THE EFFECT OF ACID HYDROLYSIS ON GRANULAR MORPHOLOGY AND PHYSICOCHEMICAL PROPERTIES OF NATIVE CEREAL STARCH GRANULES

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by

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Abstract

This study examined the composition (moisture, lipid content, ash content, nitrogen content, amylose content, and starch damage), granule morphology, physicochemical characteristics (X-ray diffraction pattern, degree of crystallinity, degree of swelling, amylose leaching, gelatinization parameters), and the average degree of polymerization (DP) of cereal starches (normal maize, waxy maize, amylomaize V, and VII, rice and oat) in their native state and after acid hydrolysis (2.2N HCl at 35°C).

The moisture contents of the cereals starches were in the range 10.4 -12.2% (dry basis). The total lipid content in normal maize, waxy maize, amylomaize V, amylomaize VII, rice and oat starches was 0.86, 0.01, 1.21, 1.49, 1.01, and 1.13%, respectively. The surface (unbound) lipids constituted 0.06%, 0.01%, 0.04%, 0.11%, 0.03% and 0.11% of the total lipid in normal maize, waxy maize, amylomaize V, amylomaize VII, rice and oat starches, respectively. The bound lipid content in normal maize, amylomaize V, amylomaize VII, rice and oat starches was 0.76%, 1.16%, 1.33%, 0.98%, and 1.01%, respectively. However, waxy maize was devoid of bound lipid. The ash and nitrogen contents of the cereal starches were in the range 0.09 - 0.45% and 0.01 - 0.05% respectively. The apparent amylose content was 23.7%, 1.1%, 49.0%, 66.9%, 15.2%, and 20.9% in normal maize, waxy maize, amylomaize V, amylomaize VII, rice, and oat starches, respectively. The total amylose content was 26.5%, 1.1%, 61.9%, 78.4%, 20.6%, and 29.3%, respectively, in normal maize, waxy maize, amylomaize V, amylomaize VII, rice and oat starches. The percentage of total

amylose complexed by native lipids was 10.6%, 20.8%, 14.7%, 26.2%, and 28.7%, in normal maize, waxy maize, amylomaize V, amylomaize VII, rice and oat respectively. The extent of starch damage was low in all starches (0.3 - 0.7%).

The average granule diameter of native normal maize, waxy maize, amylomaize V, amylomaize VII, rice and oat starches ranged from 7-8, 5-15, 4-16, 6-13, 2-7, and 5-12 μm respectively. Granules of native rice and oat starches were polygonal to irregular in shape. Maize starches granule were polygonal to irregular in shape. Except for native oat starch, the surface of other native cereal starch granules were covered with fissures or pores of varying diameter.

The average degree of polymerization (DP) in native normal maize, waxy maize, amylomaize V, amylomaize VII, rice and oat starches were, 813, 685, 1247, 1285, 1389, and 708, respectively.

Native normal maize, waxy maize, rice, and oat starches, exhibited the typical 'A'-type X-ray spectrum of cereal starches. Amylomaize V and VII starches exhibited a 'B'-type X-ray spectrum. The relative crystallinity of the native starches followed the order: waxy maize (49%) > rice (36%) > normal maize (34%) > oat (32)> amylomaize (19%)> amylomaize VII (16%).

The swelling factor (SF) of native starches followed the order: waxy maize> normal maize> oat> rice> amylomaize V> amylomaize VII. Amylose leaching

(AML) of native starches followed the order: rice> normal maize> oat> amylomaize VII> amylomaize V.

The gelatinization transition temperatures range (T_C-T_O) for native starches followed the order; amylomaize VII> amylomaize V> rice> waxy maize> normal maize> oat. The gelatinization enthalpy (ΔH) of the native starches followed the order: waxy maize> rice> normal maize> amylomaize VII> amylomaize V> oat.

All starches exhibited a two stage hydrolysis pattern. A relatively high rate was observed during the first 6 days, followed by a slower rate thereafter. At the end of the 8th day of hydrolysis (corresponding mainly to the degradation of the amorphous region of the granule), normal maize, amylomaize V, amylomaize VII, waxy maize, rice and oat starches were hydrolyzed to the extent of 61.1, 32.6, 28.5, 68.1, 62.0, and 64.4% respectively. Between the 9th and 15th day (corresponding mainly to the degradation of starch crystallites) the increase in the extent of hydrolysis was more pronounced in normal maize (9.3%) than in oat (8.3%), rice (8.1%), waxy maize (7.2%), amylomaize V (3.0%), and amylomaize VII (3.1%) starches. At the end of the 15th day, normal maize, amylomaize V, amylomaize VII, waxy maize, rice and oat starches were hydrolyzed to the extent of 73.4, 37.0, 32.3, 77.3, 75.3, and 72.9%, respectively.

In all starches, the number and size of pores on the granule surface increased after acid hydrolysis. In addition, granules were either deformed or fragmented.

In all acid treated starches, DP decreased rapidly during the first 48h. The extent of decrease in DP beyond 48h, followed the order: amylomaize VII > amylomaize V > normal maize > rice ~ waxy maize > oat.

Acid hydrolysis increased X-ray intensities, but had no influence on the X-ray diffraction pattern. Acid treatment decreased (7.4%) the relative crystallinity (RC) of waxy maize starch, but increased (7.7-8.9%) RC of the other starches.

Granule swelling was monitored by changes in swelling factor (SF). Acid hydrolysis for 24h increased the SF (rice> amylomaize V> amylomaize VII> oat> normal maize). However, granule swelling was not detectable in waxy maize starch. A decrease in SF occurred after 2 days hydrolysis in oat and rice starches, but after 3 days for maize starches. Granular swelling was not detectable in oat, rice and normal maize starches after the 4^{th}, 5^{th}, and 8^{th}, day of hydrolysis, respectively. However, amylomaize V, and VII showed measurable SF even after the 8^{th} day of hydrolysis. Hydrolysis for 24h, increased amylose leaching (AML) in all starches (oat> normal maize> amylomaize V> amylomaize VII> rice). Thereafter, AML decreased gradually. The extent of this decrease followed the order: oat> normal maize> rice> amylomaize V> amylomaize VII.

In all starches, To (onset temperature of gelatinization) decreased, but Tp (mid-point temperature of gelatinization), Tc (conclusion temperature of gelatinization) and Tc-To (gelatinization temperature range) increased with acid hydrolysis. The extent of increase in T_C-T_O (after 24h hydrolysis) followed the order: waxy maize> amylomaize V> normal maize> oat> rice. The extent of increase in ΔH (enthalpy

of gelatinization) after 24h hydrolysis, followed the order: amylomaize V> rice> oat> normal maize> waxy maize.

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Chapter 1

1. Introduction

Acid hydrolysis has been used to modify starch granule structure and produce "soluble" starch for many years (Kirchoff, 1811). Treatment of native potato starch in water with 15% (1.7M) sulfuric acid for 30 days at room temperature results in an acid-resistant fraction (Nageli amylodextrin) that is readily soluble in hot water (Nageli, 1874). Nageli amylodextrin has been shown to be a mixture of low molecular, linear, and branched dextrins, with an average degree of polymerization (DP) of 25-30 (Nageli, 1874). However, treatment of starch with 2.2N HCl at elevated temperatures (30°-40°C) produces lintnerized starch (Lintner, 1886). Lintnerized starch as been shown to consist of the following factions: A) linear amylose chains with a degree of polymerization (DP) between 13-15 (Biliaderis *et al.*, 1981, Robin *et al.*, 1975, Watanabe and French, 1980); B) singly branched amylose chains [DP 25] (Biliaderis *et al.*, 1981, Robin *et al.*, 1975, Watanabe and French, 1980); C) multiply branched chain whose branching ratio and DP are functions of hydrolysis rate (Watanabe and French, 1980, Jacobs *et al.*, 1998); D) retrograded amylose (Morrison *et al.*, 1993a), and E) amylose-lipid complexes [only in lipid containing starches] (Morrison *et al.*, 1993a). Hydrolysis of potato starch by 2.2N HCl has been shown to consist of two stages (Robin *et al.*, 1974, 1975): an initial fast step (due to hydrolysis of the amorphous regions of the granule) followed by a second slower rate [due to hydrolysis of the crystalline regions] (Robin *et al.*, 1974, 1975). Similar patterns of hydrolysis has also been observed for cereal and legume starches (Maningat

and Juliano, 1979, Biliaderis *et al.*, 1981, Muhr *et al.*, 1984, Inouchi *et al.*, 1987, Shi and Seib, 1992, Hoover and Vasanthan, 1994b, Vasanthan and Bhatty, 1996, Jane *et al.*, 1997, Jacobs *et al.*, 1998, Shi *et al.*, 1998). Evidence to suggest a preferential attack on amorphous domains within the granule comes from transmission electron microscopy observations of acid hydrolyzed starches (Mussulmam and Wagner, 1968). These authors (Mussulmam and Wagner 1968) observed a preferential etching of amorphous growth rings from normal and waxy maize starches treated with 7% HCl at room temperature for 35 days. To account for the slower hydrolysis rate of the crystalline domains of the starch granule, two hypothesis (Kainuma and French, 1971, French, 1984) have been proposed. Firstly, the dense packing of starch chains within the starch crystallites does not readily allow the penetration H_3O^+ into the regions. Secondly, the chair→ half chair transformation (required for hydrolysis of the glucosidic bond) occurs very slowly due to immobilization of the sugar conformation within the starch crystallites).

