The method of starch isolation from the ground hull-less barley grains was based on that described by Wu *et al.* (1979) with some modifications. Ground barley flour was blended with 0.06M NaOH (1:20 w/v) in a Waring blender for 3min at low speed. The slurry was stirred at room temperature (20°C) for 6h and then centrifuged at 7,500xg for 15min. The residue was blended with 0.06M NaOH (1:20 w/v) and stirred for 12h. The

slurry was passed through two layers of cheesecloth (additional 0.06M NaOH was used during the filtration). The filtrate was centrifuged at 7,500xg for 15min. The starch residue was washed twice with 0.06M NaOH, centrifuged at 7,500xg for 15min, neutralized with 1M HCl, and recovered by centrifugation at 7,500xg for 15min. The brown layer on the top of the starch layer in the centrifuge bottle was scraped with a spatula and recovered by washing and gravity settling and added back to the main stock starch. The crude starch was then washed four times with distilled water. Finally, the starch was air dried and screened through a No. 60 mesh sieve (W.S. Tyler, USA).

3.2.2 Granule morphology

Granule morphology of native and annealed starches was studied by SEM. Starch samples were mounted on circular aluminum stubs with double sticky tape and then coated with 20nm of gold and examined and photographed in a Hitachi (S570, Nissei Sangyo Inc., Rexdale, ON, Canada) scanning electron microscope at an accelerating potential of 5kV.

3.2.3 Chemical composition

3.2.3.1 Moisture content

Quantitative estimation of moisture was performed according to standard AACC (American Association of Cereal Chemists, 2000) procedures. Pre-weighed (3-4 g) starch samples were dried in a forced air oven (Fisher Isotemp 615G, Fisher Scientific,

Nepean, ON, Canada) 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

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., USA) and allowed to stand until it became a cotton-like substance and free of carbonaceous matter (~12 h). 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 (AACC, 2000).

3.2.3.3 Nitrogen content

The nitrogen content was determined according to the micro-Kjeldahl method. Samples (0.3g, db) were weighed on nitrogen free paper and placed in the digestion tubes of a Buchi 430 (Buchi Laboratorimus-Technik AG, Flawill/Schweiz, Switzerland) digester. 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 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 (AACC, 2000). Percentage nitrogen was calculated as follows:

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

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 hard glass test tubes (calibrated at the 5mL level) and gently heated with concentrated sulfuric acid (0.3mL) 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 (20 μL, 30% w/v) was added (10 μL at a time) to hit the walls of the tubes just above the acid, and the tubes well shaken. The tubes were then gently boiled for 30s. The solutions were allowed to slowly cool to room temperature, and the volume was made up to 3.6mL with distilled water. For assay, sodium sulfite solution (0.1mL, 33% w/v) was added with stirring followed by 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). As standard curve was prepared using known amounts of NaH_2PO_4.

3.2.3.5 Lipid content

3.2.3.5.1 Surface lipids

Surface lipids were extracted at room temperature (25-27 °C) by mixing starch (5g, db) with 100mL of 2:1 chloroform/methanol under vigorous agitation in a wrist action shaker for 1h. The solution was then filtered (Whatman No. 4 filter paper) into a 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 (Rotavapor – 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 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, 1992). The extracted solvent was evaporated using the rotary evaporator and the remained crude lipid residue was purified using the method of Bligh and Dyer (1959) before quantification.

3.2.3.5.3 Lipid purification (Bligh & Dyer method)

The crude lipid extracts were purified by extraction with chloroform/methanol/water (1:2:0.8, v/v/v) and forming a biphasic system (chloroform/methanol/water, 1:1:0.9, v/v/v) by addition of chloroform and water at room temperature in a separatory funnel. The chloroform layer was then diluted with benzene and brought to dryness on the rotary evaporator followed by drying at 60°C for 1h in a forced air oven. The dried lipid was cooled to room temperature in a desiccator.

3.2.3.6 Amylose content

Apparent and total amylose contents were determined by the method of Chrastil (1987).

3.2.3.6.1 Apparent amylose

1M NaOH (2mL) and distilled water (4mL) were added to the starch (20mg, db) in a screw cap tube. 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 the 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.

3.2.3.6.2 Total amylose

The total amylose content of starch samples 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 overestimation of apparent and total amylose content, amylose content was calculated from a standard curve prepared using mixtures of pure potato amylose and amylopectin (over the range 0-100% amylose) (Appendix A.1).

3.2.4 Starch damage

The starch damage was estimated following the AACC (2000) standard procedure. Starch samples (1g, db) were digested with fungal α-amylase from *Aspergillus oryzae* (0.05g) having a specific activity of 50-200 units/mg in a water bath at 30°C for 15 min. At the end of incubation, the enzyme action was terminated by adding 3mL of 3.68N sulfuric acid and 2 mL of sodium tungstate ($Na_2WO_4.2H_2O$, 12% (w/v)). The mixture was allowed to stand for 2min. and then filtered through a Whatman No. 4 filter paper. The amount of reducing sugars present in the filtrate was determined using the method of Nelson (1944). The percentage starch damage was calculated as follows:

$$\text{Starch Damage (\%)} = \frac{M \times 1.64 \times 100}{W \times 1.05} \%$$

Where; M – mg maltose equivalent in the digest

W – mg of starch (db)

1.05 – molecular weight conversion of starch to maltose

1.64 – the reciprocal of the mean percentage maltose yield from gelatinized starch. This is an empirical factor, which assumes, under the conditions of the experiment, a maximum hydrolysis of 16%.

3.2.4.1 Determination of reducing sugar

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

Materials:

Reagent-A: 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, diluted to one liter and filtered.

Reagent-B: Cupric sulfate.$5H_2O$ (30.0g) was dissolved in 200.0mL of water and two drops of concentrated sulfuric acid were added.

Reagent-C: Ammonium molybdate (25.0g) was dissolved in water (450.0mL) to which concentrated sulfuric acid (21.0mL) was added; sodium arsenate.$7H_2O$ (3.0g) was dissolved separately in 25.0mL 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:

One mL of reagent mixture (freshly prepared by mixing 25 parts of reagent-A with 1 parts of reagent-B) was added to 2mL of sugar solution and heated for 20 min. in a boiling water bath. The tubes were cooled rapidly in cold water, and 1mL of reagent-C

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 to 10mL with distilled water and the absorbance was measured at 540nm. A reagent blank was prepared using water instead of sugar solution. The standard curve (Appendix A.2) was established with maltose (to calculate the maltose equivalents in the digest).

3.2.5 Molecular characterization of amylopectin

Starch (100mg) was dispersed in distilled water (9.0mL) and heated in a boiling water bath for 1hr. After being cooled to room temperature, sodium acetate buffer (1mL, pH 3.5) and isoamylase (1mL, 30,000U) were added to the above starch solution in sequence. The mixture was then incubated in a shaker bath at 40°C for 48hr to complete the debranching reaction. The debranched starch solution was boiled for 5min (for enzyme inactivation), cooled, and then 10mL of the solution was filtered through a Sep-Pak C-18 cartridge at a flow rate of 1mL/min. The filtrate was then placed inside a 30K macrosep concentrator and centrifuged at 5,000xg for 30min. The 30K Macro-sep filtrate (2mL) was loaded onto a Sephadex G10 desalting cdumn (1.8 x 13cm) and a flow rate of 0.7mL/min was maintained as 1mL fractions were collected. Sample fractions (No 10 to 17) were combined and made up to 10 mL. This sample solution was used for matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis following the procedure of Wang *et al.* (1999).

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 10mL screw-capped tubes, distilled water (5mL) was added and 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 rapidly to 20°C, blue dextran (0.5mL, 5mg/mL) (Pharmacia, MW 2 x 10^6,) was added and the contents mixed well. The tubes were then centrifuged at 2,000xg for 5min and the absorbance of the supernatant 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.

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;

$$FW = 5.5\ (A_r/A_s) - 0.5$$

Where A_r and A_s are the absorbance of the reference and sample, respectively.

The initial volume of starch (V_0) of weight W (in mg) is;

$$V_0 = W/1{,}400$$

And the volume of absorbed intragranular water (V_1) is thus;

$$V_1 = 5.0 - FW$$

Hence the volume of the swollen starch granule (V_2) is;

$$V_2 = V_0 + V_1$$

And $SF = V_2 / V_0$

This can also be expressed by the single equation;

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

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

3.2.7 Extent of amylose leaching (AML)

Starches (20mg, db) were heated with 10mL of distilled water at 50-90°C in volume calibrated sealed tubes for 10min. The tubes were then cooled to room temperature and centrifuged at 2,000xg for 10min. Amylose content of the supernatant liquid (1.0mL) was withdrawn and its amylose content determined by the method of Chrastil (1987). Amylose leaching was expressed as percentage of amylose leached per 100g of starch.

3.2.8 Differential scanning calorimetry (DSC)

Gelatinization parameters of native and annealed starches were measured using a Seiko 210 differential scanning calorimeter (Seiko Instruments Inc., Chiba Japan) equipped with a thermal analysis data station and data recording software. Water (11μL) was added with a microsyringe to starch (3.0mg) in the DSC pans, which were then sealed, reweighed and allowed to stand overnight at room temperature before DSC analysis. The scanning temperature range and the heating rates were 25-150°C and

10°C/min, respectively. In all measurements, the 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 to avoid condensation. The transition temperatures reported are the onset (To), peak (Tp) and conclusion (Tc). The enthalpy of gelatinization (ΔH) and the enthalpy of melting of the amylose-lipid complex (ΔH_{CX}) 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 of dry starch (Appendix A.3). Three replicates per sample were analyzed.

3.2.9 X-ray diffraction

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 40mA; scanning range 3-35°; scan speed 1°/min; step time 0.02s; divergence slit width 1°; scatter slit width 1° and receiving slit width 0.6mm. RC 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 percentage RC. The proportion of

the B-polymorphic content of native and annealed starches was calculated by determining the area under the diffraction peak at 5.4° 2θ to the summed up area of all the peaks of the diffractogram. (Davydova *et al.*, 1995). The moisture content of all starch samples for X-ray diffraction was adjusted to ~19% by being kept in a desiccator over saturated $BaCl_2$ solution (25°C, $a_\omega = 0.9$) for 2 weeks (Barron *et al.*, 2000).

3.2.10 Acid hydrolysis

Native and annealed starches were hydrolyzed in triplicate with 2.2 N HCl at 35°C (1g starch / 40mL acid) for periods ranging from 1 to 18 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). The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial starch.

3.2.11 Annealing

Starch samples (15g, db) were weighed into glass containers and the moisture content was brought to 75% by adding the appropriate amount of distilled water. The sealed samples were heated at 50°C for 72h in a thermostatically controlled water bath. Samples were centrifuged (2,000xg) and supernatant was decanted (soluble carbohydrates were not detected in the supernatant). The annealed starches were washed

once with deionized water and air-dried. The resulting starches were referred to as one step annealed starches.

3.2.12 Statistical analysis

All determinations were replicated three times, mean values and standard deviations were reported. Analysis of variance (ANOVA) was performed by Tukey's HSD test ($P < 0.05$) using statistical software SPSS 12.0 for windows (SPSS Inc. Chicago, IL, USA)

Chapter 4. Results and discussion

4.1 Isolation and chemical composition

Chemical composition of hull-less barley starches is given in Table 4.1. Average yield of isolated starches was 46.5% of the total grain weight (dry basis). The low values for nitrogen (0.03 - 0.09%) and ash (0.10 - 0.30%) contents showed that the starches were of high purity. Free lipids (obtained by extraction with chloroform-methanol) ranged from 0.04 to 0.13%. However, variations in bound lipid content (obtained by extraction of chloroform-methanol residues with hot 1-propanol-water) were higher (0.10 - 0.72%). The free and bound lipids were in the range reported for other barley starches (Li *et al.*, 2001a; Suh *et al.*, 2004). The bound lipid content followed the order: normal (CDC McGwire ~ SR 93102) ~ high-amylose (SB 94897 ~ SB 94893) > waxy (CDC Fibar < HB 364). Li *et al.* (2001a) have shown by studies on normal, waxy and high-amylose barley starches, that a strong correlation ($r = 0.92$, $P < 0.01$) exists between the bound lipid content and total amylose content, however, a similar trend was not observed in this study (Table 4.1). The apparent and total amylose content of the starches ranged from 0.00 - 31.04% and 0.00 - 55.33%, respectively (Table 4.1). With the exception of SB 94893 starch, the apparent and total amylose content of the other starches were within the range reported for other barley starches (Vasanthan & Bhatty, 1996; Song & Jane, 2000; Yoshimoto *et al.*, 2000; You & Izydorczyk, 2002). The total

Table 4.1. Chemical composition of hull-less barley starches[1]

Barley Cultivar	Moisture (%)	Ash (%)	Nitrogen (%)	Total Phosphorous (%)	Amylose content (%)[2]		Lipid (%)	
					Apparent	Total	CM[3]	PW[4]
CDC Fibar	13.14±0.09[a]	0.10±0.01[a]	0.08±0.02[a]	0.024±0.001[a]	0.00±0.00[a]	0.00±0.00[a]	0.04±0.01[a]	0.10±0.01[a]
HB 364	13.78±0.09[b]	0.16±0.00[b]	0.03±0.02[b]	0.026±0.002[a]	3.88±0.08[b]	7.80±0.08[b]	0.06±0.01[a]	0.32±0.00[b]
CDC McGwire	10.06±0.09[c]	0.09±0.00[a]	0.03±0.01[b]	0.056±0.003[b]	23.32±0.14[c]	32.30±0.24[c]	0.13±0.03[b]	0.72±0.02[c]
SR 93102	7.82±0.04[d]	0.12±0.02[a]	0.05±0.02[a,b]	0.053±0.004[b]	22.10±0.23[d]	33.57±0.12[d]	0.04±0.02[a]	0.71±0.05[c]
SB 94897	9.41±0.06[e]	0.21±0.01[c]	0.09±0.01[a]	0.058±0.002[b]	27.74±0.01[e]	43.71±0.05[e]	0.04±0.01[a]	0.67±0.04[c]
SB 94893	9.27±0.02[e]	0.30±0.01[d]	0.05±0.02[a,b]	0.060±0.003[b]	31.04±0.14[f]	55.33±0.25[f]	0.05±0.01[a]	0.63±0.04[c]

[1]All data reported on dry basis and represent the mean of three determinations. Means within a column with different superscripts are significantly different ($p<0.05$).

[2]Apparent and total amylose determined by I_2 binding before and after removal of bound lipids, respectively.

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

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

phosphorus content, which represents the sum of the phosphorus content of phospholipids and inorganic phosphate ranged from 0.024 to 0.060%. This was within the range reported by Tester (1997b) and Song and Jane (2000) for starches from other barley cultivars. There was no significant difference in phosphorus content among CDC McGwire, SR 93102, SB 94897 and SB 94893 starches. However, the phosphorus content of the two waxy cultivars (CDC Fibar < HB 364) was significantly lower than that of the other cultivars (Table 4.1). In all barley starches, starch damage was negligible (<0.004%).

4.2 Granule morphology

The hull-less barley starches consisted of a mixture of large (spherical, disc and lenticular shaped) and small (irregularly shaped) granules. Most of these granules were present as clusters (Figures 4.1-4.6). Large pores were present on the surface of spherical and lenticular granules of the waxy starches (CDC Fibar, [Figure 4.1C], HB 364 [Figure 4.2C], whereas small pores were present on normal (CDC McGwire [Figure 4.3C], SR 93102 [Figure 4.4C]) and high-amylose (SB 94897 [Figure 4.5C], SB 94893 [Figure 4.6C]) starches. Presence of surface pores has also been shown in granules of maize, wheat, rye, barley, sorghum, millet and innala starches (Fannon *et al.*, 1993; Li *et al.*, 2001a; Jayakody *et al.*, 2005). Fannon *et al.*, (1993) have shown that pores are normal, real, anatomical features of the native granule structure and are not artifacts produced by the isolation, specimen preparation or observation techniques. The pores are formed by tube like channels (present in the granule matrix) that open to the

Figure 4.1. Scanning electron micrographs of native and annealed CDC Fibar starches.

A) Native x 800

B) Annealed x 800

C) Native x 3,000

D) Annealed x 3,000

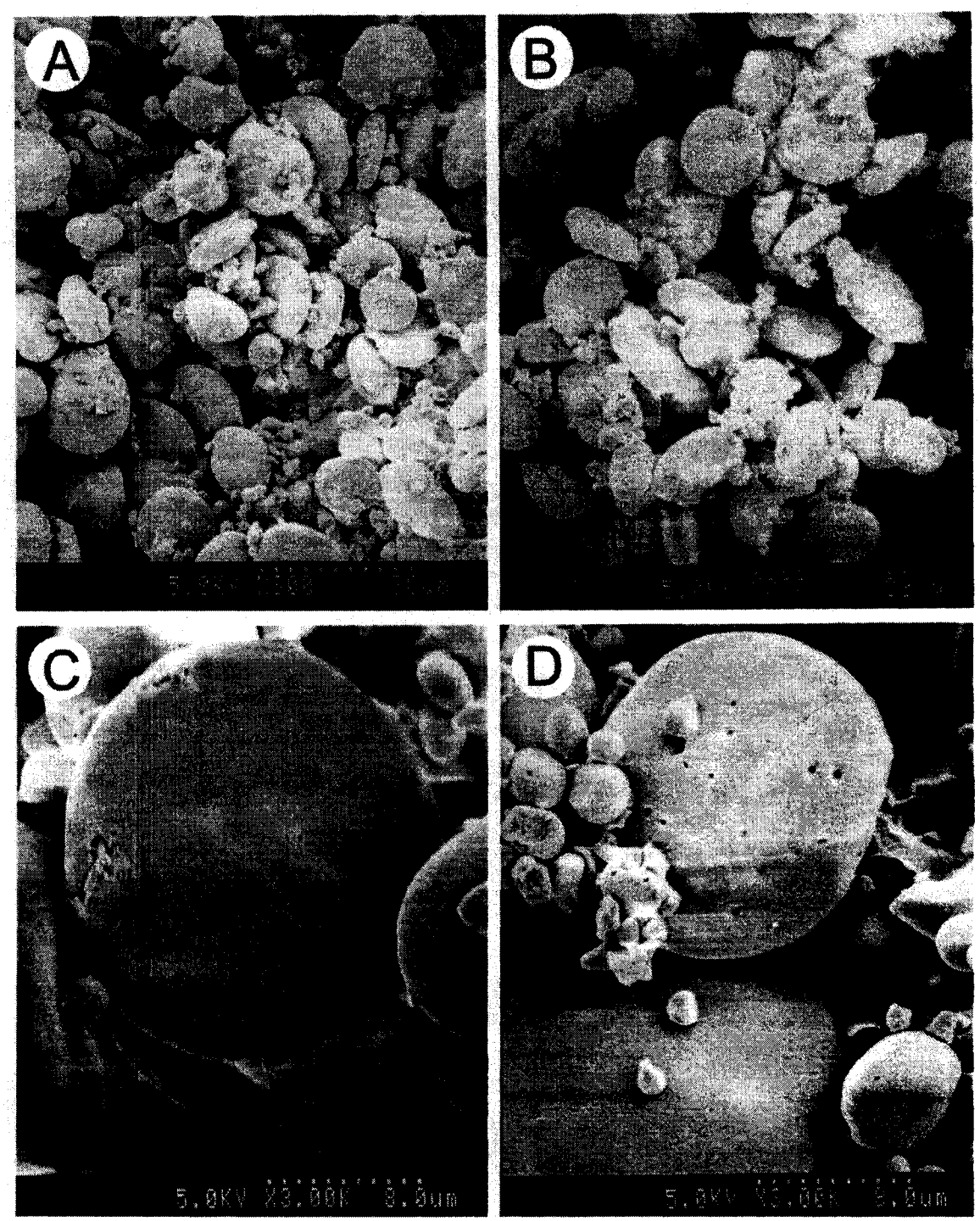

A
B
C
D
5.0KV X3.00K 8.0um
5.0KV X3.00K 8.0um

Figure 4.2. Scanning electron micrographs of native and annealed HB 364 starches.