The differences in the rate and extent of hydrolysis among and between cereal, tuber and legume starches has been attributed to differences in granule size (Jane *et al.*, 1997, Vasanthan and Bhatty, 1996, Singh and Ali, 2000), extent of starch chain interactions (Hoover *et al.*, 1993) within the amorphous and crystalline domains of the granule, extent of phosphorylation (Jane *et al.*, 1997), amount of α (1→6) linkages (Inouchi *et al.*, 1987), lipid-complexed amylose chains (Morrison *et al.*, 1993a) and the extent of distribution of α (1→6) linkages between the amorphous and crystalline domains (Jane *et al.*, 1997). It is also

likely, that acid hydrolysis could be influenced by the number and size of pores on the granular surface. However, this aspect has not been investigated. Most of the reported data on acid treated starches have been on the solubilization pattern and on the structure of the acid treated residues of potato and wheat starches (Jacobs *et al.*, 1998, Morrison *et al.*, 1993a, Jane *et al.*, 1997, Hoover and Vasanthan, 1994b, Inouchi *et al.*, 1987, Vasanthan and Bhatty, 1996). Furthermore, none of the earlier researchers have attempted to explain in detail the solubilization profiles of native starches and the physicochemical properties (granular swelling, amylose leaching, gelatinization parameters, X-ray pattern and relative crysalinity) of the acid treated residues. Moreover, there is a dearth of information on changes to the average degree of polymerization (DP) and to the granule surface during the time course of acid hydrolysis.

The objective of this study was three fold: 1) To investigate the granule morphology and the physicochemical properties such as average degree of polymerization (DP), swelling factor, amylose leaching, X-ray diffraction pattern, relative crystallinity and gelatinization parameters of native normal maize, waxy maize, amylomaize V, amylomaize VII, rice and oat starches; 2) To investigate the susceptibility of the above native starches towards hydrolysis by 2.2N HCl at 35°C and 3) To investigate how morphological and physicochemical properties change at different time periods of acid hydrolysis.

Chapter 2

2. Review of Literature

2.1 Starch - General introduction

Starch is nature's chief way to store energy as carbohydrate in green plants. It is the major storage polysaccharide of edible parts of plants, for example in cereal grains (maize, rice, oat, millet, sorghum, barley, wheat) tubers (potatoes, coco yam, diascorea spp.), roots (sweet potatoes, cassava, arrowroots,) pulses (peas, beans), stem (sago) and plantains (Sanchez-Castillo *et al.* 2000, Swinkels, 1985a). Starch is among the most abundant bio-polymers on the earth, second only to cellulose (Jane *et al*, 1994 and Jane, 1997, Hizukuri, 1996, Hancock and Tarbet 2000). It is a major source of energy for humans many species of animals, and microorganisms. In higher plants, starch is synthesized in amyloplast and stored in the form of granules in various parts of the plant such as seeds, grains, roots, tuber, rhizomes, bulbs, stem (pith), leaves, fruit (banana), pollen, woody tissues, flowers, pericarp, cotyledons, and embryo (Shannon and Garwood, 1984, Lineback, 1984, Hizukuri, 1996). Starch is also found in lower plants such as mosses, ferns, algae, bacteria and protozoa (Badenhuizen, 1965, Shannon and Garwood, 1984). The physicochemical characteristics, granular size and morphology are essentially typical to the biological origin of the starch (Swinkels, 1985a, Badenhuizen, 1963).

2.2 Uses of starches

It has been estimated that by the year 2000 more than 900 million metric tonnes of starch will be produced worldwide from cereals alone (Zobel, 1992). Starch from high-amylose genotypes containing the amylose-extender allele (*ae*) could be used as an important source of resistant starch that serves as dietary fiber (Yue and Waring, 1998, Garcia-Alonso *et al.*, 1998). High-amylose starch can also be used in food coatings, where it forms a barrier to prevent moisture loss and fat uptake during frying (Van Patten and Freck, 1972). In addition, genotypes containing various combinations of the recessive alleles such as dull (*du*), horny (*h*), sugary-2 (*su2*), and amylose-extender (*ae*) are useful in the production of starch jelly candy because cooking temperatures can be reduced to 116°C from the conventional cooking temperature of 170°C (Furcsik and Mauro, 1990). Starch's main use is as a thickener, but it also functions as an adhesive, binder, encapsulating agent, film former, filler (surimi gel), and gelling agent, with numerous other applications both in the food and non-food applications (Mauro, 1996).

In its native granular form, starch has few uses. To release the polymer properties, granule disruption and sometimes also modification are necessary. These can be achieved by chemical (acid modified e.g. glucose syrup) and/or physical processes (pyroconvertion e.g. British gum). Starches from maize, rice, potato, wheat, cassava (tapioca or yucca), sago palm, sorghum, barely, and other grains and roots serve as raw materials for the manufacture of hydrolystate products world wide (Roller, 1996). It has been estimated that about 70% of the

world production of maize starch is converted into glucose-containing sweeteners such as glucose syrups (Schenck and Hebeda, 1992, Howling, 1992). Starch has been subjected to acid treatment in order to produce sweet materials ("soluble starch") since 1811 (Kirchoff), which finally led to the commercial production of dextrose (D-glucose) (BeMiller, 1965, Wurzburg, 1987, Robyt *et al.*, 1996). Glucose syrups are largely composed of dextrose and have a dextrose equivalent (DE) values between 20 and 80 (Howling, 1992). Dextrose equivalent is a measure of the percentage of glucosidic bonds that are hydrolyzed. Syrups can be made with acid hydrolysis alone. However, at about 40 DE, side reactions start to be important and dark-coloured (undesirable) syrups are obtained (Hoseney, 1994).

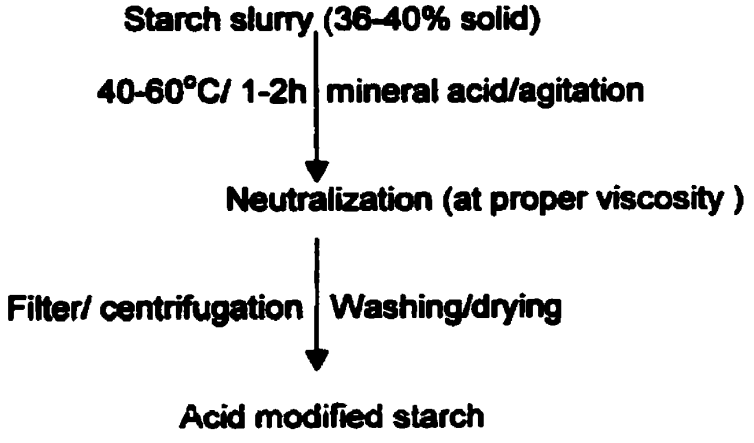
Low-DE syrups, called dextrins. Dextrins are made with acid or a combination of acid and α-amylase (Hoseney, 1994). Dextrins are bland and nonsweet carbohydrates, and their gel-forming capacity mimics the texture and mouthfeel of fat (Chun *et al.*, 1997). Maize starch (corn starch) maltodextrin, tapioca dextrin, potato starch maltodextrin, β-glucan amylodextrin from oat, and potato-based modified starch are widely used as fat replacers in food products such as margarines, salad dressing, frozen desserts, and bakery products (Haumann, 1986, Hudnall *et al.*, 1991, Sobczynska and Setser, 1991, Alexander 1992, Niemann and Whistler, 1992, Chronakis, 1998). Furthermore, maltodextrins are applied, as spray-drying aids for flavors and seasonings, carriers for synthetic sweeteners, flavor enhances, and bulking agents (Wang and Wang, 2000). The small granule rice starch and the small size of wheat starch are also capable of

forming similar highly absorbent starch aggregates (Alexander, 1996). Chun *et al.*, (1997) have shown that rice amylodextrins prepared by hydrolyzing rice starch in acidic (4% HCl) alcohol (70%) solutions at 78-80°C was readily solubilized with warm water (50°C). Emulsion prepared by replacing a portion of the oil (used in the formulation of a mayonnaise-type emulsion) with rice amylodextrin, exhibited small and uniform droplets and displayed high viscosity and stability. This suggests that amylodextrins could be used as fat replacers (Chun *et al.*, 1997).

To produce high-DE syrups, glucoamylase must be used. Glucoamylase produces glucose from the nonreducing end of the starch chain and can hydrolyzed both α- (1→4) and α- (1→6) bonds. High-DE syrups do contain high levels of glucose and thus are relatively sweet (Hoseney, 1994).

2.2.1 Acid modified starches

During the process of acid modification, the acid hydrolyzes the glucosidic linkages, shortening the chain length. Acid modified starches are produced as shown below (Wurzburg 1987, Howling, 1992).



Acid modification alters a number of physicochemical properties of starch. The primary objective in acid modification is to reduce the hot paste viscosity of starch. The hot pastes of acid-modified starches are relatively clear fluids, more Newtonian in behavior and less thixtropic (Wurzburg, 1987). For instance, acid converted waxy maize starches are widely used as adhesives in the production of remoistening gum tapes. The low viscosity starches may be desirable as textile warp sizing agents to increase yarn strength and abrasion resistance in the weaving operation, paper surface sizing, paper coating, and detergents (Solarek, 1987). Acid-modified starches substituted with cationic and sulfo-succinate groups yield improved performance in high alum paper stock systems (Solarek, 1987). Some other industrial uses of acid treated starches are as follows: (a) as a premodification stage for the production of cationic and amphoteric starches (Solarek, 1987); (b) for preparation of starch gum candies (Solarek, 1987) (d) for manufacture of gypsum board for dry wall construction (Solarek, 1987).

Nonfood interest in starches is also growing, especially for high-amylose starch which are used increasingly in the production of biodegradable packaging materials (Fergason, 1994a). The use of starch as a replacement for carbon black has been studied in detail (Otey and Doane, 1984). Cross-linked starch xanthate has been incorporated into rubber to provide reinforcement to the same extent as medium grades of carbon black (Otey and Doane, 1984). Ethanol produced by fermentation of starch hydrolyzates may be used as a motor fuel when blended with gasoline at a level of 10% (Watson, 1984). It has been

reported that the total substitution potential of starchy products for detergents lies around 50-60% and 65-75% of powder and liquid, respectively (Koch *et al.*, 1993).

2.3 Starch granule morphology

All starches are distributed in plants in the form of granules, these differ in size and shape according to botanical source in which they are assembled, and many different forms are found in nature [Jane *et al.*, 1994] (**Table** 2.1). The size of starch granules, vary from 0.5 to 175 μm in diameter (Zobel, 1988a, Zobel, 1992, Biliaderis, 1998). Leaf starches, which are produced directly by photosynthesis, are generally the smallest (less than 1μm) whereas the root starches may be as large as 175 μm (Hizukuri, 1986, Biliaderis,1998).

Oat starch displays small irregularly shaped, polygonal granules, having an average granule diameter of 2-15μm and are aggregated in the native state (Hoover and Vasanthan, 1992, Jane *et al.*, 1994). These granules are characterized by a smooth surface (Hoover and Vasanthan, 1992). Oat starch granules are not embedded in a continuous protein matrix (Zhou *et al.*, 1998). Oat starch has little commercial value since starch granules cannot easily be separated from the other components of the grain (Zhou *et al.*, 1998).

Rice starch has the smallest granules of all commercially available starches (Swinkels 1985b, Jayakody, 1991). Rice starch occurs as compound granules tightly bound in a protein matrix. The granule is very small with diameter of

Table 2.1 Granular shapes and sizes of starches from various botanical sources

Source of starch	Granule shape	Granule size (μm)	Reference
Cereal			
Normal maize	-	5-20	Jane *et al.*, 1994
Normal maize	round or polyhedral	15	Lineback, 1984, Hoseney, 1994
Normal maize (wild)	round or polyhedral	30	Buleon *et al.* 1998
Normal maize	round or polyhedral	3-26	Blanshard 1987
Normal maize	round, polygonal	2-30	Swinkels 1985b
Amylomaize V	-	10-15	Jane *et al.*, 1994
Amylomaize V	round irregular sausage shaped	25	Lineback 1984
Amylomaize VII	-	6-15	Jane *et al.*, 1994
Amylomaize VII	highly elongated irregular filament	5-25	Buleon *et al.* 1998
Waxy maize	-	5-18	Jane *et al.*, 1994
Waxy maize	round, polygonal	2-30	Swinkels, 1985b
Waxy maize	round	15	Hoseney, 1994
Waxy maize	-	12-15	Mussulman and Wagner, 1968
Rice (mature)	polyhedral	3-5	Champagne, 1996
Rice (individual granule)	polygonal	2-13	Hoseney, 1994
Rice (individual granule)	polygonal	3-8	Jane *et al.*, 1994, Linrback 1984
Oat (individual granule)	polyhedral (sometimes ovoid/hemispherical)	3-10	Linrback, 1984, Hoseney, 1994
Oat	compound	2-15	Jane *et al.*, 1994
Oat	compound	15	Buleon *et al.* 1998
Oat	polyhedral to irregular	6-10	Hoover and Vasanthan, 1992

Cont.,

Sorghum	round	35	Lineback, 1984
Wheat	lenticular or round	20-35	Hoseney, 1994
Barley	round or elliptical	20-25	Lineback, 1984
Root and tuber			
Potato normal (wild)	large oval	40	Buleon *et al.* 1998
Potato	large oval	40	Lineback, 1984
Tapioca	truncated, spherical, oval	5-25	Jane *et al.*, 1994
Legume			
Pea *RR* (wild)	oval	30	Buleon *et al.* 1998
Pea *rr*	compound	50	Buleon *et al.* 1998
Pea *rbrb*	round	20	Buleon *et al.* 1998
Pea *rr rbrb*	compound	-	Buleon *et al.*,1998
Chick pea	spherical, oval	10-27	Jane *et al.*, 1994
Cowpae	spherical, oval	10-35	Jane *et al.*, 1994
Mung bean	oval, irregular	10-27	Jane *et al.*, 1994
Lentil	ellipsoidal	10-20	Jane *et al.*, 1994

3-8 μm, polygonal and irregularly shaped (Swinkels, 1985b, Hoseney, 1994, Jane *et al.*, 1994, Champagne, 1996). The amyloplast contains 20-60 of the small granules forming a spherical to ellipsoidal cluster, varying from 7 to 39 μm in diameter (Champagne, 1996).

Normal maize has irregularly shaped granules with a number of faces (polyhedric) and relatively sharp edges. The granules vary in size between 3 and 26μm (Swinkels, 1985b, Jane *et al.*, 1994). Maize starch granules can be categorized by morphology into four groups: spherical angular, dimpled, and irregular (Fannon *et al.*, 1992). The amylose extender (*ae*) allele genes in amylomaize has been shown to result in both greatly elongated granules and granules with protrusions. The number of protrusions increases with the *ae* gene dosage (Katz *et al.*, 1993). As the amount of amylose increases (amylose 27-70%) in the maize starches, the granules loose their polygonal shapes. Amylomaize starches (amylomaize-V and -VII) have relatively smooth irregularly shaped granules (Jane *et al.*, 1994). Waxy maize starch has irregularly shaped granules similar to the granules of normal maize starch but with a varied polygonal appearance in which the individual faces are not as specific and have rough surfaces (Jane *et al.*, 1994).

2.3.1 Pores and fissures on starch granules

The small openings which are randomly distributed over the surface of starch granules are called "holes", "pin holes", "microscopic pores" or "cavities" (Fannon *et al.*, 1992, Baldwin, 1994). These features were first reported using

scanning electron microscopy (SEM) by Hall and Sayre (1970). Fannon *et al.*, (1992, 1993) have observed (using transmission electron microscopy and SEM) surface pores and interior channels in maize, sorghum, and millet starches. Furthermore surface pores have also been observed along the equatorial groove of wheat, rye and barley starch granules (Evers and McDermott, 1970, Evers *et al.*, 1971, Dronzek *et al.*, 1972, Hood and Liboff, 1983). The surface pores and interior channels are believed to be naturally occurring features of the starch granule structure, with the pores being the external openings of the interior channels. The interior channels in the starch granule were reported to be "serpentine" i.e. to have a tortuous path, and to be roughly in the radial direction (Fannon *et al.*, 1993). The presence of these holes may affect the properties of the starch and has implications for the internal structure of the starch granule (Whistler *et al.*, 1959, Fannon *et al.*, 1992). Whistler *et al.*, (1959) and Whistler and Spencer (1960) further reported that the chemical reactivity of maize starch granules is greatest at the granular surface and at the surface of cavities, indicating that both holes and surfaces could be important sites of chemical and enzymic attack. Furthermore, it has been postulated that these cavities may connect with the interior granule hole and allow macromolecules, including enzymes, direct access to the granule interior (Whistler *et al.*, 1955, Whistler and Spencer, 1960, Gallant *et al.*, 1972, Fannon *et al.*, 1992). Cavities have been observed in normal maize and waxy maize starch granules (Huber and BeMiller, 1997). Water and ions can enter granules so easily that maize starch gives a complete exchange of all hydroxyl groups within approximately 60 minutes after

being placed in deuterium oxide (D_2O) (Taylor *et al.*, 1961). Pores on maize starch are usually observed on smooth spherical granules and often occur in clusters (Fannon *et al.*, 1992).