A) Native x 800

B) Annealed x 800

C) Native x 3,000

D) Annealed x 3,000

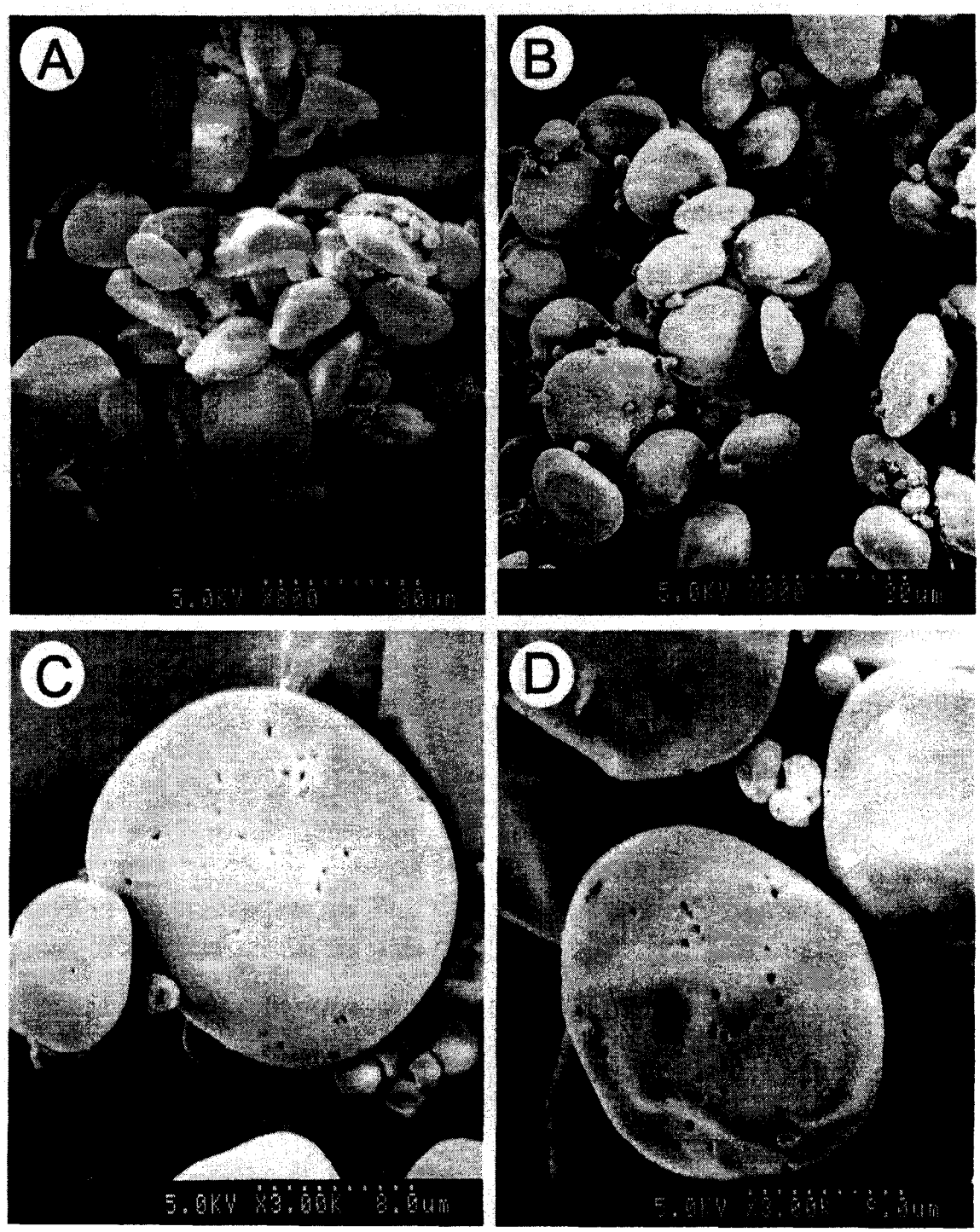

A
B
C
D
5.0kV X3.00K 8.0um

Figure 4.3. Scanning electron micrographs of native and annealed CDC McGwire starches.

A) Native x 800

B) Annealed x 800

C) Native x 3,000

D) Annealed x 3,000

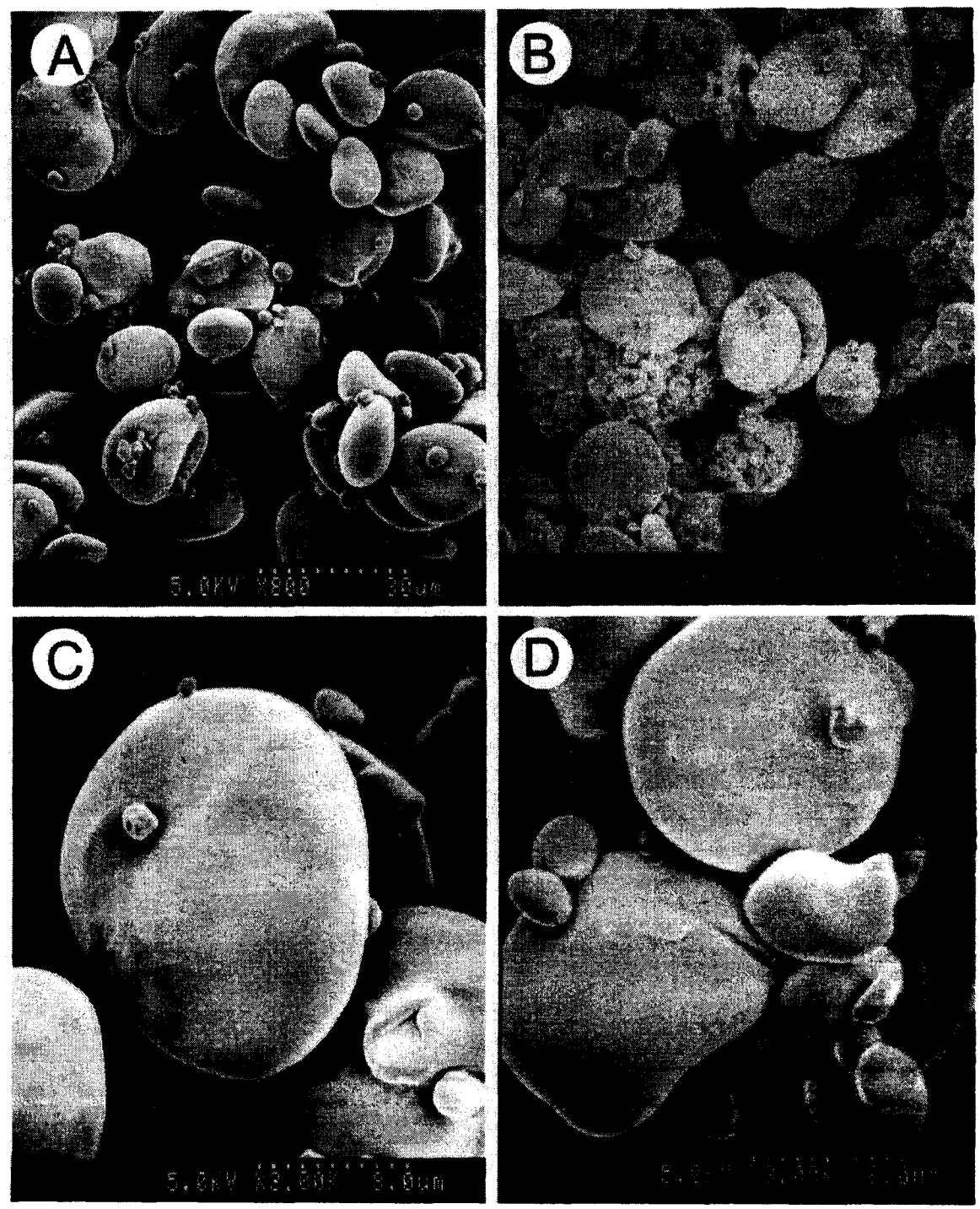

A
B
C
D

Figure 4.3. Scanning electron micrographs of native and annealed CDC McGwire starches.

A) Native x 800

B) Annealed x 800

C) Native x 3,000

D) Annealed x 3,000

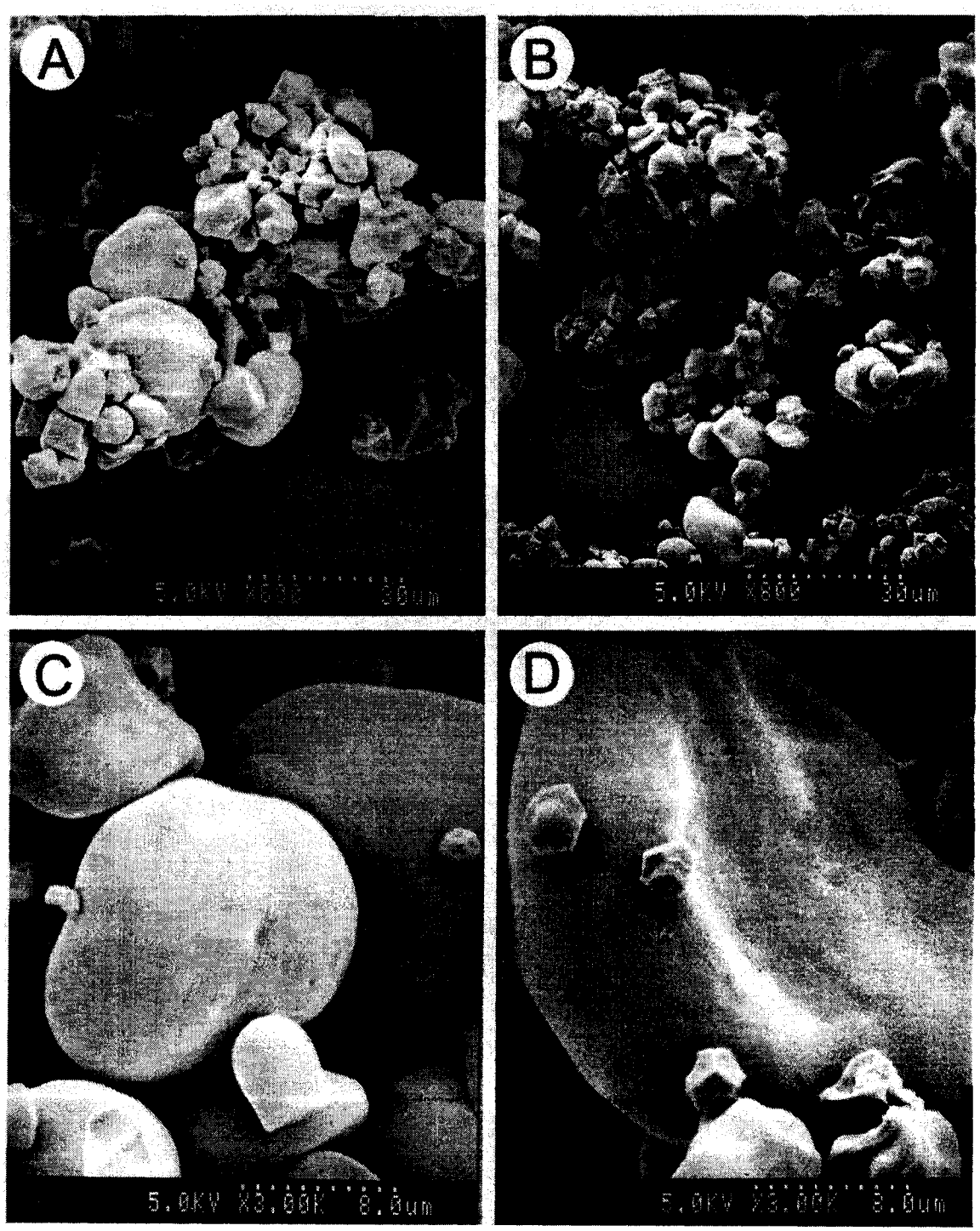

A
B
C
D

Figure 4.4. Scanning electron micrographs of native and annealed SR 93102 starches.

A) Native x 800

B) Annealed x 800

C) Native x 3,000

D) Annealed x 3,000

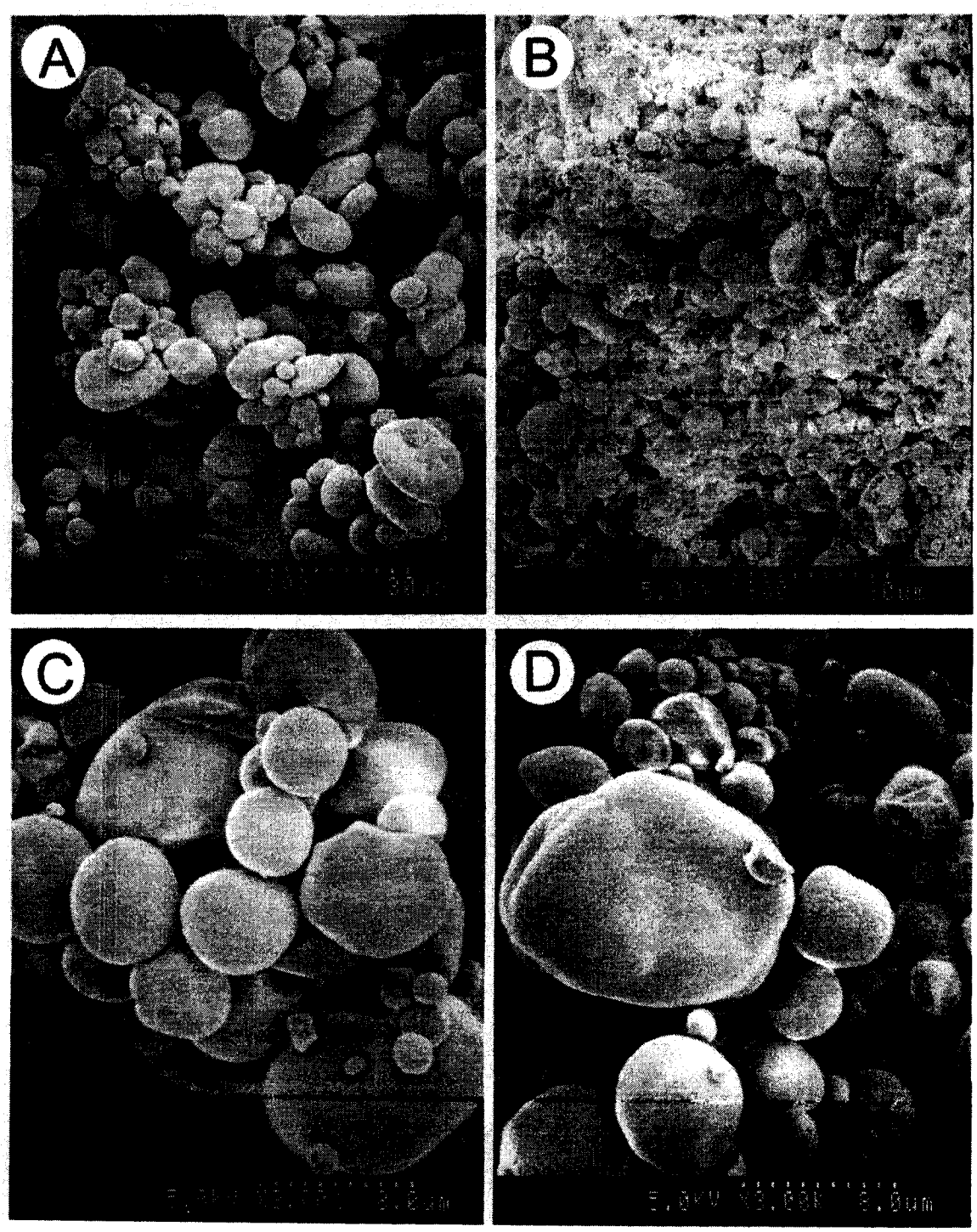

A
B
C
D

Figure 4.5. Scanning electron micrographs of native and annealed SB 94897 starches.

A) Native x 800

B) Annealed x 800

C) Native x 3,000

D) Annealed x 3,000

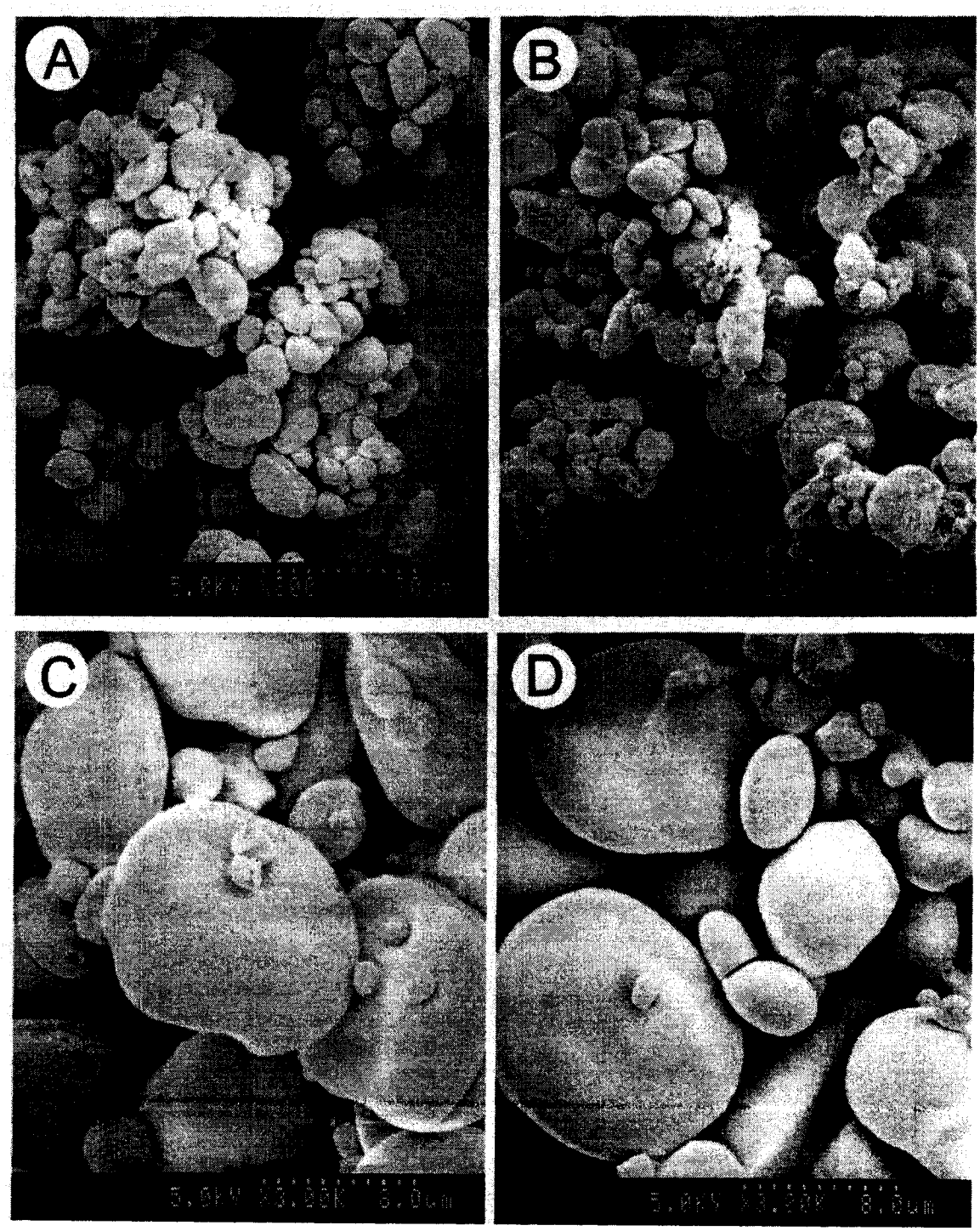

A
B
C
D

Figure 4.6. Scanning electron micrographs of native and annealed SB 94893 starches.

A) Native x 800

B) Annealed x 800

C) Native x 3,000

D) Annealed x 3,000

external surface (Fannon *et al.*, 1993; Fannon & BeMiller, 1992). The pore size in CDC Fibar (Figure 4.1C & D) and HB 364 (Figure 4.2C & D) increased slightly on annealing. However, pore size in granules of the other barley starches remained unchanged on annealing (Figures 4.3-4.6).

4.3 Amylopectin structure

The chain length distribution of debranched amylopectins of the barley starches determined by MALD1-MS, are presented in Table 4.2. Normal and high-amylose starches had nearly similar average chain length (CL), branch points and chain length distribution. The highest peak in the MALDI-MS spectra occurred at DP 12 in SR 93102, SB 94897 and SB 94893 starches, whereas the corresponding DP values for CDC McGwire were at 11.0 and 12.0. There was no significant difference between the two waxy (CDC Fibar, HB 364) starches with respect to peak DP, CL, branch points, and chain length distribution. The waxy starches differed from normal and high-amylose starches in exhibiting the highest peak at DP 5.0, a slightly lower proportion of long DP>35 chains and a slightly higher proportion of DP 5-17 chains. Minor differences in amylopectin structure among other barley cultivars of varying amylose content has also been reported by Tester & Morrison (1992), Czuchajowska *et. al.* (1998), Song and Jane (2000), and Yoshimoto *et al.* (2000).

Table 4.2. Chain length distribution of debranched amylopectin of hull-less barley starches.[a]

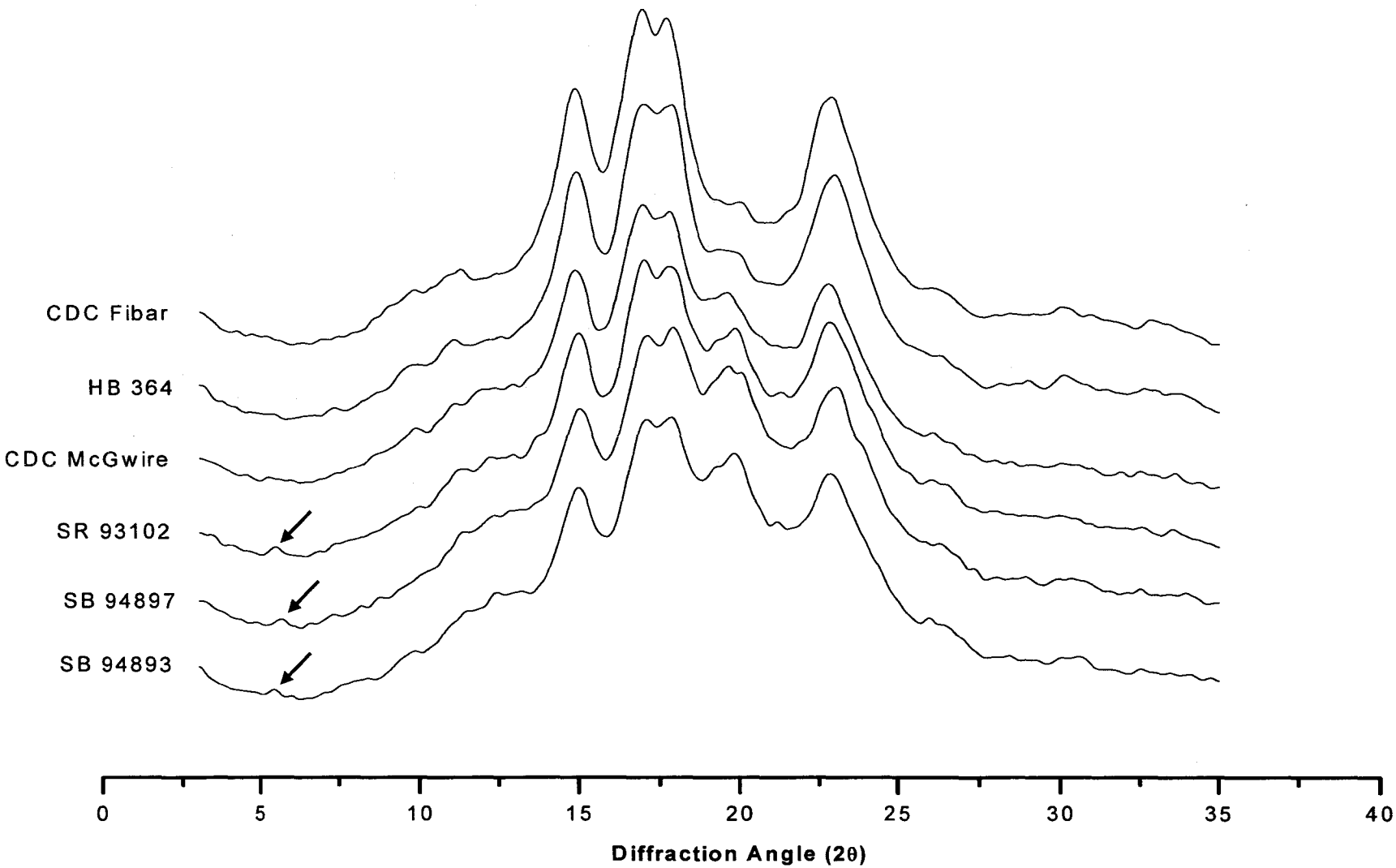
Barley cultivar	Peak DP[b]	CL[c]	BP[d]	Chain length (% distribution)		
				DP 5-17	DP 18-34	DP 35-67
CDC Fibar	5	17.1	5.8	64.3	29.3	6.4
HB 364	5	16.2	6.2	67.5	27.8	4.6
CDC McGwire	11, 12	19.0	5.3	56.6	35.9	7.5
SR 93102	12	20.4	4.9	53.8	34.2	12
SB 94897	12	20.2	5.3	52.6	36.1	10.6
SB 94893	12	19.1	5	56.9	35	8.2
LSD ($p<0.05$)[e]		1.4	0.4	7	5.4	2.1

[a]Determined by MALDI-MS.
[b]Degree of polymerization.
[c]Average chain length.
[d]Branch points.
[e]Least significant difference.

4.4 X-ray diffraction

Native CDC Fibar, HB 364 and CDC McGwire exhibited the typical 'A'-type X-ray patterns of cereal starches (Figure 4.7). The most intense peaks corresponded to Bragg angles (2θ) 15, 17, 18.1 and 23.5°. In addition, a weak peak at 20°2θ representing crystalline V-amylose-lipid complexes (Zobel, 1988) was present in all barley starches (Figure 4.7). The presence of this peak in the waxy cultivar (CDC Fibar), which had a 0% amylose content (Table 4.1), was rather surprising since its bound lipid content was only 0.10% (Table 1). The V-complex in CDC Fibar may have been formed between the outer branches of amylopectin and the native lipids. Vasanthan and Bhatty (1996) also reported a weak peak at 20°2θ for a waxy barley cultivar (SB 89528, 3.2% amylose) which had a bound lipid content of 0.4%. The intensity of the peak at 20°2θ in all native starches increased with increase in amylose content (Figure 4.7). Native SR 93102, SB 94897, and SB 94893 starches exhibited a mixed 'A+B'-type X-ray pattern (Figure 4.7). A small peak centered at 5.6°2θ was indicative of the presence of 'B'-type crystals (Figure 4.7) in the above starches. Suh *et al.* (2004) have also shown the existence of 'B'-type crystals in Franubet barley starch (23.1% amylose). However, many other barley starches have been shown to exhibit only the 'A'- type X-ray pattern (Vasanthan & Bhatty, 1996; Czuchajowska *et al.*, 1998; Song & Jane, 2000; Yoshimoto *et al.*, 2002). In all native barley starches, with an increase in amylose content, the intensities of the major peaks became progressively weaker (Figure 4.7), whereas, the peak centered at 20°2θ (Figure 4.7) became progressively stronger (indicative of an increase in the

Figure 4.7 X-ray diffraction patterns of native barley starches. The arrow points to the peak that represents the 'B' type unit cells.


CDC Fibar
HB 364
CDC McGwire
SR 93102
SB 94897
SB 94893
0
5
10
15
20
25
30
35
40
Diffraction Angle (2θ)

proportion of lipid complexed amylose chains). A similar trend has also been reported for maize starches of varying amylose content (Cheetham & Tao, 1998a). The results suggest that the transition of the X-ray pattern from a pure 'A'-type (CDC Fibar, HB 364, CDC McGwire) to a mixed 'A + B'-type pattern (SR 93102, SB 94897 and SB 94893) occurs at approximately 33.0% amylose. In maize starches, this transition has been shown to occur at 40% amylose (Cheetham & Tao, 1998a). The barley starches (SR 93102, SB 94897, SB 94893) exhibiting the 'A+B'-type X-ray pattern (Figure 4.7) retained many of the peaks characteristic of 'A'-type starches which could be attributed to their low content (Table 4.3) of 'B'-type unit cells (0.07-0.11%). Legume starches have also been shown to exhibit a mixed 'A+B'-type X-ray pattern (Zhou *et al.*, 2004) with 'B'-polymorphic contents in the range of 27.1-37.5%.

The RC of the native barley starches ranged from 37.0 to 44.3% (Table 4.3), while that of CDC McGwire (37.0%) differed significantly from those of the other barley starches (39.8 - 44.3%) and there was no significant difference in RC among HB 364, SR 93102 and SB 94897, and between CDC Fibar and SB 94893 starches. However, the RC of CDC Fibar was significantly different from those of HB 364, CDC McGwire, SR 93102 and SB 94897 starches (Table 4.3). It is difficult to compare the RC of the above six barley starches with those reported for maize starches (RC 17.6 - 21.8%, [Cheetham & Tao, 1998a]) and for starches from other barley cultivars (RC 23.7 – 44.8% [Song & Jane, 2000; Tang *et al.*, 2000; Qi *et al.*, 2004]) due to differences in the moisture content of the analyzed samples. Cheetham and Tao (1998a) have shown that in maize starches,

the RC decreases progressively with increase in amylose content. However in this study, the RC of the native barley starches showed no correlation with amylose content. For instance, there was no significant difference in RC between CDC Fibar (waxy) and SB 94893 (high-amylose) starches and between HB 364 (waxy) and SB 94897 (high-amylose) starches. Granule crystallinity has been shown to be influenced by: 1) the amount of double helices that are organized into a crystalline array, 2) amylose content, 3) crystallite size, and 4) extent of disruption of amylopectin crystallites by amylose (Jenkins, 1994; Jenkins & Donald, 1995; Cheetham & Tao, 1998a; Jayakody & Hoover, 2002). Jenkins (1994) has shown by small angle X-ray scattering studies on normal maize, waxy maize and high-amylose starches, that amylose acts to disrupt the packing of the amylopectin double helices within the crystalline lamellae. Jenkins and Donald (1995) have suggested that the disrupting effect of amylose on amylopectin structure could be due to co-crystallization of a portion of an amylose chain into a hybrid amylose/amylopectin helix within the crystalline lamellae and/or to penetration of amylose into the amorphous regions where the $\alpha(1\rightarrow 6)$ branch points are located. This could very well explain the large decrease in RC with increase in amylose content observed by Cheetham and Tao (1998a) for maize starches of varying amylose content. However, the marginal differences in RC observed among the native, waxy and high-amylose barley starches (Table 4.3) suggest that amylose is not co-crystallized with amylopectin within native granules of high-amylose barley starches. It is likely, that the variations in RC observed among the barley starches probably reflect the interplay of the

following factors: 1) crystallite size, 2) crystallite orientation, and 3) amount of crystalline V-amylose-lipid complexes.

The 'A'- type X-ray pattern of CDC Fibar, HB 364 and CDC McGwire remained unchanged on annealing (Figure 4.8a-c). Similar findings have been reported for wheat, potato, pea, oat, and lentil starches (Gough & Pybus, 1971; Stute, 1992; Hoover & Vasanthan, 1994a; Jacobs & Delcour, 1998). However, the 'A+B'- type X-ray pattern of native SR 93102, SB 94897 and SB 94893 starches resembled more closely the 'A'- type X-ray pattern on annealing (Figure 4.8d-f). Changes to the polymorphic structure ('A + B' $\rightarrow$ 'A') on annealing have also been shown to occur during annealing of sweet potato starch (Genkina *et al.*, 2004b). The X-ray intensity of all barley starches increased slightly on annealing (Figure 4.8a-f) with the most pronounced increase shown by the V-amylose-lipid complex peak, centered at 20°2θ. It is highly unlikely that this is due to complexing of unbound lipids with amylose helices for the following reasons: 1) only trace amounts of unbound lipids are present in the granules of all native starches (Table 4.1), and 2) the enthalpy of melting of the V-amylose-lipid complex (ΔH_{CX}) remains unchanged on annealing in CDC McGwire, SR 93102 and SB 94897 starches (Table 4.4). We postulate, that the increase in intensity at 20°2θ on annealing, may have been due to enhanced ordering of lipid molecules that were present as V-amylose-lipid complexes within granules of the native starches. The RC of the high-amylose (SB 94897, SB 94893) starches remained unchanged on annealing (Table 4.3). However, RC increased in normal (CDC McGwire, SR 93102) and waxy starches (CDC Fibar, HB 364). The extent of this increase followed the order: CDC McGwire > SR 93102 > CDC Fibar >

Figure 4.8. X-ray diffraction patterns of native and annealed barley starches. The arrow points to the peak that represents the 'B' type unit cells.