Surface pores or fissures have been observed over the entire granular surface of maize starches (Fannon *et al.*, 1992). Huber and BeMiller (1997) showed the presence of an internal cavity at the hilum of normal maize and waxy maize starch. Channels connect these cavities to the granular surface. However, surface pores or fissures have been not reported on the surface of oats starches (Fannon *et al.*, 1992, Hoover and Vasanthan, 1992, Jane *et al.*, 1994). Cavities were also shown to be present at the hilum of rice starch (Baldwin *et al.*,1994). Fannon *et al.*, (1993) have postulated that all starch granules contain pores and channels that are unobserved either because they are covered over with sputter coating materials or because they are too small to be observed by the electron microscope, yet large enough for passage for water, reagents, and macromolecules. Whistler (1959) reported that the percentage of cavitated granules was consistent, regardless of the drying method used, including freeze-drying, which indicates that pores and channels into the granule are not an artifact of drying, although they could be altered or enlarged by drying. However, the biological origin of the canals is not known.

2.3.2 Growth rings or shell

The amyloplast grows by apposition (centrifugal deposition of successive layers). There is now conclusive evidence that starch granules do not arise as a

coacervate by rapid crystallization, but that they grow over an extended period of time (May and Buttrose1959) by apposition (Badenhuizen and Dutton, 1956). The new ring (layer or shell) deposited on the outside of the previous ring varies in thickness, depending upon the amount of carbohydrate available at that time. These rings represent concentric shells or layers of alternating high and low refractive index, density, crystallinity and resistance or susceptibility to chemical and enzymatic attack. The regions between the dense rings are apparently more amorphous and are more evident after treatment of the starch with dilute acid or enzymes (Lineback, 1984, Hoseney, 1994). However, Buttrose (1960) pointed out that some endogenous rhythm may regulate transport of carbohydrate substrate to starch-synthesizing tissue, resulting in fluctuations in density of packing of molecules.

2.3.3 Structure of starch granule

The botanical point of origin of the starch granule is known as the hilum (Jane *et al.*, 1994, Hancock and Tarbet, 2000). Starch granules from various botanical sources may have very similar basic chemical compositions yet still exhibit wide differences in physicochemical properties, due not only to factors such as the amylopectin-to-amylose ratio, but perhaps equally important, the arrangement of these components in the amorphous and crystalline domains of the granules (Villwock *et al.*,1999).

Differences have been reported between the exterior and interior of granules which would indicate that the relatively inert nature of native starch is most likely

to be due to a different arrangement of the polysaccharide at the granule surface to the interior (Evers *et al.*, 1971). Polarization optic studies show it to be a spherocrystal with the chains of glucose residues oriented radially, the radial orientation being confirmed by X-ray analysis (Kreger, 1951). The visibility of rings, referred to above, has been shown to be due to a regular decrease in refractive index from the inside to the outside of each shell, with a sudden discontinuous rise at the boundary of the next outer shell. Distinct knowledge of how the branched and unbranched molecules are distributed and crystallized is lacking.

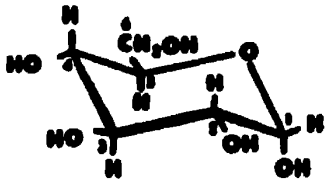
2.4 Structure of starch and components

2.4.1 Major components

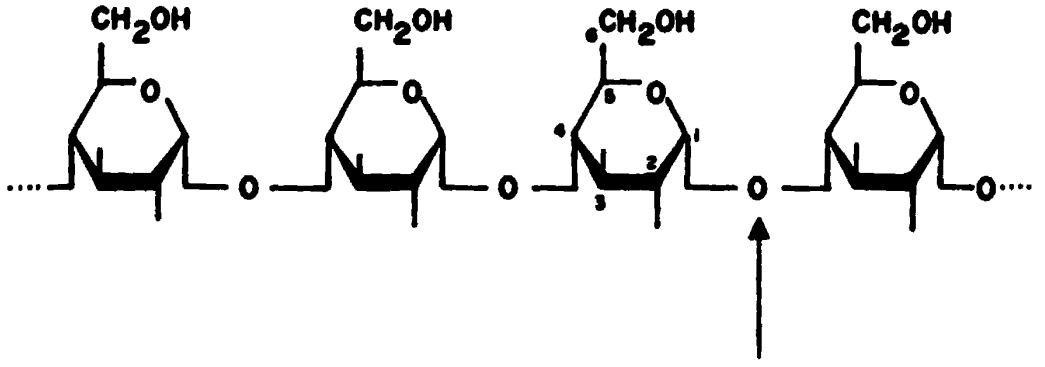
2.4.1.1 Amylose

Amylose is found with molecular weights ranging from 1x 10^5 to 2x10^6 g/mol and with the number of glucose residues per molecule (DP) ranging from 930 to 4920 (Young, 1984, Galliard and Bowler, 1987, Hoover, 1995). Chemical and biochemical evidence have indicated that the amylose chains of starch are composed of D-glucose units, linked by α-(1→4) bonds (**Figure** 2-1). Most commercial starches contain about 25% amylose, but the amylose content of starch granules varies with the botanical source (**Table** 2.2). Amylose is considered to be essentially linear, but it also contains a few branches. this has been confirmed by β-amylase and gel-permeation chromatography and high-performance size-exclusion chromatography of many starches from sources (Hizukuri *et al.*, 1981, Takeda *et al.*, 1984). The incomplete enzymic hydrolysis

Figure 2-1 Schematic diagram of α-D glucose and linear-chain structure of amylose



α-D-Glucose



α-(1→4) glycosidic linkage

Table 2.2 Amylose content of starches from different botanical sources

Starch source	Amylose (%)	Reference
Cereal		
Normal maize	26.2	Tester, 1997a
Normal maize	26.5-27.5	Shi *et al.*, 1998
Normal maize	28.0	Cheetham and Tao, 1988
Normal maize	28.6	Qian *et al*, 1998
Normal maize	29.4	Jane *et al.*, 1999
Normal maize	29.9	Hoover and Manuel, 1996
Normal maize	30.0	Morrison *et al* .,1993a
Maize	40.0	Cheetham and Tao, 1988
Amylomaize V	52.0	Jane *et al*, 1999
Amylomaize V	56.0	Cheetham and Tao, 1988
Amylomaize V	56.4-57.2	Shi *et al.*, 1998
Amylomaize V	65.5	Hoover and Manuel, 1996
Amylomaize V	65.0	Cheetham and Tao, 1988
Amylomaize VII	60.0-73.0	Buleon *et al.*, 1998
Amylomaize VII	68.0	Jane *et al*, 1999
Amylomaize VII	69.4-72.6	Shi *et al.*, 1998
High amylomaize	84.0	Cheetham and Tao, 1988
Newly developed low amylopectin starch	87.7-92.1	Shi *et al.*, 1998
Waxy maize (Amioca)	0.2-1.2	Shi *et al.*, 1998
Waxy maize	0.5	Buleon *et al.*, 1998
Waxy maize	0.8	Tester, 1997a
Waxy maize	1.2	Hoover and Manuel, 1996