A) Native and annealed CDC Fibar

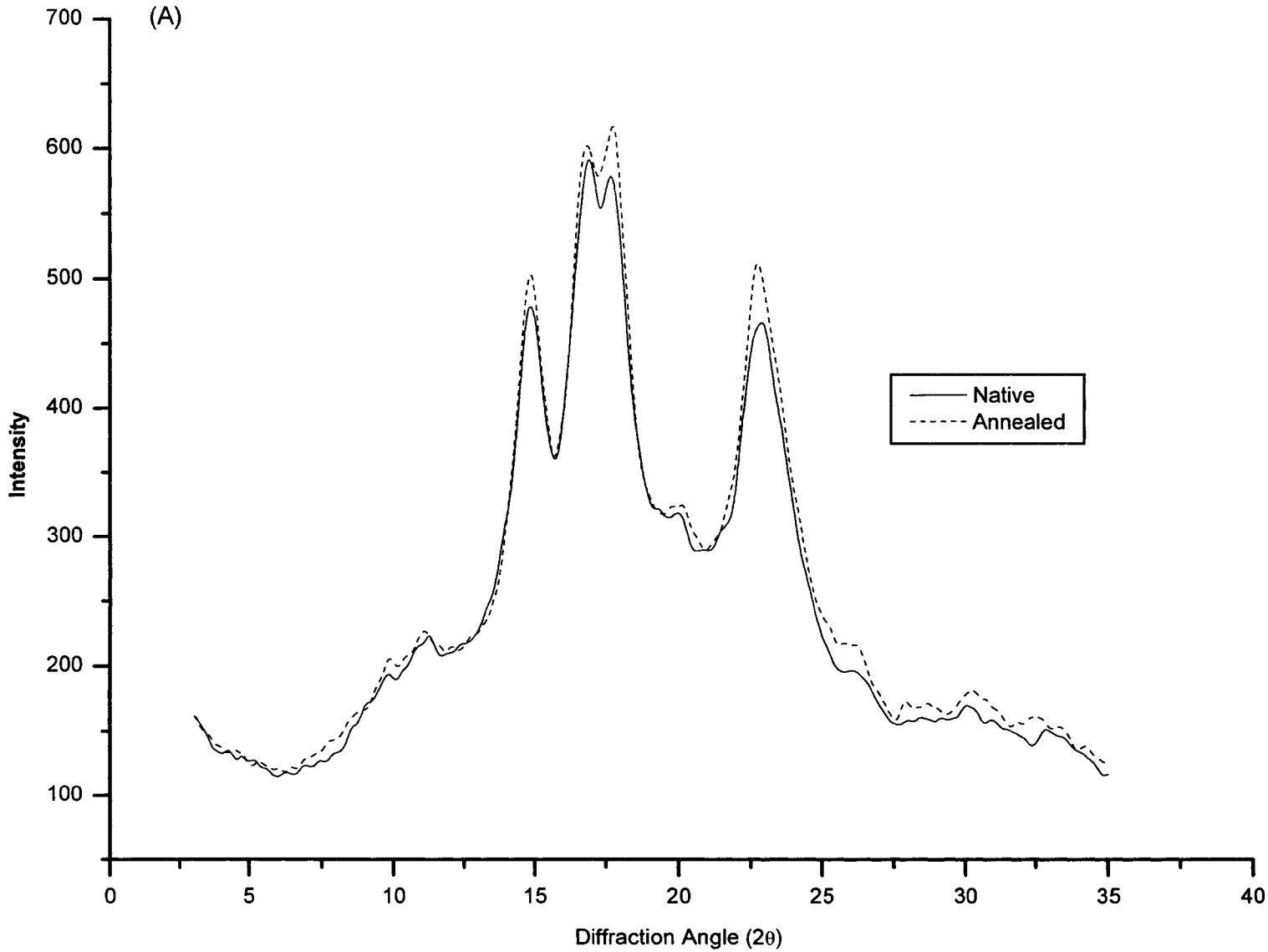
B) Native and annealed HB 364

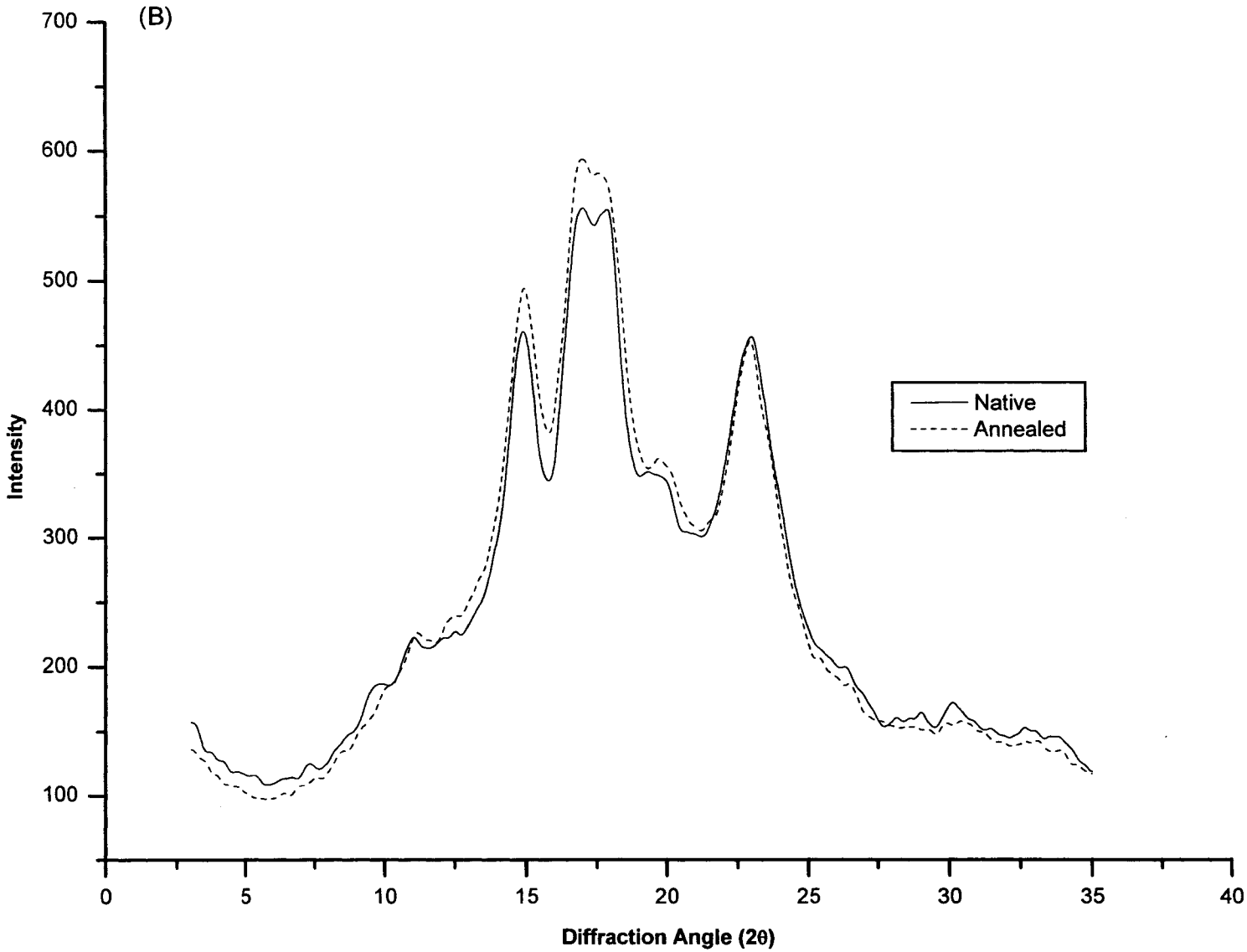
C) Native and annealed CDC McGwire

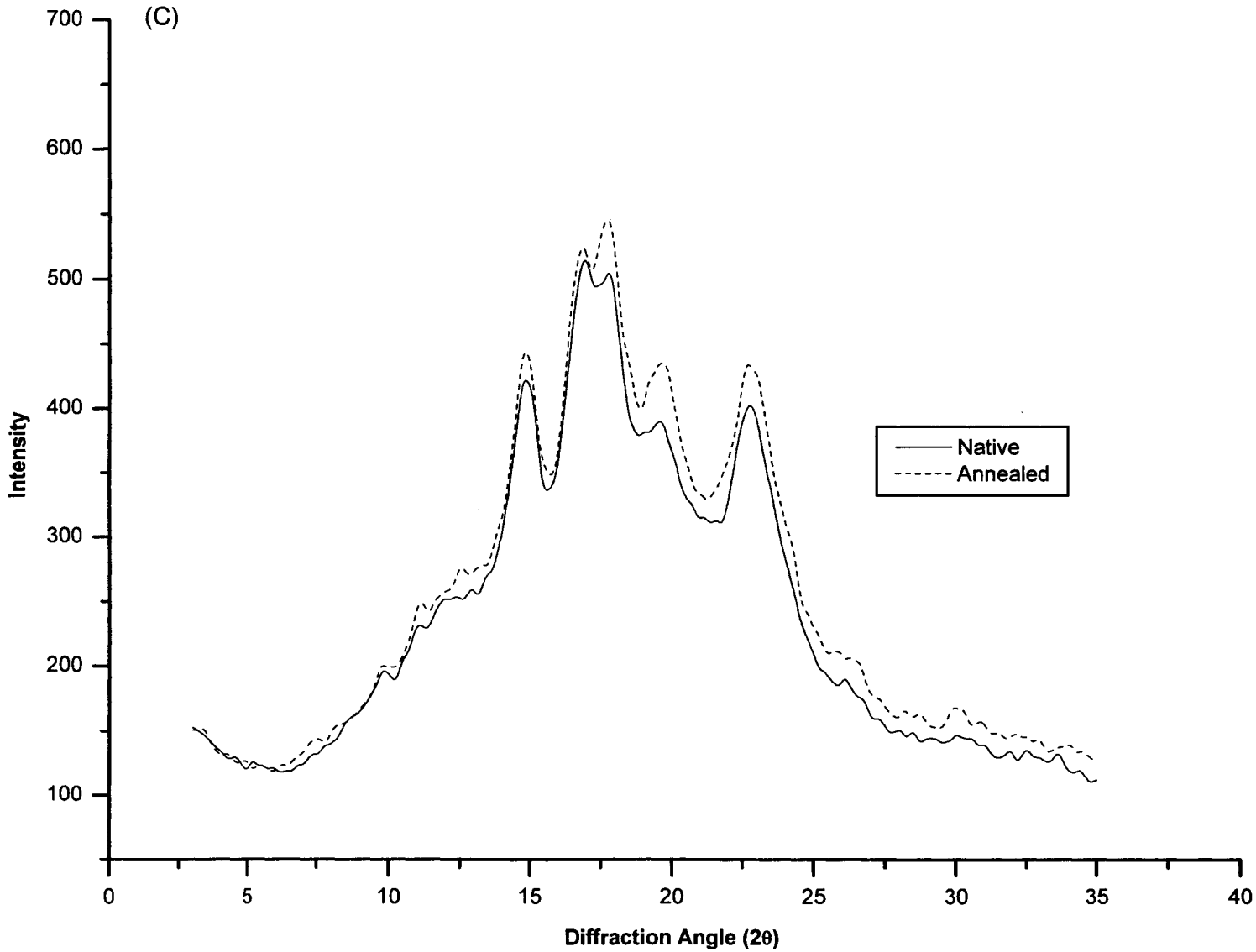
D) Native and annealed SR 93102

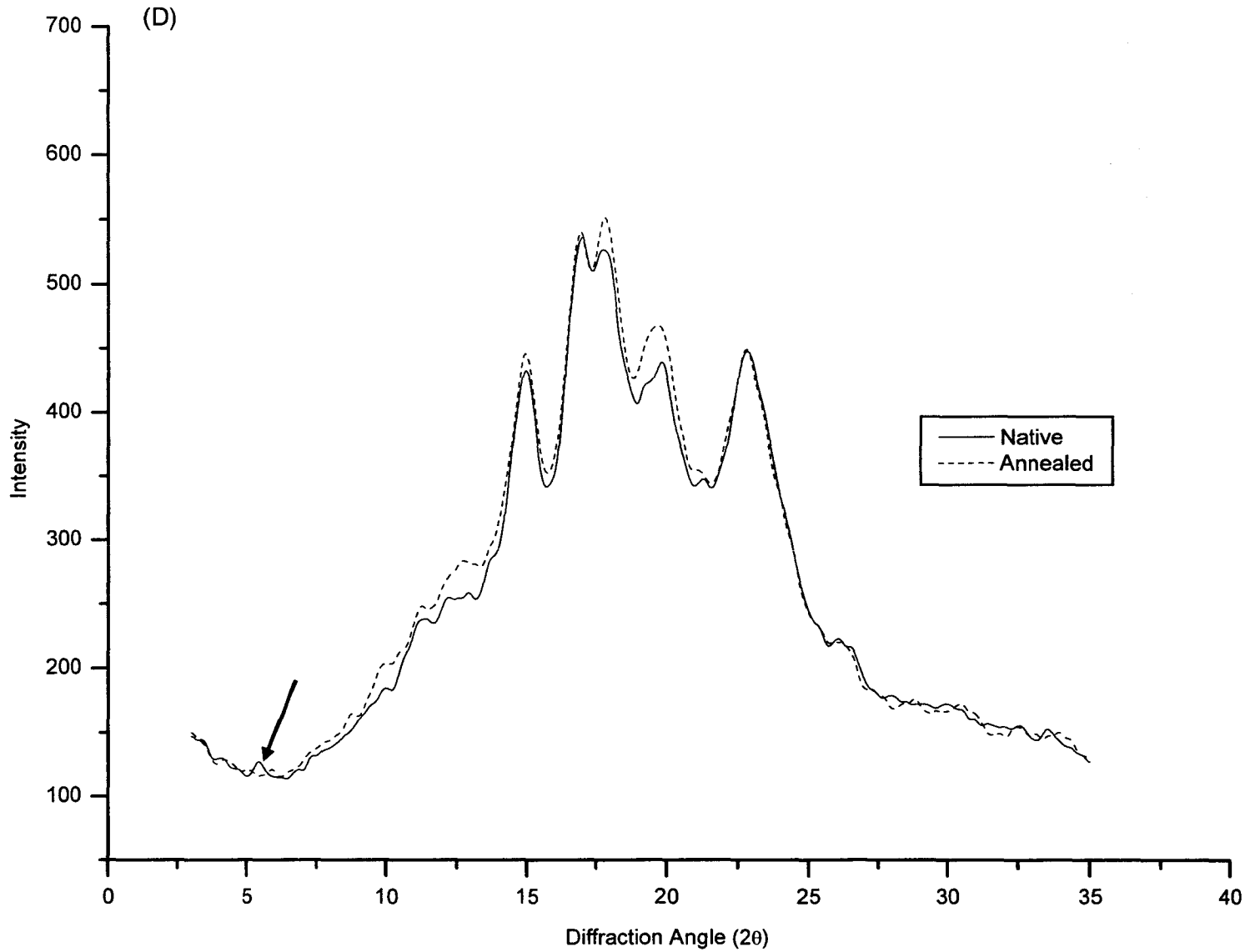
E) Native and annealed SB 94897

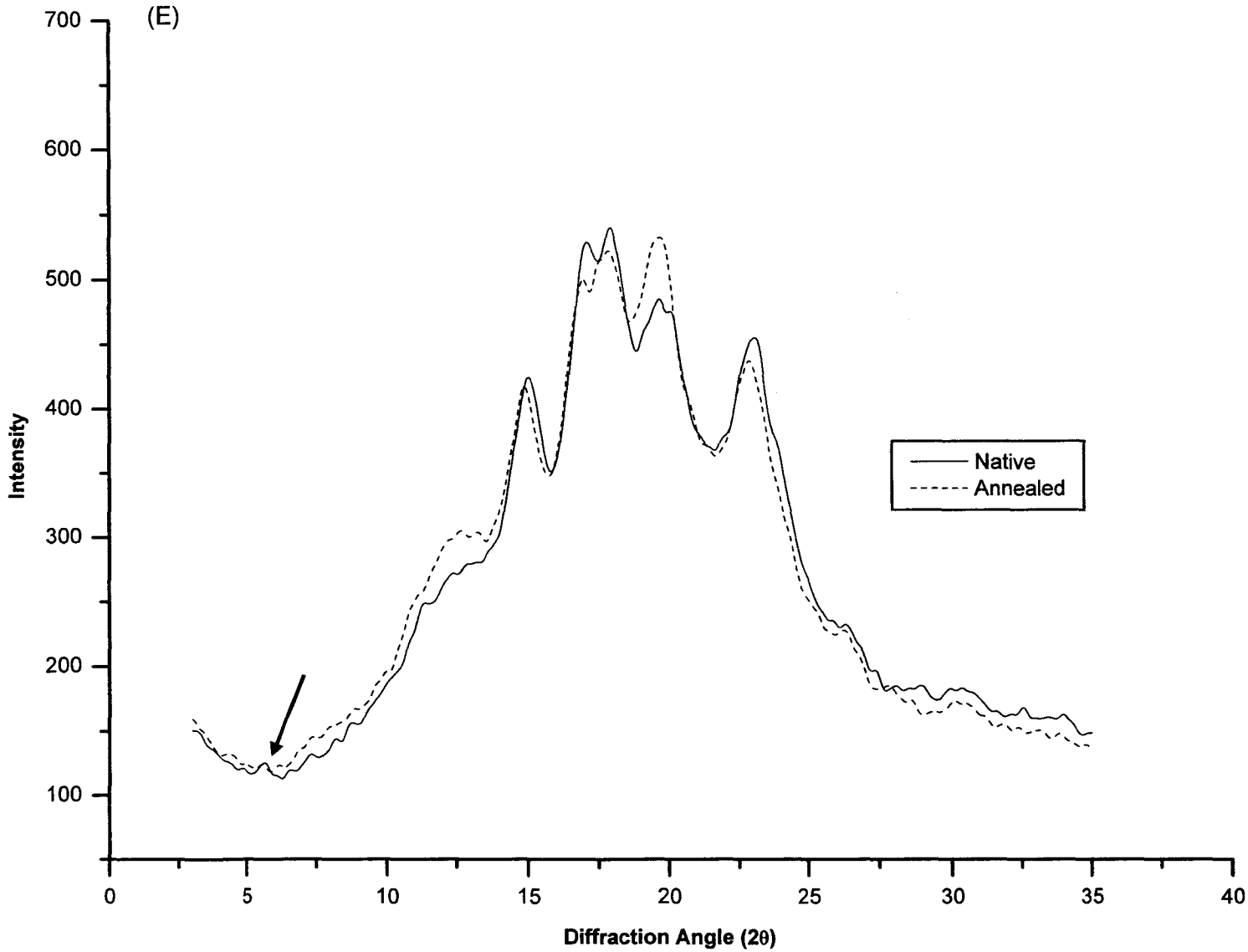
F) Native and annealed SB 94893

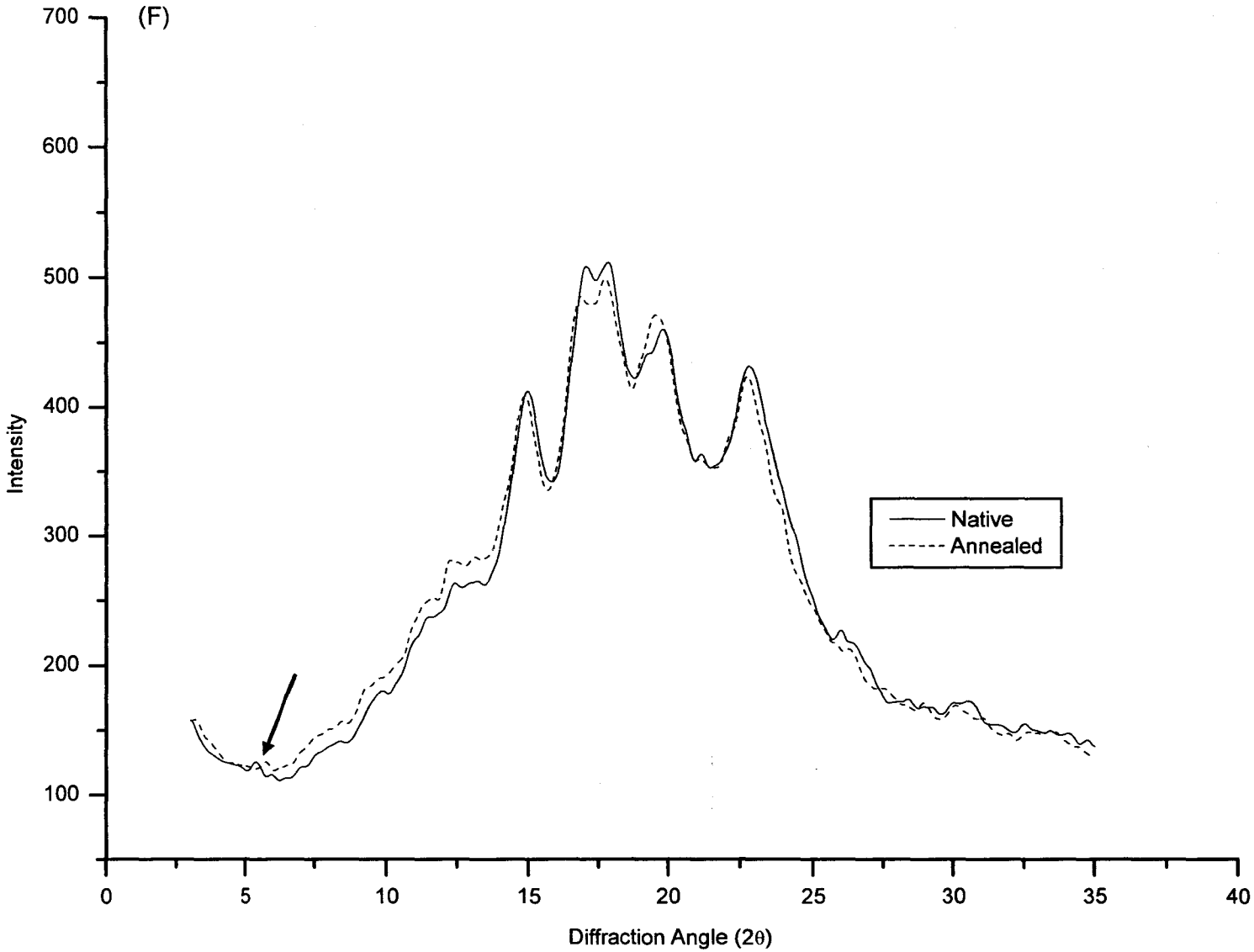

(A)
700
600
500
400
300
200
100
Intensity
0
5
10
15
20
25
30
35
40
Diffraction Angle (2θ)
Native
Annealed


(B)
700
600
500
400
300
200
100
Intensity
0
5
10
15
20
25
30
35
40
Diffraction Angle (2θ)
Native
Annealed


(C)
700
600
500
400
300
200
100
Intensity
0
5
10
15
20
25
30
35
40
Diffraction Angle (2θ)
Native
Annealed


(D)
700
600
500
400
300
200
100
Intensity
0
5
10
15
20
25
30
35
40
Diffraction Angle (2θ)
Native
Annealed


(E)
700
600
500
400
300
200
100
Intensity
0
5
10
15
20
25
30
35
40
Diffraction Angle (2θ)
Native
Annealed

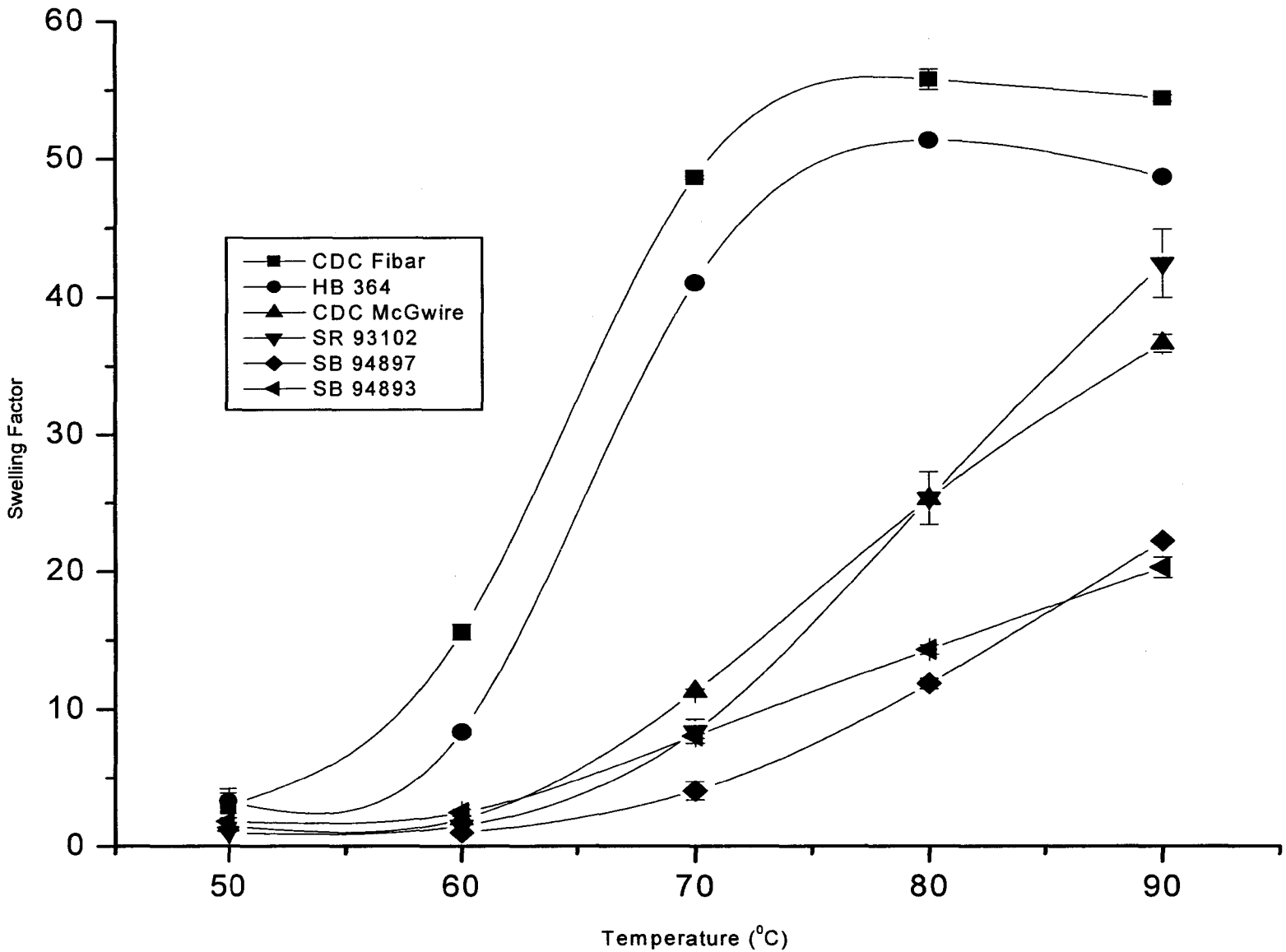

(F)
700
600
500
400
300
200
100
Intensity
0
5
10
15
20
25
30
35
40
Diffraction Angle (2θ)
Native
Annealed

HB 364. The increase in RC on annealing may have been influenced by the interplay of the following factors: 1) amylopectin content, 2) changes in orientation of the starch crystallites, 3) crystallite perfection, and 4) enhanced ordering of the crystalline V-amylose-lipid complex. It is likely, that changes to RC on annealing are influenced to a large extent by amylopectin content, since the RC of the high-amylose starches (SB 94897, SB 94893) remained unchanged on annealing (Table 4.3).

4.5 Swelling factor (SF)

The SF of native barley starches heated in the temperature range of 50-90°C are presented in Figure 4.9. The SF (50-90°C) of the native starches followed the order: CDC Fibar (0% amylose) > HB 364 (7.80% amylose) > CDC McGwire (32.30% amylose) ~ SR 93102 (33.57% amylose) > SB 94897 (43.71% amylose) ~ SB 94893 (55.33%) amylose. The decrease in SF with increase in amylose content has also been reported for maize starches (waxy > normal > amylomaize V > amylomaize VII) (Jayakody & Hoover, 2002; Tester *et al.*, 2000). The SF of both native waxy barley starches (CDC Fibar, HB 364) increased rapidly in the temperature range 60-70°C. Thereafter, the increase was gradual and at 90°C, SF decreased slightly. However, in the native normal barley starches (CDC McGwire, SR 93102), the SF increased rapidly in the temperature range 60-90°C, whereas, in the native high-amylose barley starches (SB 94897, SB 94893), the increase in SF in the temperature range 60-90°C was gradual. SF

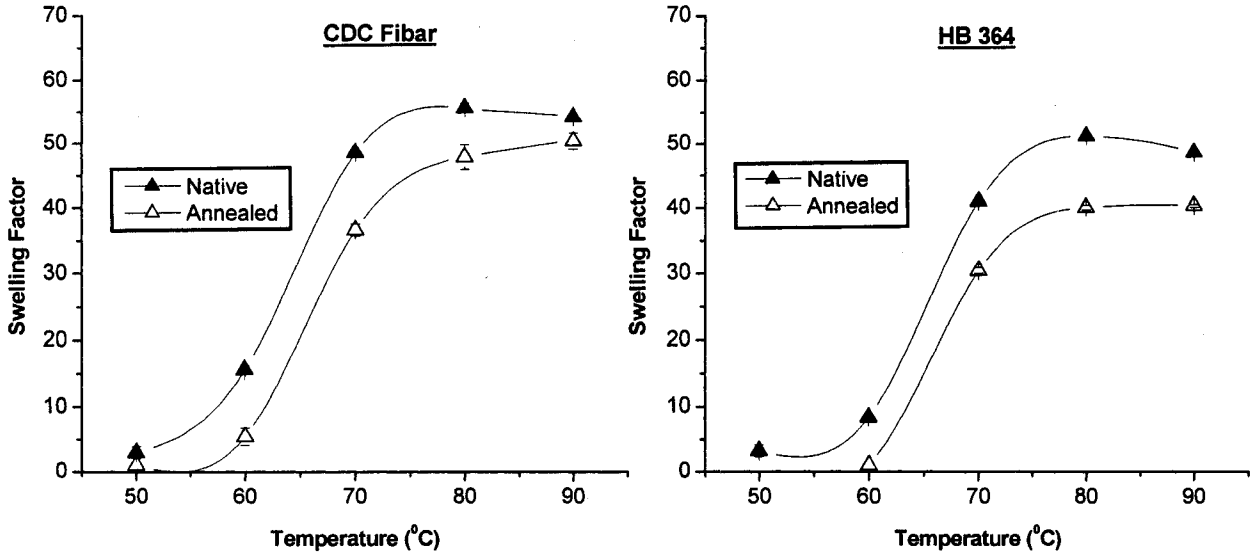
Figure 4.9 Swelling factor of native barley starches in the temperature range 50-90°C


60
50
40
30
20
10
0
Swelling Factor
CDC Fibar
HB 364
CDC McGwire
SR 93102
SB 94897
SB 94893
50
60
70
80
90
Temperature (°C)

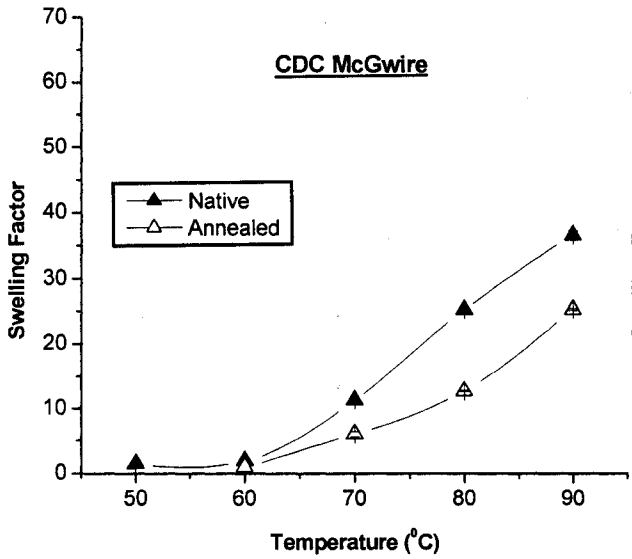
has been shown to be influenced by: 1) V-amylose-lipid complexes (Maningat & Juliano, 1980; Tester & Morrison, 1990a; Tester *et al.*, 1993), 2) amylose content (Morrison *et al.*, 1993b; Sasaki & Matsuki, 1998; Tester *et al.*, 2000), 3) amylopectin structure (Sasaki & Matsuki, 1998; Shi & Seib, 1992; Tester *et al.*, 1993), and 4) extent of interaction between starch chains in the native granule (Hoover & Manuel, 1996; Tester *et al.*, 2000). In this study, the SF of native barley starches is probably influenced by the interplay of factors 1, 2, and 4, since the differences in amylopectin structure among the barley starches was only marginal (Table 4.2).

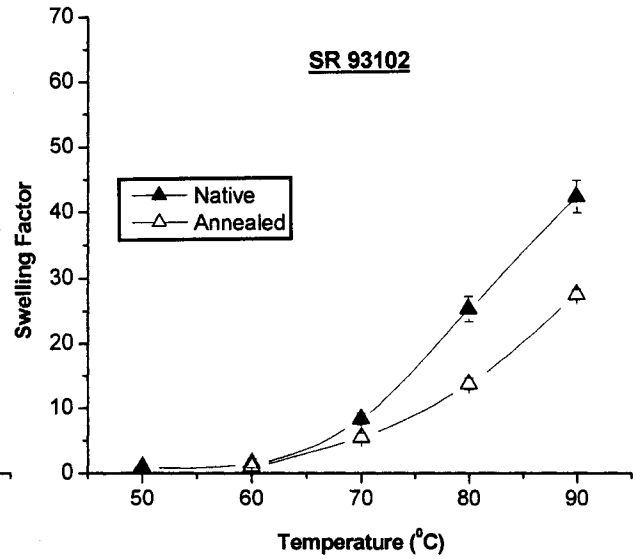
In all barley starches, the SF of annealed starches was lower than their native counterparts (Figure 4.10). The extent of this reduction (at all temperatures) followed the order: normal > waxy > high-amylose. The reduction in SF in the waxy starches on annealing is mainly due to perfection of starch crystallites (crystallite perfection will reduce the extent of hydration of the amorphous regions). Whereas, in the normal and high-amylose barley starches, the interplay of crystallite perfection and amylose-amylose interactions on annealing may have been responsible for the reduction in SF. The extent of this reduction is of a lower order of magnitude in the high-amylose barley starches due to their lower amylopectin content (Table 4.1). Decreased granular swelling on annealing has also been observed in wheat (Lorenz & Kulp, 1978; Hoover & Vasanthan, 1994a,b), potato (Kuge & Kitamura, 1985; Hoover & Vasanthan 1994 a,b), and oat and lentil (Hoover & Vasanthan, 1994a,b) starches. Surprisingly, there are no reports on the effect of annealing on the SF of maize starches of varying amylose content.

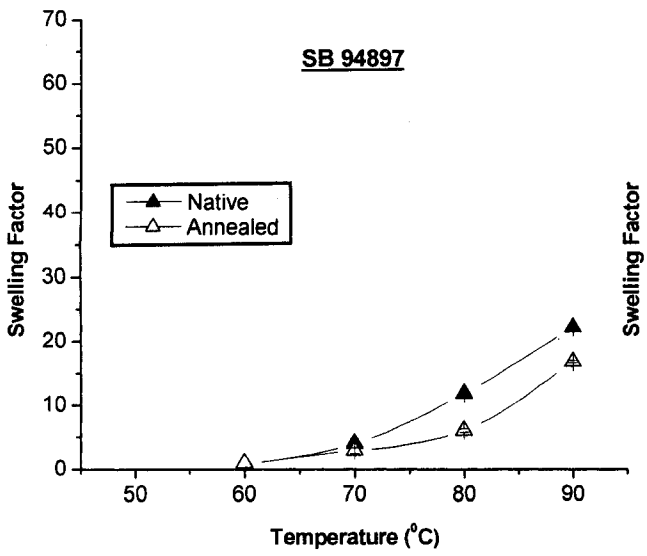
Figure 4.10 Swelling factor of native and annealed barley starches in the temperature range 50-90°C.