Cont.,

Rice (waxy)	0.3-1.1	Morrison and Azudin, 1987
Rice (non waxy)	6.6-28.6	Morrison and Azudin, 1987
Rice	17.30	Tester, 1997a
Rice (soft)	18.4-22.5	Ong and Blanshard, 1995
Rice	20.0	Morrison *et al.* 1993a
Rice	21.3	Biliaderis and Tonogni, 1991
Rice (hard)	22.4-29.5	Ong and Blanshard, 1995
Rice	25.0	Tester, 1997a
Oat	19.4	Hoover and Vasanthan, 1992,1994a,b
Oat	25-29	Morrison *et al.*, 1984
Oat	27-29	Gudmundsson and Eliasson, 1989
Oat	27-30	Tester and Karkalas, 1996
Oat	29.2	Shamekh *et al.*, 1994
Oat	30.3-33.6	Hartunian-Sowa and White, 1992
Root and tuber		
Potato (wild)	18-21	Buleon *et al.*, 1998
Potato	21.1-25.1	Vasanthan *et al.*, 1999
Potato	24.7	Hoover *et al.*, 1994
Legume		
Pea RR (wild)	33-46	Buleon *et al.*, 1998
Pea rr	66-76	Buleon *et al.*, 1998
Lentil	38.0	Hoover and Vasanthan, 1994a

indicates that a certain degree of branching is present in amylose. Amyloses from different sources contain, on average, 2-8 branch points per molecule, the side chains ranging in length from 4 to > 100 glucose units (Hizukuri *et al.*, 1981, Takeda *et al.*, 1984). The extent of branching depends on the origin of amylose (Takeda *et al.*, 1987) and increases with the molecular size of amylose from a particular source (Greenwood and Thompson, 1959, Banks and Greenwood, 1975). In spite of its slight branching, amylose chains behave essentially like a linear polymer, forming films and complexes with ligands (Biliaderis, 1998). The conformation of amylose in solution has been the subject of controversy. The conformation has been shown to vary from helical to an interrupted helix to a random coil. In alkaline solution (KOH), and in dimethylsulfoxide (Me_2SO) amylose probably has an expanded coil conformation, while in water and neutral aqueous potassium chloride solutions it is a random coil with short, loose helical segments (Banks and Greenwood, 1971). Jane and Robyt (1985) identified (using ^{13}C NMR) expanded and compact helical conformations in aqueous amylose solutions in the absence and presence of complexing agents, respectively.

2.4.1.1.1 Genotype variation and environmental effect on amylose content

The amylose content of the starch granules varies with the botanical source of the starch, physiological maturity, and climatic, and soil conditions during grain development (Morrison *et al.*, 1984, Asaoka *et al.*, 1985, Morrison and Azudin, 1987, Campbell *et al.*, 1994, 1995). For instance high temperatures decrease the amylose content of rice, whereas cool temperatures have the opposite effect

(Champagne, 1996). Amylose concentration in starch granules reportedly increases with increasing physiological maturity of the amyloplast from normal maize, amylomaize and many other varieties, except waxy (*wx*) genotypes (Boyer *et al.*, 1976, Yun and Matheson, 1992). Yun and Matheson (1992) reported that amylose molecular size increases with maturation of normal maize starch, but decreases with that of amylose extender genotypes (*ae*) maize. Furthermore, it has been reported that amylose extender (*ae*) containing genotypes had reduced granule size when compared to normal or to waxy (*wx*) maize starches (Katz *et al.*, 1993).

2.4.1.1.2 Location of amylose and co-crystallization of amylose

Comparison of the amylose content in starch, at different stages of maturity has shown that amylose is more concentrated at the periphery of the granule (Boyer *et al.*, 1976). Jane *et al.*, (1992) have shown (by cross-linking studies on corn and potato starches) that amylose is interspersed among amylopectin molecules.

The effects of varying amylose content on the internal granular structure of normal, waxy and amylomaize starches were studied by Jenkins and Donald (1995). They showed that in each case although the amylopectin cluster size remained constant, increasing the amylose content had the effect of increasing the size of the crystalline portion of the cluster (**Figure** 2-2). The above authors postulated that amylose acts to disrupts the packing of amylopectin chains within the crystalline lamellae (**Figure** 2-3). Supporting evidence for this hypothesis was provided by the apparent reduction in crystalline lamella electron density with increasing amylose content.

Figure 2-2 Effect of varying amylose content on the electron-density profiles representing the internal structure of maize starches (Jenkins and Donald, 1997, reproduced with permission)

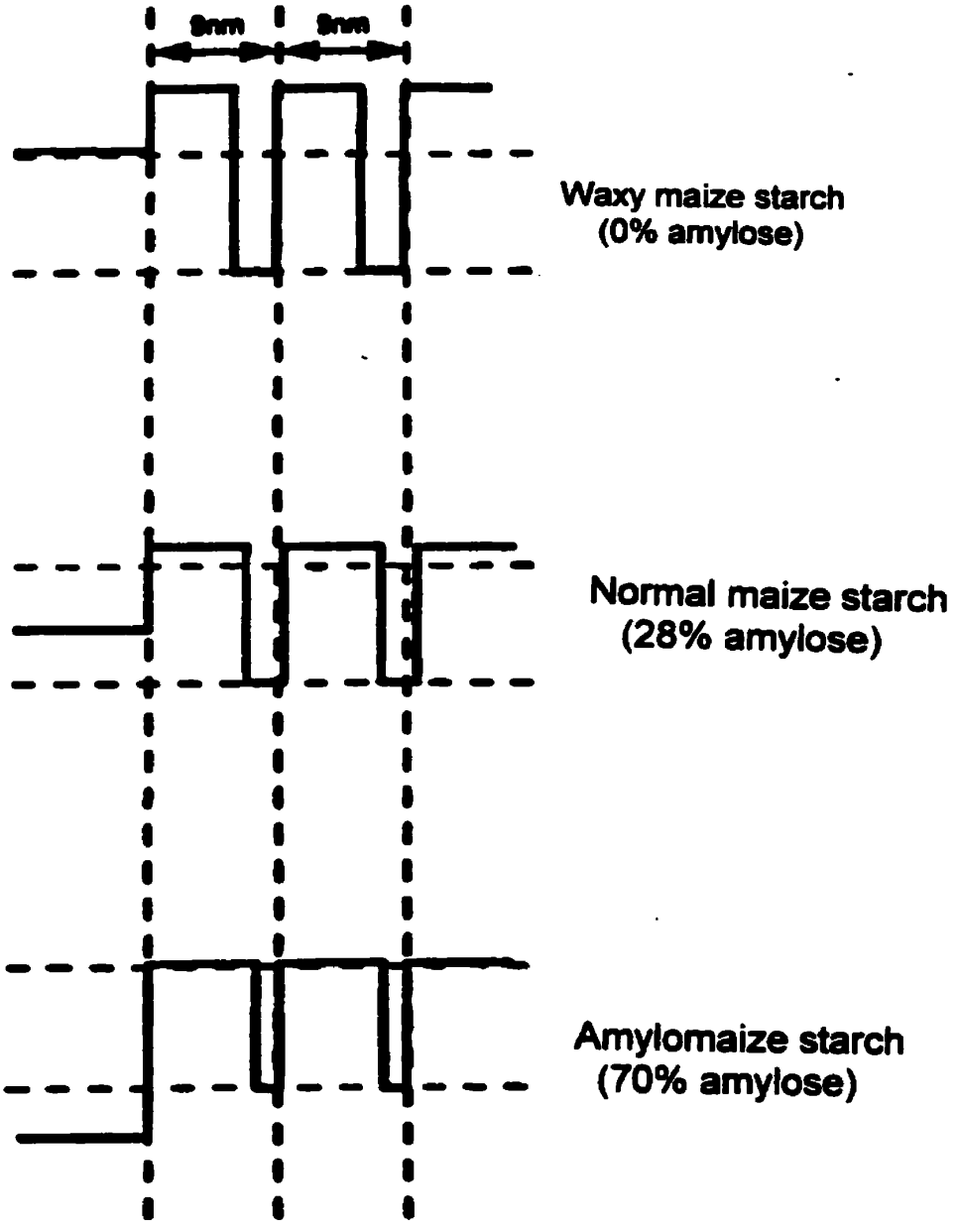
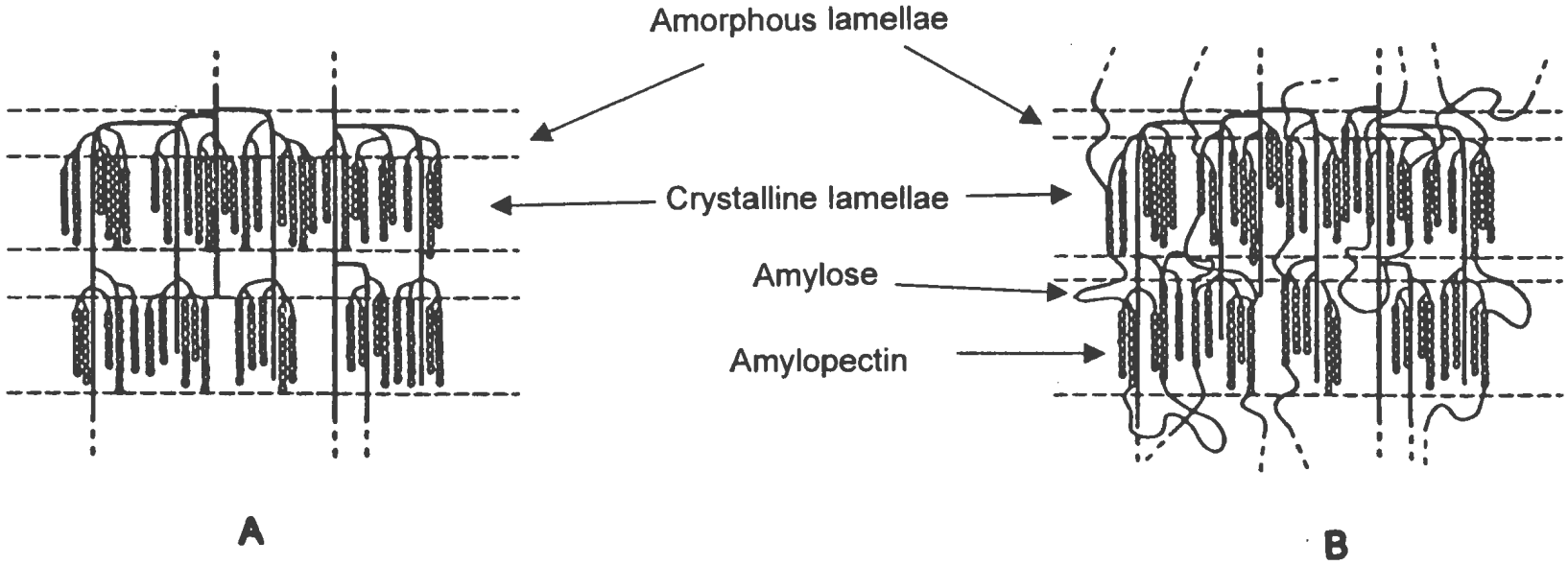