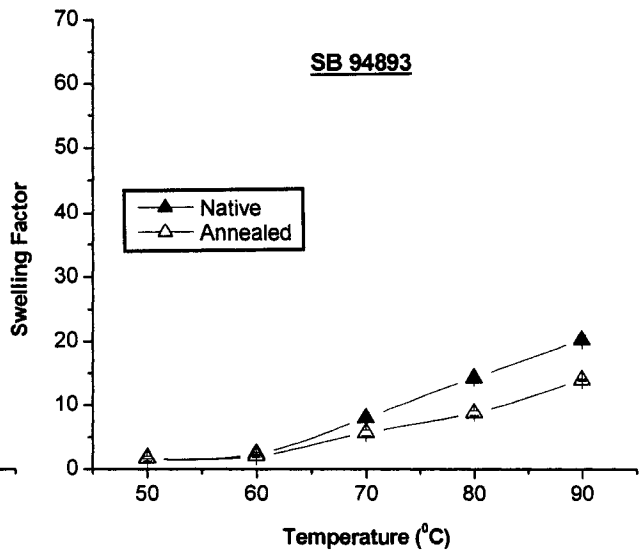

CDC Fibar
Native
Annealed
Swelling Factor
Temperature (°C)
0
10
20
30
40
50
60
70
50
60
70
80
90

HB 364
Native
Annealed
Swelling Factor
Temperature (°C)
0
10
20
30
40
50
60
70
50
60
70
80
90


CDC McGwire
Native
Annealed
Swelling Factor
Temperature (°C)
0
10
20
30
40
50
60
70
50
60
70
80
90


SR 93102
Native
Annealed
Swelling Factor
Temperature (°C)
0
10
20
30
40
50
60
70
50
60
70
80
90

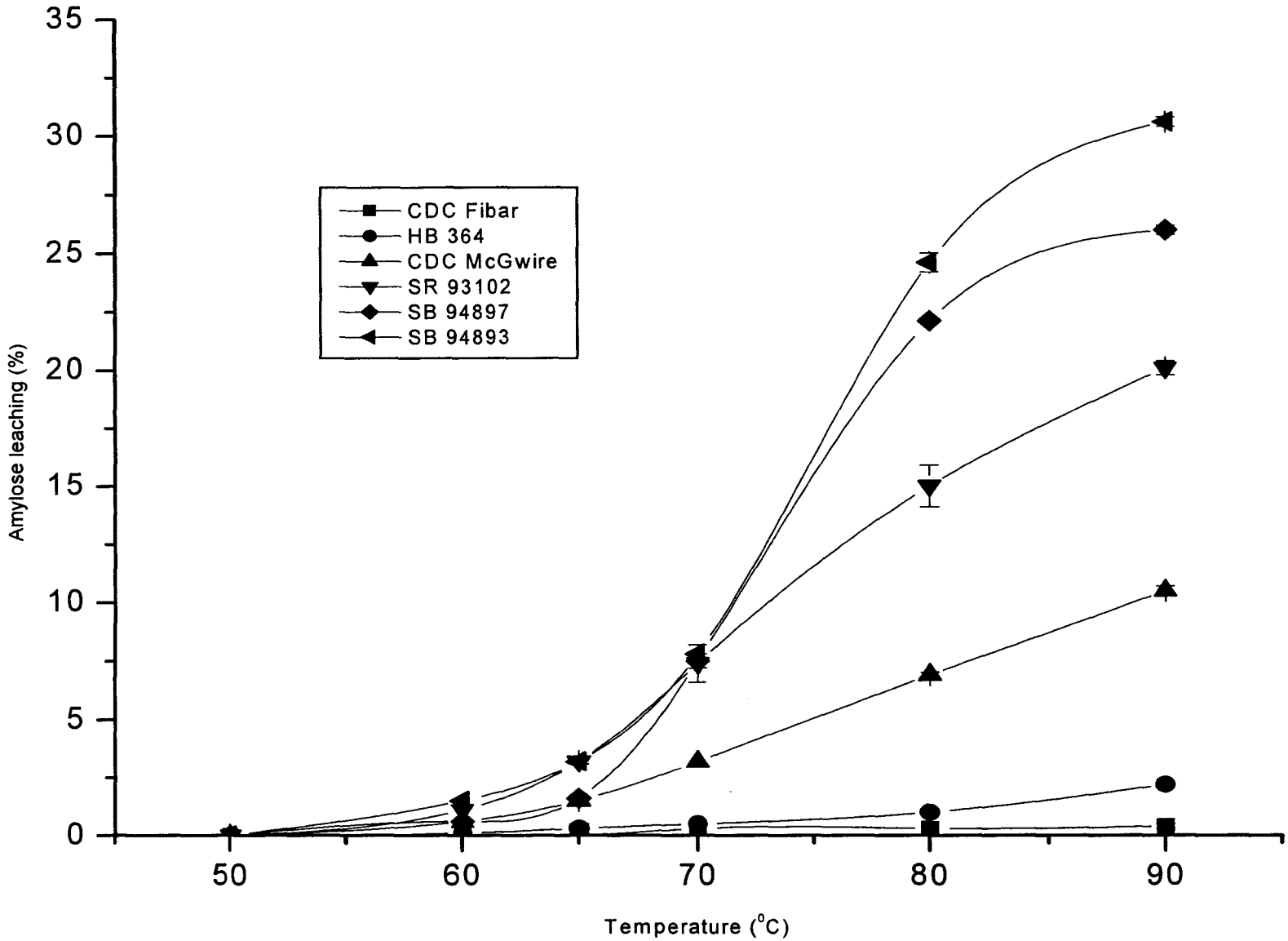

SB 94897
Native
Annealed
Swelling Factor
Temperature (°C)
0
10
20
30
40
50
60
70
50
60
70
80
90


SB 94893
Native
Annealed
Swelling Factor
Temperature (°C)
0
10
20
30
40
50
60
70
50
60
70
80
90

4.6 Amylose leaching (AML)

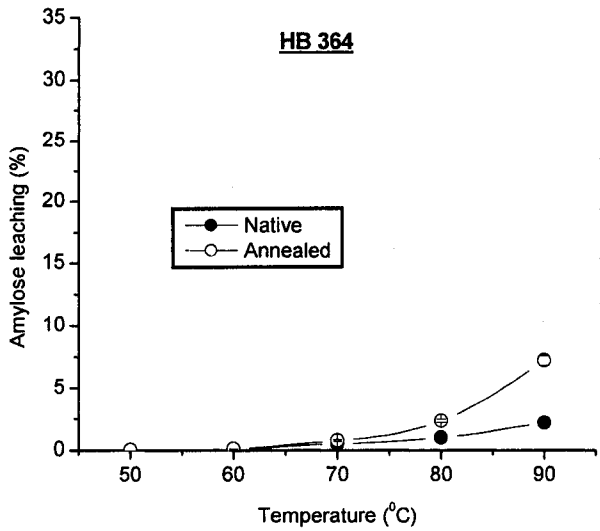
The extent of AML in native starches in the temperature range of 50-90°C is presented in Figure 4.11 and shows that AML increased with rise in temperature (SB 94893 > SB 94897 > SR 93102 > CDC McGwire > HB 364). No AML was detected in native CDC Fibar starch. The extent of AML in the native starches at 60°C, followed the order: CDC McGwire > SB 94893 > SR 93102. However, AML was not detected at the above temperature in native HB 364 and SB 94897 starches. In all native starches, AML increased dramatically in the temperature range of 70- 90°C. The extent of AML has been shown (Hoover & Vasanthan, 1994a; Nakazawa & Wang, 2003) to be influenced by: 1) total amylose content, 2) amount of lipid complexed amylose chains, and 3) extent to which amylose chains are associated with each other and/or with the outer branches of amylopectin. The higher degree of AML exhibited by native SB 94897 and SB 94893 (SB 94897 < SB 94893) starches reflect their higher amylose content. The difference in the extent of AML between native CDC McGwire and SR 93102 (CDC McGwire < SR 93102) can be attributed to differences in the extent of interaction between amylose chains in the amorphous regions of these starches. This seems plausible, since the amylose content of both starches are comparable (Table 4.1), and the amount of lipid complexed amylose chains are higher in native SR 93102 starch (Figure 4.7). Thus, the lower extent of AML in native CDC McGwire starch indicates that interactions between amylose chains within the native granules of CDC McGwire are much stronger than SR 93102. The low degree of AML shown by native HB 364 starch reflects its low amylose content (Table 4.1).

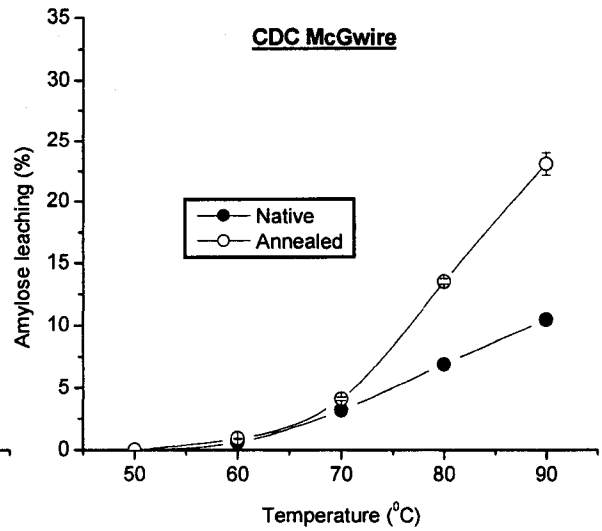
Figure 4.11 Amylose leaching of native starches in the temperature range 50-90°C.

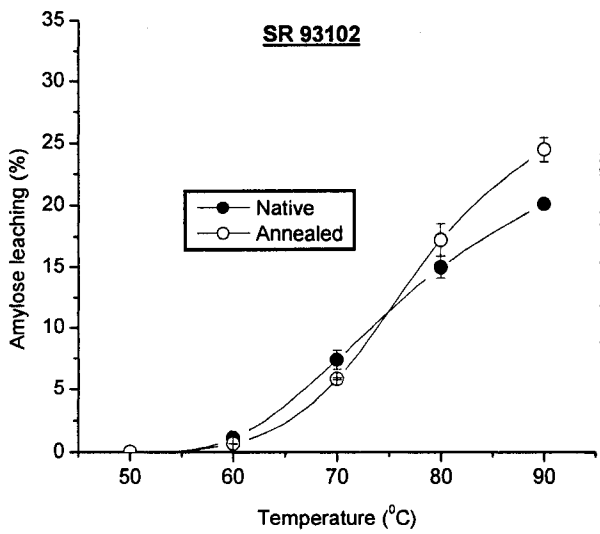

35
30
25
20
15
10
5
0
Amylose leaching (%)
CDC Fibar
HB 364
CDC McGwire
SR 93102
SB 94897
SB 94893
50
60
70
80
90
Temperature (°C)

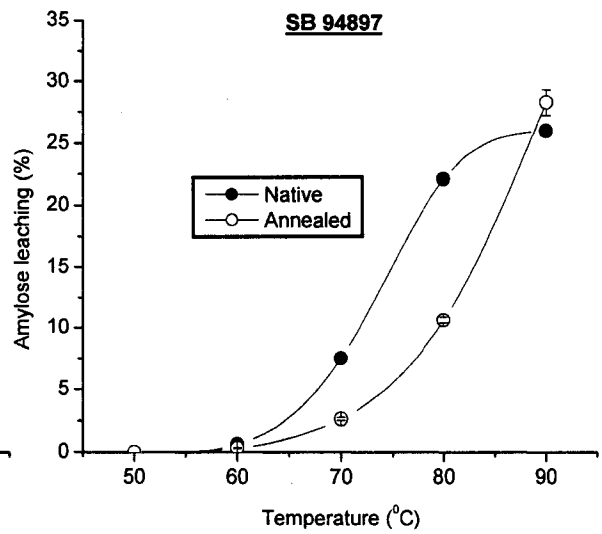
At all temperatures, the extent of AML in annealed SR 93102, SB 94897 and SB 94893 starches were significantly lower than their native counterparts (Figure 4.12). The extent of this decrease followed the order: SB 94893 > SB 94897 > SR 93102. There was no significant difference in the extent of AML between native and annealed HB 364 starches at temperatures below 80°C. However, at 90°C, the extent of AML was higher in annealed than in native HB 364 starch (Figure 4.12). Native and annealed CDC McGwire starches exhibited nearly the same degree of AML at temperatures below 70°C, however, in the temperature range 70-90°C, AML was more extensive in annealed CDC McGwire (Figure 4.12). The results for HB 364 and CDC McGwire was rather surprising, since annealing has been shown by several researchers to reduce AML at all temperatures below 100°C (Lorenz & Kulp, 1978; Kuge & Kitamura 1985; Hoover & Vasanthan 1994a,b; Gomez *et al.*, 2004), in wheat, potato, oat, lentil and cassava starches. The decrease in AML on annealing has been attributed to: 1) interaction between amylose chains, 2) decrease in granular swelling, and 3) increase in V-amylose-lipid content (Hoover & Vasanthan, 1994a,b; Tester *et al.*, 2000). As shown earlier, annealing decreased granular swelling in HB 364 and CDC McGwire starches (Figure 4.10) and increased significantly the amount of V-amylose-lipid complex chains in the latter (Figure 4.8b,c). Therefore, theoretically, the above annealed starches should have exhibited decreased AML at all temperatures. This reversal in AML (annealed > native) can be explained as follows: In native HB 364 and CDC McGwire starches, some amylose chains may have been associated with amylopectin chains within the crystalline

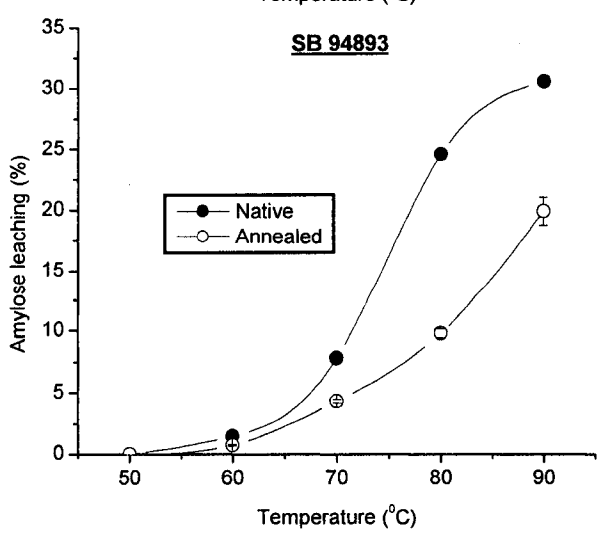
Figure 4.12 Amylose leaching of native and annealed starches in the temperature range 50-90°C.


HB 364
Native
Annealed
Amylose leaching (%)
Temperature (°C)
0
5
10
15
20
25
30
35
50
60
70
80
90


CDC McGwire
Native
Annealed
Amylose leaching (%)
Temperature (°C)
0
5
10
15
20
25
30
35
50
60
70
80
90


SR 93102
Native
Annealed
Amylose leaching (%)
Temperature (°C)
0
5
10
15
20
25
30
35
50
60
70
80
90


SB 94897
Native
Annealed
Amylose leaching (%)
Temperature (°C)
0
5
10
15
20
25
30
35
50
60
70
80
90


SB 94893
Native
Annealed
Amylose leaching (%)
Temperature (°C)
0
5
10
15
20
25
30
35
50
60
70
80
90

lamellae. These associations may have been of a very low order of magnitude, since it involves interaction of long amylose chains with the short exterior chains of amylopectin. It is likely, that these weak interactions are disrupted during reorganization of starch chains during annealing and consequently, more AML would occur in annealed than in the native starches. The difference in AML between native and annealed starches is greater in CDC McGwire (Figure 4.12) due to its higher amylose content (greater opportunity for interaction with amylopectin). The additional amylose chains (that were associated with amylopectin in the native granule) that leach out in annealed HB 364 and CDC McGwire starches, are probably of a larger molecular size than unassociated amylose chains, since they are leached out only at higher temperatures (Figure 4.12).

4.7 Gelatinization

The gelatinization transition temperatures (onset [To], midpoint [Tp], conclusion [Tc]), gelatinization transition temperature range (Tc-To), gelatinization enthalpy (ΔH), amylose-lipid complex melting peak (Tp_{CX}) and enthalpy of melting of the V-amylose-lipid complex peak (ΔH_{CX}) of native and annealed barley starches are presented in Table 4.4. All native barley starches exhibited comparable To values. However, they differed with respect to Tp (SB 94893 ~ SB 94897 > SR 93102 ~ CDC McGwire ~ HB 364 ~ CDC Fibar) and Tc (CDC Fibar > SB 94893 > SB 94897 > HB 364 > SR 93102 > CDC McGwire). (Tc - To) of the native starches followed the order: CDC Fibar > SB 94893 > SB 94897 > SB 93102 ~ HB 364 > CDC McGwire (Table 4.4). ΔH of the native starches followed the order: CDC Fibar > HB 364 > CDC McGwire > SR 93102 > SB 94897 >

Table 4.4. Gelatinization characteristics[1] of native and annealed hull-less barely starches.

Starch source	Gelatinization transition parameters					Amylose-lipid complex transition parameters	
	To (°C)[2]	Tp (°C)[2]	Tc (°C)[2]	(Tc-To) (°C)[2]	ΔH (J/g)[3]	Tp_{CX}(°C)[4]	ΔH_{CX}(J/g)[5]
CDC Fibar							
Native	59.3±0.1[a]	64.9±0.1[a]	81.8±0.1[a]	22.5±0.1[a]	13.0±0.3[a]	...[6]	...[6]
Annealed	66.2±0.0[b]	70.2±0.2[b]	82.9±0.1[b]	16.7±0.1[b]	13.4±0.1[a]	...[6]	...[6]
HB 364							
Native	61.3±0.1[a]	65.5±0.0[a]	75.6±0.1[a]	13.3±0.2[a]	11.4±0.0[a]	...[6]	...[6]
Annealed	67.1±0.1[b]	69.9±0.0[b]	76.7±0.1[b]	9.6±0.0[b]	11.6±0.0[a]	...[6]	...[6]
CDC McGwire							
Native	61.4±0.0[a]	65.3±0.1[a]	72.8±0.1[a]	11.4±0.1[a]	10.0±0.1[a]	88.4±0.4[a]	0.4±0.0[a]
Annealed	66.7±0.0[b]	69.8±0.1[b]	77.0±0.1[b]	10.3±0.1[b]	10.0±0.2[a]	87.1±0.6[a]	0.5±0.2[a]
SR 93102							
Native	60.5±0.1[a]	65.2±0.3[a]	73.7±0.0[a]	13.3±0.1[a]	9.0±0.2[a]	89.1±0.0[a]	0.8±0.1[a]
Annealed	66.4±0.1[b]	69.5±0.1[b]	77.2±0.2[b]	10.8±0.1[b]	9.1±0.3[a]	87.1±0.1[b]	0.9±0.1[a]
SB 94897							
Native	62.4±0.3[a]	68.8±0.2[a]	76.3±0.1[a]	14.0±0.2[a]	6.6±0.1[a]	88.8±0.2[a]	1.5±0.2[a]
Annealed	67.4±0.1[b]	72.6±0.0[b]	78.7±0.1[b]	11.3±0.1[b]	6.5±0.2[a]	87.5±0.1[b]	1.7±0.1[a]
SB 94893							
Native	61.0±0.1[a]	67.9±0.1[a]	76.6±0.2[a]	15.6±0.1[a]	6.0±0.1[a]	84.9±1.6	1.8±0.3
Annealed	67.9±0.0[b]	72.1±0.1[b]	79.0±0.0[b]	11.1±0.0[b]	7.7±0.1[b]	...[6]	...[6]

[1]Starch: water ratio is, 1:3 (w/w, db); All data reported on dry basis and represent the mean of three determinations; Means within a column with different superscripts (between native and annealed starch of each cultivar) are significantly different ($p<0.05$).

[2]To, Tp, Tc and (Tc-To) represent the onset, peak, conclusion and the gelatinization temperature range, respectively.

[3]Enthalpy of gelatinization.

[4]Tp_{CX} represents the peak melting temperature of the V-amylose-lipid complex.

[5]ΔH_{CX} represents the enthalpy of melting of the V-amylose-lipid complex

[6]Not detected.

SB 94893 (Table 4.4). Song & Jane (2000) have also shown by studies on other barley cultivars, that differences in To are marginal among normal (29.5% amylose), high-amylose (43.4% amylose) and waxy (9.1% amylose) barley starches. However, significant differences were shown to exist with respect to Tp (high-amylose > normal > waxy) and ΔH (waxy > normal > high- amylose). No data was reported for Tc. In maize starches (Tester *et al.*, 2000; Jayakody & Hoover, 2002) differences in To, Tp, Tc, (Tc – To) and ΔH among normal, waxy and high-amylose starches do not follow the same trend exhibited by barley starches. This could be attributed to the higher amylose content of the high-amylose maize starches (61.5 - 78.3%) and to larger differences in amylopectin structure among waxy, normal, and high-amylose maize starches (Cheetham & Tao, 1998a,b). The higher (Tc – To) exhibited by native CDC Fibar can be attributed to its higher amylopectin content (100%) and/or to a higher degree of variation in crystalline stability. The higher ΔH exhibited by native CDC Fibar starch reflects its higher amylopectin content.

Native CDC Fibar and HB 364 starches with bound lipid contents of 0.10 and 0.32%, respectively (Table 4.1), did not exhibit a V-amylose-lipid complex melting transition peak (Table 4.4). However, native CDC McGwire, SR 93102, SB 94897 and SB 94893 starches with bound lipid contents in the range of 0.63 - 0.72% (Table 4.1) exhibited V-amylose-lipid complex melting peak (Tp_{CX}) in the range 84.9 – 89.1°C (Table 4.4). With the exception of native SB 94893 starch, the Tp_{CX} of the starches was within the range (88.0 - 102.7°C) reported for other barley starches (Tester *et al.*, 1993; Yoshimoto *et al.*, 2000, 2002; Qi *et al.*, 2004). Kugimiya and Donovan (1981) have

shown that ΔH_{CX} can be used as a measure of the amount of V-amylose-lipid complexes present in the granule. The ΔH_{CX} values of the native barley starches, indicate that the amount of lipid complexed amylose chains in the native starches follow the order: SB 94893 ~ SB 94897 > SR 93102 > CDC McGwire. This order was in fairly close agreement with that obtained from X-ray diffraction data (Figure 4.7). The DSC (Table 4.4) and X-ray (Figure 4.7) data for the V-amylose-lipid complexes in the native starches indicates that the amount of V-amylose-lipid complexes cannot be ascertained from the bound lipid content (Table 4.1) since there was no significant differences among the above starches with respect to their bound lipid content. It is likely, that some of the bound lipids may have been present trapped in the spaces between amylose and amylopectin and/or linked via ionic or hydrogen bonding to the starch components. The ΔH_{CX}, values for the native starches were within the range (0.3 - 3.0 J/g) reported for starches from other barley cultivars (Tester *et al.*, 1993; Yoshimoto *et al.*, 2002). In comparison with ΔH_{CX}, variations with respect to Tp_{CX} among the starches were only minor (Table 4.4). This suggests that the same type of lipids are probably associated with the amylose helix in native SB 94893, SB 94897, SR 93102, and CDC McGwire starches. The absence of Tp_{CX} and ΔH_{CX} for native CDC Fibar and HB 364 starches, suggests that the amount of lipid complexed amylose chains may have been too small to be detectable by DSC.

In all barley starches, annealing increased To, Tp and Tc and decreased (Tc – To) (Table 4.4). Such changes on annealing have been shown to be typical for all starches irrespective of their molecular structure or amylose content (Knutson, 1990; Hoover &

Vasanthan, 1994a; Jacobs & Delcour, 1998; Tester *et al.*, 1998, 2000; Kiseleva *et al.*, 2004) and has been attributed to perfection of the crystalline structure. The ΔH of SB 94893 starch increased significantly on annealing, whereas ΔH of the other barley starches remained unchanged (Table 4.4). The constancy of ΔH pre- and post-annealing in the waxy (CDC Fibar, HB 364), normal (CDC McGwire, SR 93102), and high-amylose (SB 94897) starches suggest that no new double helices are formed on annealing. The constancy of ΔH pre- and post-annealing has also been reported for normal and waxy maize starches (Tester *et al.*, 2000). The increase in ΔH shown by SB 94893 (55.3% amylose), suggests that when the 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 may occur between amylose-amylose and/or amylose-amylopectin chains leading to the formation of new double helices. This seems plausible, since Tester *et al.* (2000) have shown using ^{13}C-CP/MAS-NMR, that the double helical content of amylomaize starch (61.3% amylose) increased by 11% after single step annealing. Annealing had a marginal effect on the V-amylose-lipid complex melting endotherm of CDC McGwire, SR 93102 and SB 94897 starches. However, no endotherm was visible in annealed SB 94893 starch even at 150°C. This suggests, that due to its high amylose content, some free amylose chains of SB 94893 starch may have interacted (via hydrogen bonding) with single V-amylose-lipid complex helices during annealing, thereby modifying its melting temperature. Jacobs *et al.*, (1998a,b) have also shown that annealing does not influence the V-amylose-lipid complex melting endotherm of normal wheat starch.

4.8 Acid hydrolysis

The solubilization patterns of native, and native and annealed starches are presented in Figure 4.13 and Figure 4.14, respectively. Two phases were observed during acid hydrolysis of native and annealed starches; the first rapid phase, corresponding to hydrolysis of the amorphous domains (1-12 days), and the second slow phase (12-18 days) corresponding to hydrolysis of the crystalline domains. At the end of the 13th day of hydrolysis, native CDC Fibar, HB 364, CDC McGwire, SR 93102, SB 94897, and SB 94893 starches were hydrolyzed to the extent of 45.9, 45.0, 36.0, 39.5, 31.0, and 30.0%, respectively (Figure 4.13). At the end of the 18th day of hydrolysis, native CDC Fibar, HB 364, CDC McGwire, SR 93102, SB 94897, and SB 94893 starches were hydrolyzed to the extent of 52.0, 50.0, 44.0, 45.0, 41.0, and 38.0%, respectively (Figure 4.13). Differences in the extent of hydrolysis among native maize starches have also been shown to follow the order: waxy > normal > high-amylose (Jayakody & Hoover, 2002; Nakazawa & Wang, 2003). Difference in the extent of acid hydrolysis between starches has been attributed to the interplay of the following factors: 1) presence of lipid complexed segments of single V-amylose helices (Morrison *et al.*, 1993c), 2) packing arrangement of starch chains in the amorphous and crystalline regions of the granule (Hoover & Manuel, 1996; Hoover, 2000), and 3) presence of pores on the granule surface (Jayakody & Hoover, 2002). The higher extent of hydrolysis exhibited by the native waxy barley (CDC Fibar, HB 364) starches (Figure 4.13) can be attributed to their lower amylose content (Table 4.1) and larger surface pores (Figure 4.1C & 4.2C).

Figure 4.13 Acid hydrolysis of native barley starches.

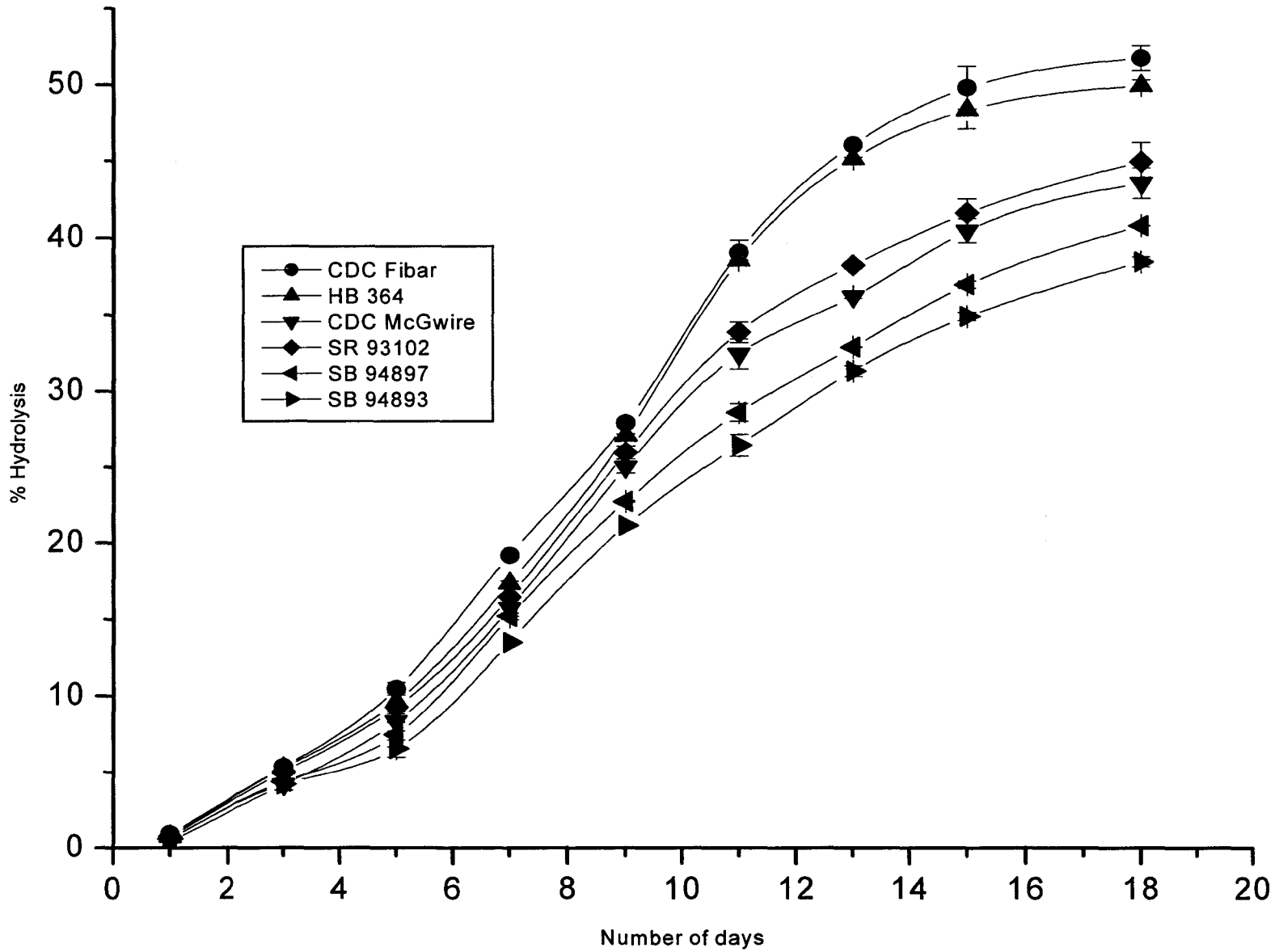
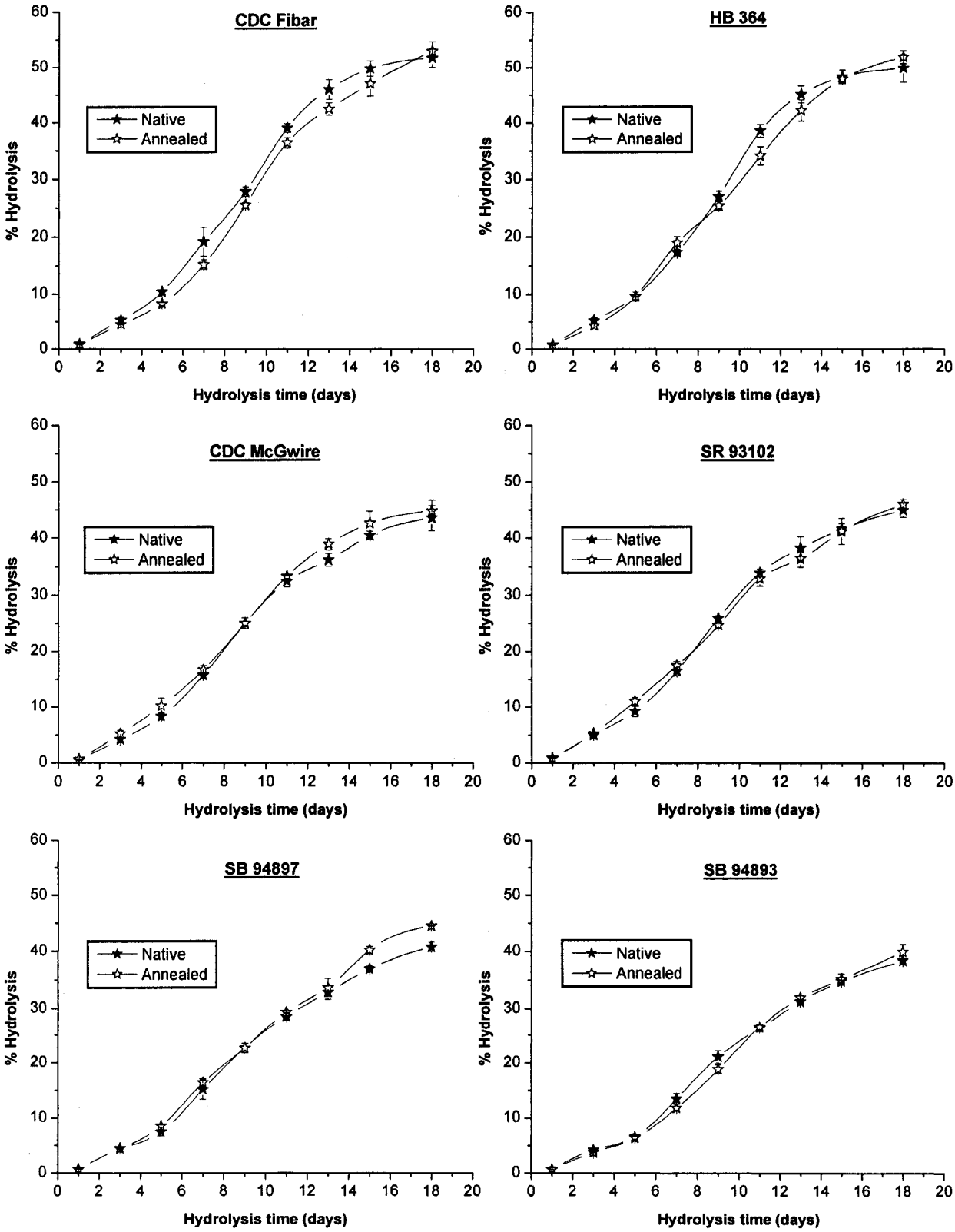

% Hydrolysis
Number of days
CDC Fibar
HB 364
CDC McGwire
SR 93102
SB 94897
SB 94893
0
10
20
30
40
50
0
2
4
6
8
10
12
14
16
18
20

Figure 4.14 Acid hydrolysis of native and annealed barley starches.


CDC Fibar
HB 364
CDC McGwire
SR 93102
SB 94897
SB 94893
Native
Annealed
% Hydrolysis
Hydrolysis time (days)

The native high-amylose starches (SB 94897, SB 94893) are hydrolyzed to a lesser extent than the native normal starches (CDC McGwire, SR 93102) (Figure 4.13), due to their higher amylose content (Table 1) and greater content of V-amylose-lipid complexed chains (Table 4.4, Figure 4.7).

Difference in hydrolysis between native and annealed starches was more marked in CDC Fibar than in the other starches. Between days 1 and 13, annealed CDC Fibar starch was hydrolyzed to a lesser extent than its native counterpart (Figure 4.14). However, there was no significant difference beyond the 13^{th} day (Fig. 4.14). Native and annealed starches of HB 364, CDC McGwire, SR 93102, SB 94897, and SB 94893 were however, hydrolyzed nearly to the same extent throughout the time courses of hydrolysis (Figure 4.14). The impact of annealing on acid hydrolysis has been shown to be influenced by the method used for annealing (single-step, double-step, multi-step), annealing temperature, and starch source (Hoover & Vasanthan, 1994a; Jacobs *et al.*, 1998a; Nakazawa & Wang, 2003). Annealing (single-step) has been reported to decrease the acid susceptibility of wheat, potato and lentil starches (Hoover & Vasanthan, 1994a). Jacobs *et al.* (1998a) also showed that acid susceptibility of potato starch decreased on annealing (single- and double-step), however, no differences were observed between wheat and pea starches. Tester *et al.* (1998) reported that during the first phase of acid hydrolysis, annealed (single-step) wheat starch was more extensively degraded than its native counterpart, while during the second phase, there was no difference in the extent of hydrolysis. Nakazawa and Wang (2003) showed that multi-step annealing of wheat, tapioca, potato, maize, waxy maize and amylomaize increased the acid susceptibility

during both phases of acid hydrolysis with potato starch showing the greatest and high-amylose starches showing the least changes.

Various theories have been put forward to explain the susceptibility of annealed starches towards acid hydrolysis. The decrease in acid hydrolysis on annealing has been attributed to: 1) perfection of starch crystallites, 2) formation of double helical structures between amylose chains, 3) increased resistance of α (1→6) branch points, and 4) formation of V-amylose-lipid complexes (Hoover & Vasanthan, 1994a; Jacobs *et al.*, 1998a). Jacobs *et al.* (1998a) have postulated that in native starches, some branch linkages in amylopectin exists in the form of imperfect double helices, which are hydrolyzed by acid. After annealing, these imperfect double helices might become perfect double helices and part of the crystallites, therefore, they would be less susceptible to hydrolysis by acid. 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 the enhanced order in the crystalline region (Tester *et al.*, 2000), and 2) formation of void spaces (allows penetration of H_3O^+ into the granule interior) that result from perfection of the crystalline lamellae (Nakazawa & Wang, 2003). Similarity in hydrolysis between native and annealed starches during the second phase of hydrolysis has been attributed to: 1) presence of the same amount of double helices in the crystalline regions, and 2) limited ordering of amylopectin crystallites (Tester *et al.*, 2000; Nakazawa & Wang, 2003). The above theories suggest that the impact of annealing on the rapid and slow phases of acid hydrolysis is influenced to a large extent by the changes that occur within the crystalline lamellae. The DSC results (Table 4.4) showed that

crystallite perfection on annealing occurs to a greater extent in CDC Fibar than in the other starches. This suggests that the resistance of α(1→6) branch points towards acid hydrolysis in the annealed starches would be more pronounced in CDC Fibar than in the other barley starches. This would then explain the decrease in susceptibility of CDC Fibar to acid hydrolysis (during the first 13 days) on annealing. The nearly similar hydrolysis patterns shown by native and annealed HB 364, CDC McGwire, SR 93102, SB 94897, and SB 94893 starches is indicative of the lower degree of crystalline perfection that occurs during annealing (Table 4.4). The similarity in hydrolysis patterns between native and annealed starches of all six barley cultivars during the second phase (> 13 days), suggests that no new double helices were formed between amylopectin chains during annealing. The above data suggests that formation of V-amylose-lipid complexes and interactions involving amylose chains during annealing has no significant impact on the acid hydrolysis pattern of the barley starches.