3mm
3mm
Waxy maize starch
(0% amylose)
Normal maize starch
(28% amylose)
Amylomaize starch
(70% amylose)

Figure 2-3 A possible mechanism to explain the disruption of amylopectin double helical packing by amylose. (a) Amylopectin structure with no amylose present. Small crystalline lamellar size (b) Co-crystallinity between amylose and amylopectin pulls a number of the amylopectin chains out of register (Jenkins and Donald, 1995, reproduced with permission)


Amorphous lamellae
Crystalline lamellae
Amylose
Amylopectin
A
B

The above authors put forward two mechanisms to explain the disruptive effect of amylose: 1) a co-crystallinity between amylose and amylopectin; 2) penetration of amylose into the amorphous regions of the cluster where the branch points are located). At present, insufficient evidence is available to discriminate between the two mechanisms.

2.4.1.1.3 Amylose inclusion complexes

The formation of inclusion complexes between starch chains and a hydrophobic guest molecule has long been reported (Mikus *et al.*, 1946). The long linear nature of amylose gives it some unique properties, such as the ability to form complexes with iodine, organic alcohols, or acids. Such complexes are called *clathrates* or *helical inclusion compounds* (Hoseney, 1994).

2.4.1.1.3.1 Amylose-lipid complex

Amylose in the single helical (V) conformation has 6 glucosyl residues per turn (with bulky ligands there are 7 or 8), stabilized by hydrogen bonds between the hydroxyl groups of adjacent glucosyl residues, 2-OH-----3'-OH interturn hydrogen bonds 2-OH -----6' OH and numerous intra- and intermolecular Van der Waals contacts located on the outer surface of the helix (Banks and Greenwood, 1975, Rappenecker and Zugenmaier, 1981, Blanshard, 1987, Biliaderis, 1998), and the helix cavity is effectively a hydrophobic channel. The hydrocarbon chains of the fatty acid or lipid lies inside the hydrophobic channel of the amylose helix (**Figure** 2-4) and is stabilized by Van der Waals contacts with adjacent C (5)- hydrogen of mylose, but the polar ends of the lipids are not inside the helix cavity (Godet *et al.*, 1993).

Figure 2-4 Schematic illustration of amylose-lipid complex (Adapted from Carlson *et al.*, 1979)

The 'V'- X-ray pattern of amylose-lipid complexes is found in high-amylose starches, and in starches from plants containing genes such as amylose extender and in dull or sugary starches (Zobel,1988a). Proof that amylose-lipid inclusion complexes do exist in native starch granules and that they are not artifacts formed during starch isolation was obtained by ^{13}C CP/MAS-NMR spectroscopy (Morrison *et al.*, 1993a; Morrison *et al.*, 1993b; Morrison *et al.*, 1993c).

Monoacyl lipids will induce the formation of amylose-lipid complexes during gelatinization and also restrict granular swelling (Buleon *et al.*, 1998). Furthermore, hydrocarbon chains of internal lipids suppress hydration of the starch granule amorphous regions and thereby influence granule swelling, amylose leaching, and gelatinization of starches (Lorenz, 1976, Maningat and Juliano, 1980, Goshima *et al.*, 1985, Tester and Morrison, 1990a, Vasantahan and Hoover, 1992a).

2.4.1.1.3.2 Amylose-iodine complex

The reaction between amylose and iodine has been known for over a century. Rundle and Baldwin (1943) proposed that the iodine component of the complex is present in a unidimensional array within any amylose helix with six glucose residue per turn. Teitelbaum *et al.*, (1978, 1980) studied the structure of the amylose-iodine complex using Raman and Mossbauer spectroscopy and postulated that the principal chromophore was the pentaiodide anion (I^{5-}).

2.4.1.2 Amylopectin

Amylopectin is one of the largest biomolecules found in nature (Falk *et al.*, 1996). The molecular mass of amylopectin has been reported to be several hundred thousand to as high as 10^9 Dalton (Shannon and Garwood, 1984, Colonna and Mercier,1984, Kirby, 1987, Yalpani, 1988, Hoseney, 1994, Buleon *et al.*, 1998, Hancock and Tarbet, 2000). Amylopectin molecules grow from the hilum (Hancock and Tarbet, 2000).

2.4.1.2.1 Branch linkages of amylopectin

The schematic representation of amylopectin is shown in **Figure** 2-5. Figure 2-5 illustrates the α-(1→4) backbone, α-(1→6) linked branch points, and the relatively short α-(1→4) branches that characterize this molecule. Amylopectin is a highly branched molecule and the major component of most starches. Its fine structure plays a critical role in the physicochemical properties of starch (Tester, 1997a, Jane *et al.*, 1999). The glucose units with α-(1→6) linkages are the branching points of the amylopectin molecule and make up to 4-5% of the total glucose units in amylopectin (Swinkels, 1985b, Kirby, 1987, Manners, 1989, Cura and Krisman 1990, Eliasson and Larsson, 1993, Jane, 1997, Buleon *et al.*, 1998, Biliaderis, 1998).

2.4.1.2.2 Branch chain of amylopectin and chain length

Amylopectin has three types of chains (**Figure** 2-5): A chains are unbranched and composed of glucose linked α1→4; branched B-chains, composed of glucose linked α1→4 and α1→6; and C-chains, made up similar to B-chains but

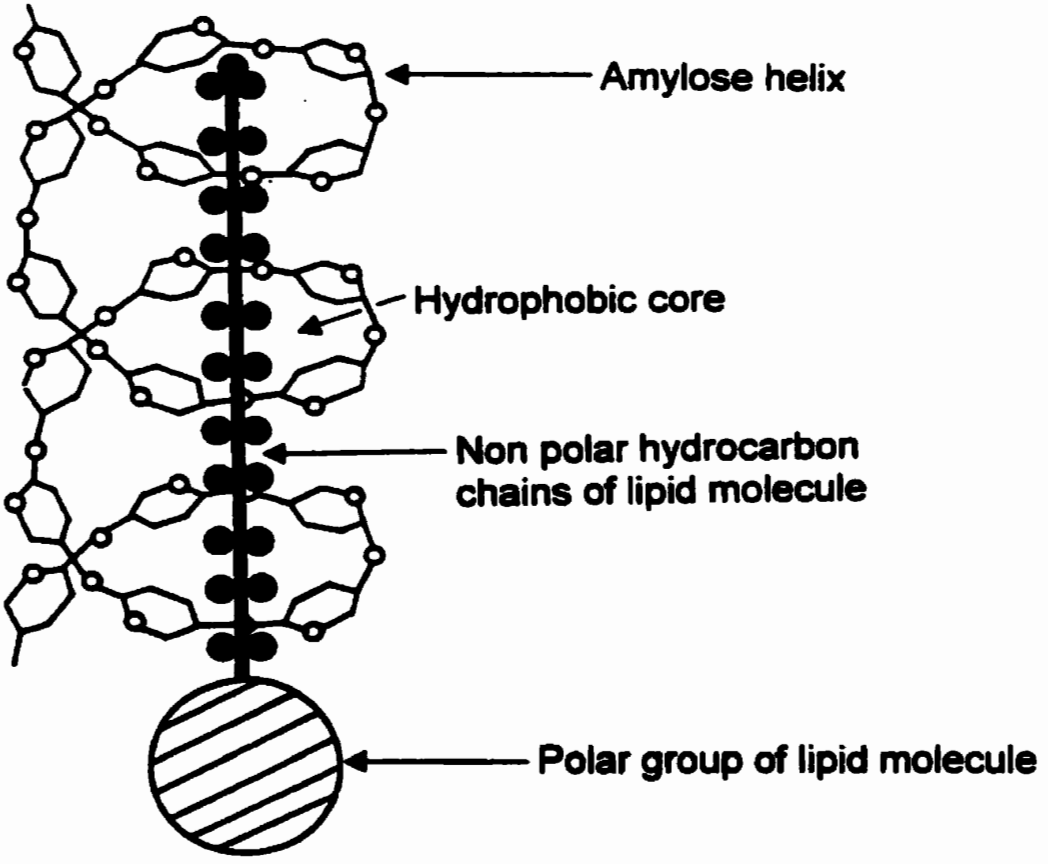
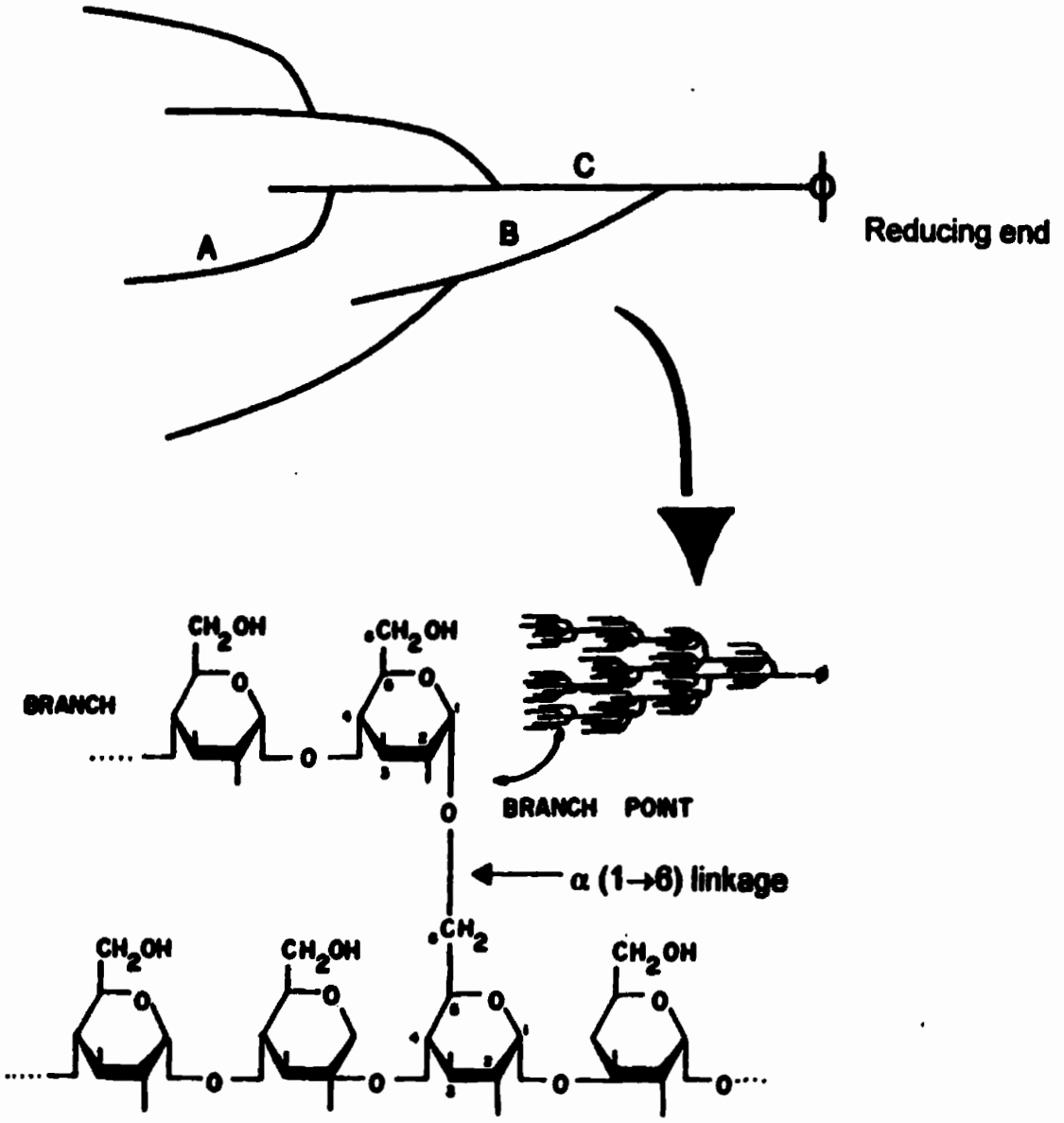

Amylose helix
Hydrophobic core
Non polar hydrocarbon chains of lipid molecule
Polar group of lipid molecule

Figure 2-5 Schematic diagram of amylopectin with a branch point at the 1→6 position


C
A
B
Reducing end
BRANCH
BRANCH POINT
α (1→6) linkage

contains the only reducing group (unbound C-1) in the molecule. The branched nature of amylopectin explains why it does not form threads or films and is unable to retrograde (Guthrie and Honeyman, 1968, Eliasson and Larsson, 1993, Yuan *et al.*, 1993). The outer A branches of the amylopectin molecules form double helices that are arranged in crystalline domains (Sarko and Wu, 1978). Branching points are arranged in clusters which are not distributed randomly throughout the macromolecule. The currently accepted amylopectin structure involves short amylopectin chains forming double helices and associating into clusters (Robin *et al.*, 1974)(**Figure** 2-6). These clusters pack together to produce a structure of alternating crystalline and amorphous lamellar composition. The ratio of A to B chains ranges from 4:1 to 9:1. The α (1→6) linkage is present at the branch points of the amorphous lamellae (Hoseney, 1994, Kirby, 1987, Eliasson and Larsson, 1993, Jenkins and Donald, 1995, Buleon *et al.*, 1998). The A-: B-chains ratio, which is also called the degree of multiple branching, is an important parameter . It has been reported, that during acid hydrolysis, H_3O^+ ions preferentially attack the long B chains of amylopectin (Inouchi *et al.*, 1987).

2.4.1.2.3 Amylopectin inclusion complexes

The ability to form inclusion complexes in amylopectin is much less pronounced than in amylose (Eliasson and Larsson, 1993). However, Jane *et al.*, (1999) pointed out that long chains of amylopectin have the capability of forming a helical complex with iodine. However, amylopectin is unable to form stable complex with iodine because of the short length of the unit chains.

Figure 2-6 Currently accepted model of amylopectin with chain clusters defined in terms of glucose residues individual chains (DP) and cluster size an Angstroms (Robin *et al.*, 1974, reproduced with permission)

Only a small amount of iodine (<0.6%) is bound forming a red-brown colour complex (λ_{max} 530-540nm).

2.4.2 Intermediate component of starch

Some starches contain a fraction that is neither amylose nor amylopectin known as the intermediate component (Lansky *et al.*, 1949, Banks and Greenwood, 1975, Eliasson and Larson, 1993, Jane *et al.*, 1999). The intermediate component is a blend of the two major fractions (Shannon and Garwood, 1984). There is some confusion regarding the structure of this fraction-whether it should be described as an anomalous amylopectin or an anomalous amylose (Banks and Greenwood, 1975,1968). In any case, its branching pattern differs from that of its "normal" counterpart (Manners, 1985). The criteria for classification of this component are the degree of branching and the molecular weight (Hizukuri, 1996). However, the intermediate component seems to cover a broad range in the degree of branching and molecular size, and accordingly it is difficult to fractionate (Hizukuri, 1996).