Summary and Conclusions

Starches from waxy (CDC Fibar, HB 364), normal (CDC McGwire, SR 93102), and high-amylose (SB 94897, SB 94893) barley grains were studied before and after single-step annealing for their structure, morphology and physicochemical properties. Starches from all genotypes consisted of a mixture of large (spherical, disk, lenticular) and small (irregular) granules. Pores were present on the granule surface of all starches. The total amylose content, the bound lipid content and the total phosphorous content ranged from 0.00 to 55.30%, 0.10 to 0.72%, and 0.024 to 0.060%, respectively. The amylopectin structure of all six starches was nearly identical. The transition of X-ray pattern from pure 'A'-type to mixed 'A+B'-type occurred at ~33% amylose. X-ray studies showed the presence of V-amylose-lipid complexes in all native starches. In all native barley starches, with an increase in amylose content, the intensities of the major X-ray peaks became progressively weaker, whereas the X-ray peak corresponding to the V-amylose-lipid complex became progressively stronger. Relative crystallinity (RC), gelatinization temperatures (To, Tp, Tc), and gelatinization temperature range (Tc-To), were not correlated with the amylose content. However, the enthalpy of gelatinization (ΔH) decreased with increase in amylose content. DSC studies also provided evidence for the presence of V-amylose-lipid complexes in non-waxy barley (CDC McGwire, SR 93102, SB 94897, SB 94893) starches. Swelling factor (SF) and amylose leaching (AML) increased with increase in heating temperature. SF decreased and AML increased with increase in amylose content. Waxy barley (CDC Fibar, HB 364) starches showed a

two-stage swelling, whereas non-waxy barley (CDC McGwire, SR 93102, SB 94897, SB 94893) starches showed a single-stage swelling. Two phases were observed during acid hydrolysis of native barley starches; the first rapid phase corresponding to hydrolysis of the amorphous domain (1-12 days), and the second slow phase (12-18 days) corresponding to hydrolysis of the crystalline domain. Extent of hydrolysis decreased with increase in amylose content.

Annealing increased the pore sizes of waxy barley (CDC Fibar, HB 364) starches. The RC of waxy (CDC Fibar, HB 364) and normal (SR 93102, CDC McGwire) barley starches increased on annealing, whereas it remained unchanged in high-amylose barley (SB 94897, SB 94893) starches. Annealing increased the intensity of X-ray peaks in all six starches. The 'A'- type X-ray pattern of CDC Fibar, HB 364, and CDC McGwire starches remained unchanged, while the 'A+B'- type X-ray pattern of SR 93102, SB 94897 and SB 94893 starches resembled more closely the 'A'- type pattern on annealing. In all starches, the X-ray intensity of the V-amylose-lipid complex peak increased on annealing. Annealing increased the To, Tp, and Tc, and decreased the (Tc-To) in all starches. The ΔH of SB 94893 starch increased on annealing, whereas it remained unchanged in the other starches. Annealed SB 94893 starch did not show the DSC endotherm for the melting of V-amylose-lipid complex. The peak temperature of the melting of V-amylose-lipid complex (Tp_{CX}) of SR 93102 and SB 94897 starches remained unchanged on annealing, whereas Tp_{CX} of CDC McGwire increased slightly. The enthalpy of the melting of V-amylose-lipid complex (ΔH_{CX}) of native and annealed CDC McGwire, SR 93102, and SB 94897 were similar. In all starches, SF decreased on

annealing. Annealing decreased AML in SR 93102, SB 94897, and SB 94893 starches in the temperature range of 50-90°C, but increased AML in HB 364 and CDC McGwire starches at higher temperatures. Annealing decreased acid hydrolysis in CDC Fibar starch during the early stages of hydrolysis. Thereafter, both native and annealed CDC Fibar starches were hydrolyzed to the same extent. However, all other starches showed no significant changes in acid hydrolysis on annealing.

The results showed that the different responses of waxy, normal, and high-amylose barley starches towards annealing were mainly influenced by differences in the amylose / amylopectin ratio and by the packing arrangement of the starch chains within the amorphous and crystalline regions of the native granule.

Directions for further research

1. A study of the surface characteristics and the internal structure of starch granules before and after annealing by AFM (atomic force microscopy) and CLSM (confocal laser scanning microscopy) may provide information with regard to the formation of pores, cavities, and changes to distribution pattern of growth rings during annealing. This study may be of importance in the food and non-food industries, since these features could influence the susceptibility of annealed starches to acid, enzyme or to reagents used in chemical modification.

2. Studies have shown that small and large granules of barley starch vary with regard to physicochemical properties, such as granular swelling, gelatinization parameters, and acid hydrolysis (Vasanthan & Bhatty, 1996; Tang *et al.*, 2001, 2002). Therefore, a study of the structure and properties of fractionated small and large barley starch granules on annealing, may provide further insights into the mechanism of annealing.

3. A study of the effect of annealing on legume starches would provide a deeper insight into the molecular mechanism of annealing, since legume starches have been shown to vary widely in their amylose content, proportion of 'B'-type crystallites, extent of interaction between starches, and relative crystallinity.

4. The effect of lipid complexing during annealing is still in dispute. A study of the interaction of tuber and legume starches (which are known to contain trace quantities of bound lipid) with added monoacylglycerols (with different chain lengths) before and after annealing may provide a wealth of information with regard to the formation of V-amylose-lipid complexes during annealing.

5. Acid hydrolysis has been used as a probe to determine the arrangement of starch chains in the amorphous and crystalline regions of the granules. However, very little work has been done to investigate how interactions that occur within the amorphous and crystalline domains of the granule during annealing would influence the entry of H_3O^+ into the granule interior. A study of the kinetics of acid hydrolysis before and after annealing together with studies on the starch residues obtained before and after annealing at different hydrolysis times, may provide information about the extent to which the starch chains reorganize during annealing. For this study, starches varying widely in amylose content within a single species (maize, barley, legume) should be used.

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Awards

1. Graduate fellowship, Memorial University of Newfoundland, St. John's, NL, Canada (from January 2004 to August 2004).

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Appendices

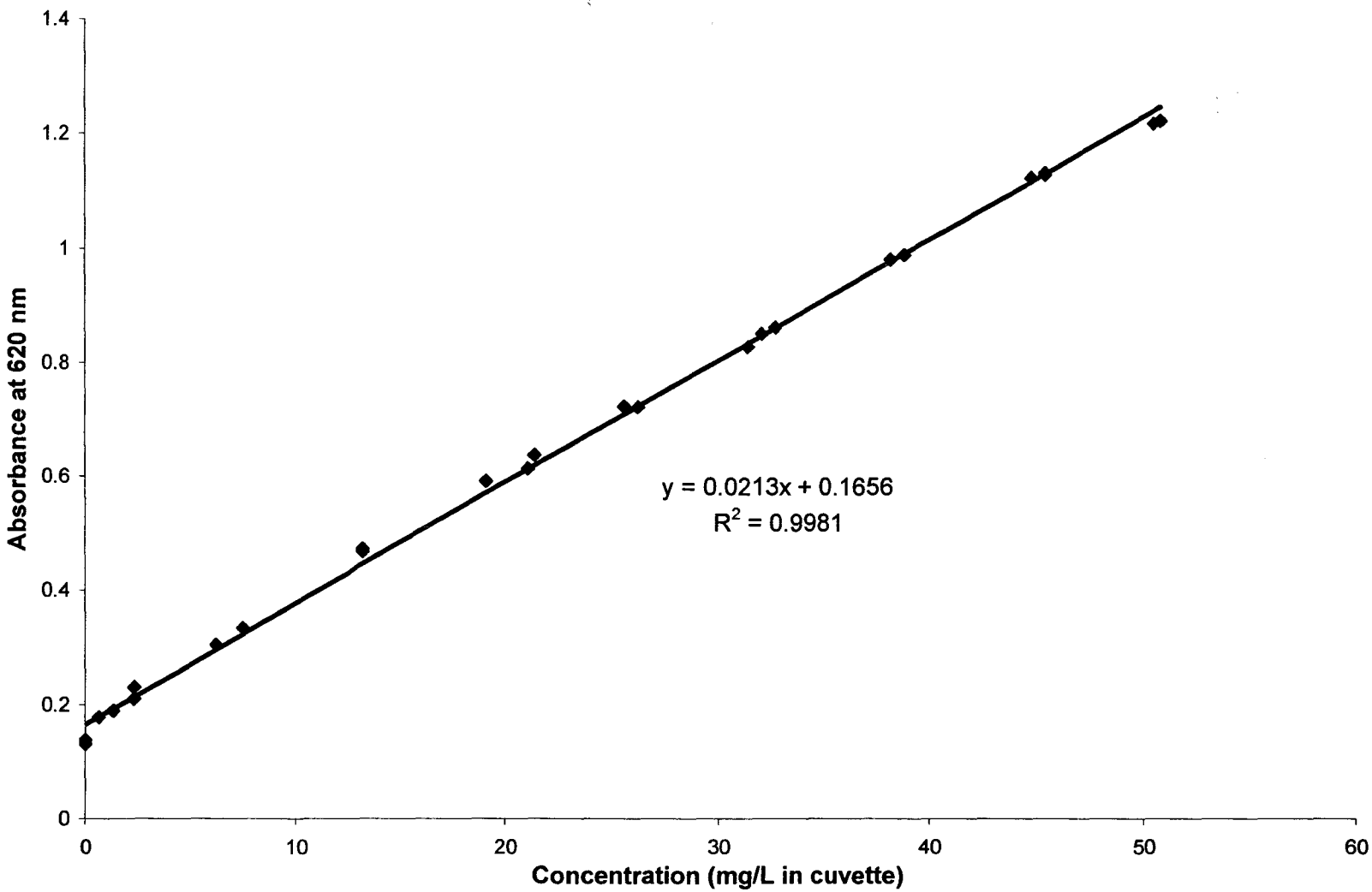

Figure A.1 Standard curve for amylose determination as glucose at 620 nm.

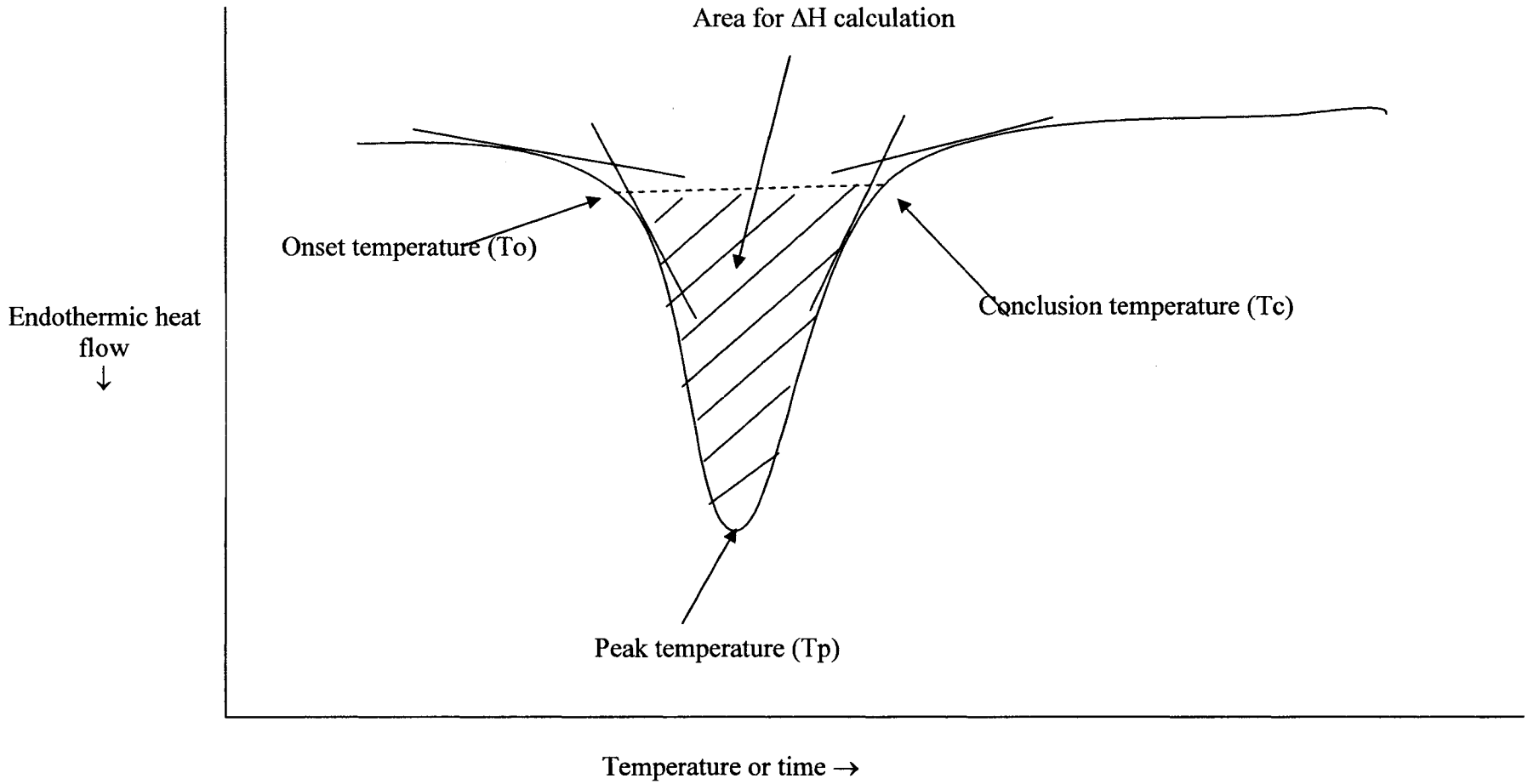

Figure A.3 Schematic illustration for the determination of gelatinization parameters.

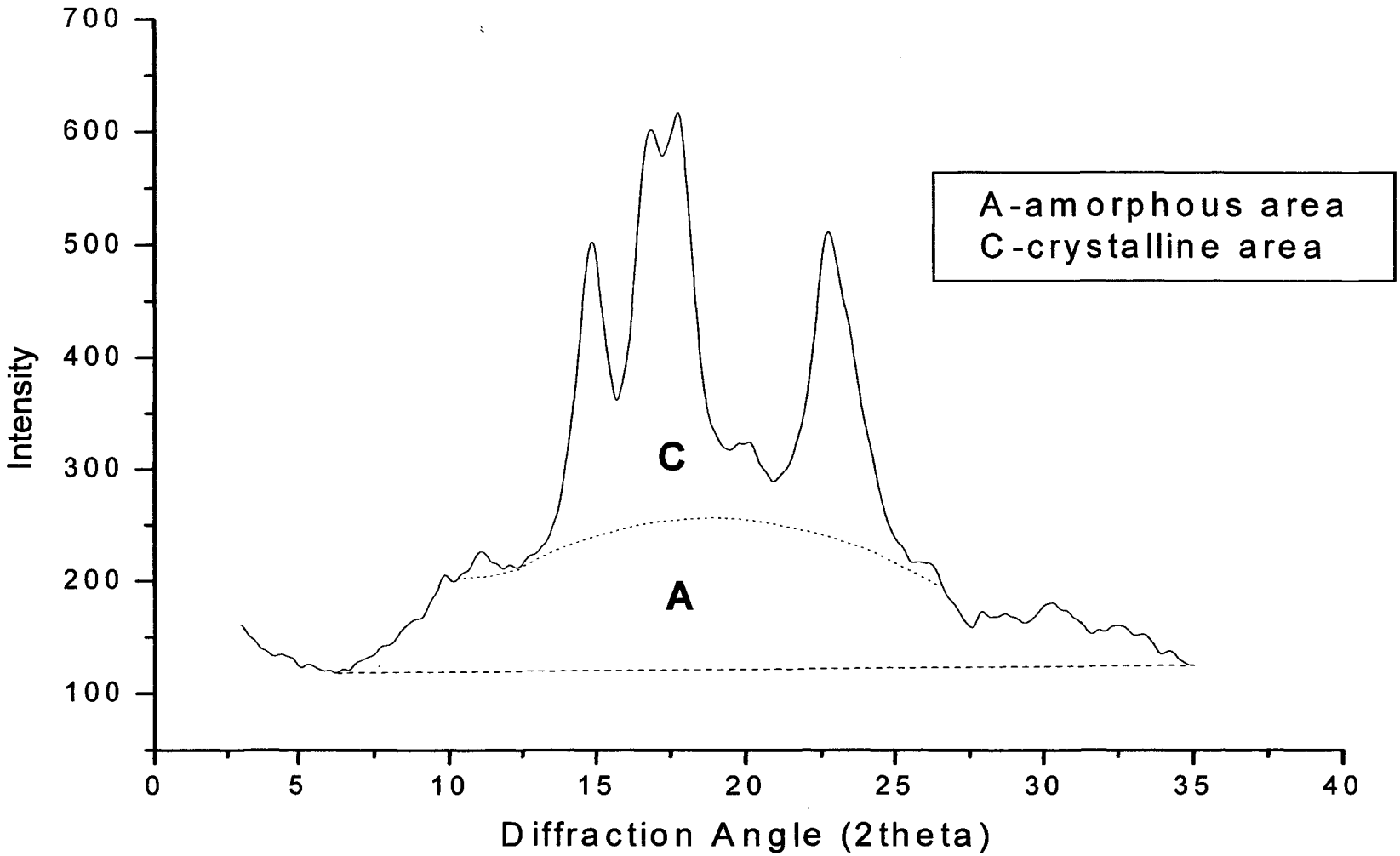

Figure A.4 Schematic illustration for relative crystallinity determination