2.4.2.1 Location and amount of intermediate material in different botanical sources

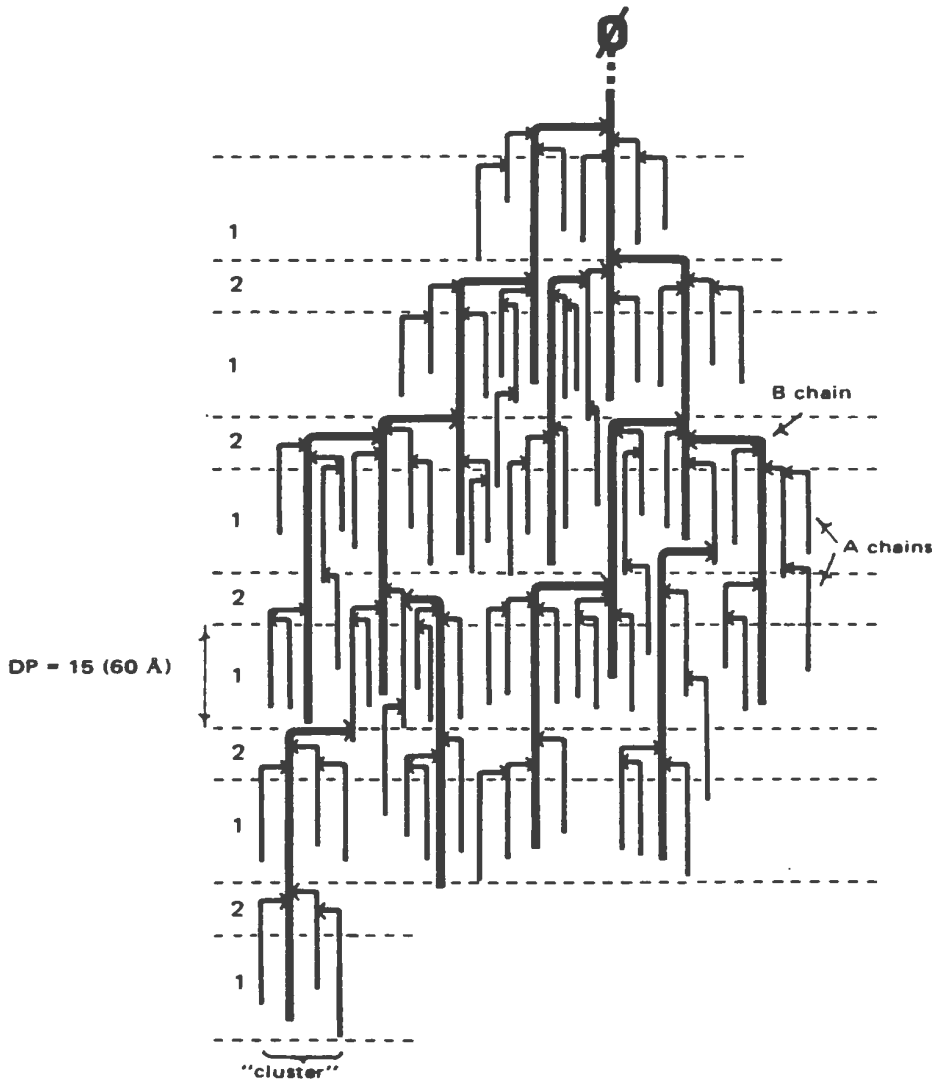
Jane and Shen (1993) reported that the intermediate component was primarily located at the periphery of the granule, indicating that the intermediate component could be amylopectin molecules whose biosynthesis had been prematurely terminated.

The intermediate material amounts to about 5-10% in most cereal starches (Greenwood, 1976, Lineback, 1984). Based on indirect evidence from iodine affinities, Lansky *et al.*, (1949) suggested that 5-7% of normal maize starch consists of intermediate material between the strictly linear and highly branched fractions. Oat starches were also reported to have around 9% intermediate materials with less highly branched and longer branch lengths than that of amylopectin (Banks and Greenwood, 1975, Wang and White, 1994a,b).

The anomalous amylopectin of amylomaize starch was shown to be a mixture of short linear amylose (DP~100) and normal amylopectin (average chain length 25) (Banks and Greenwood, 1968, Banks *et al.*, 1974). Baba *et al.*, (1987) showed that amylomaize starch contains approximately 55% intermediate material, 20% amylomaize-specific amylopectin, and 25% amylose. However, they could not properly separate and identify the structures of the intermediate material and amylomaize-specific amylopectin. Therefore, the amylopectin fraction of amylomaize starches was suggested to be a heterogeneous mixture of several kinds of molecular species, mainly intermediate materials, with minor amount of amylopectin, and branched and linear amylose with low degree of polymerization (Banks and Greenwood, 1968, Banks *et al.*, 1974, Baba *et al.*, 1987).

2.4.3 Minor components

The components present in minor quantities such as lipids, proteins, phosphorous and minerals, are generally known as minor components (Eliasson


Ø
1
2
1
B chain
2
1
A chains
2
DP = 15 (60 Å)
1
2
1
2
1
"cluster"

and Gudmundsson, 1996). These are deposited in the starch granule during development of the grain or are introduced during grain processing as contaminants (Swinkels 1985a). The minor components of starch are present either as surface materials on the granules or as internal components within the granule matrix. However, even though they are present in minor quantities, they have a dramatic effect on the physicochemical properties. Minor components associated with starches correspond to three categories, according their location (i) particulate material, composed mainly of cell-wall fragments; (ii) surface components composed mainly of enzymes, amino acids and nucleic acids; and (iii) internal components composed of lipids (Galliard and Bowler 1987, Buleon *et al.*, 1998). The exact ratio of each of the components depends on the botanical source. However, the protein and lipid are always by far the most abundant of the minor components (**Table** 2.3). Incomplete removal of contaminating substances and the presence of minor components on the surface, or within, starch granules may well be of major significance in determining the quality and functional properties of starches.

2.4.3.1 Proteins

Generally, nitrogen exists in starch granule in the form of real protein or other related forms such as peptides, amides, amino acids, enzymes and nucleic acids (Swinkels 1985a, Lineback and Rasper, 1988). The amount of protein content present in purified starch is a good indicator of starch purity. Alkali extraction appears is very effective in solubilizing protein. Therefore, careful washing of

Table 2.3 Composition (%) of some minor components of starches from various botanical sources

Starch source	Total lipids	Nitrogen	Ash	Phosphorus	Reference
Cereal					
Norma maize	0.70	0.10-0.13	0.10	0.02	Swinkels, 1985a
Normal maize	0.73	0.02	0.03	-	Hoover and Manuel, 1996
Normal maize	0.27	0.03	0.09	-	Qian *et al.*, 1998
Normal maize	0.63	0.03	0.07	-	Vasanthan *et al.*, 1997
Amylomaize V	0.95	0.03	0.02	-	Hoover and Manuel,1996
Waxy maize	0.15	0.04	0.10	0.01	Swinkels, 1985a
Waxy maize	0.22	0.02	0.02	-	Hoover and Manuel, 1996
Oat	1.13	0.05	0.03	-	Hoover and Vasanthan, 1992, 1994b
Oat	1.1-1.7	0.04-0.06	-	0.06-0.07	Gibinski *et al.*, 1993
Oat	2.1-2.5	0.14-0.15	-	0.15-0.19	Hartunian-Sowa and White, 1992
Wheat	0.80	0.07	0.20	0.06	Swinkels, 1985a
Wheat	0.70	0.04	0.02	-	Hoover *et al.*, 1994b
Wheat	0.32	0.03	0.13	-	Qian *et al.*, 1988
Root and tuber					
Potato	0.11	0.03	0.05	-	Hoover *et al.*, 1994b
Potato	0.05	0.01	0.40	0.08	Swinkels, 1985a
Tapioca	0.10	0.02	0.20	0.01	Swinkels, 1985a
Yam	0.09	0.03	0.02	-	Hoover and Vasanthan, 1994b
Legume					
Lentil	0.14	0.02	0.03	-	Hoover and Vasanthan, 1994b

crude starch with diluted alkali can reduce protein values in purified cereal starches. However, a serious problem with any starch purification procedure is selective loss of small starch granules in discarded protein and cell debris (Morrison, 1988). Approximately 10% of the starch proteins appeared to be associated with the granule surface (Galliard and Bowler, 1987). Maize and waxy maize starches contain a considerable amount of proteins (0.25-0.8%) compared to tuber and root starches (potato 0.06%, and tapioca [cassava] starch 0.01% respectively)[Swinkels, 1985a, Buleon *et al.*, 1998] (**Table** 2.3). In rice starch protein may be found on the periphery of the starch or embedded inside the granule (Yonezaki and Oshima, 1975). The high amount of proteins in the cereal starches may have the following disadvantages: form building and color formation in hydrolysates, formation of mealy flavors (Swinkels, 1985a, Galliard and Bowler, 1987), formation of Maillard products during conversion of starch to hydrolysis products (e.g. sugar syrups) (Galliard and Bowler, 1987). However, nitrogenous materials in starch hydrolysates may be unimportant, or even beneficial, if these syrups are used as fermentation substrates.

2.4.3.2 Ash (mineral)

Mineral are not distributed evenly in the grain. Most of the minerals are concentrated in the aleurone layer (Hoseney, 1994). Native starches contain mainly calcium, potassium, magnesium and sodium (Swinkles, 1985a, Galliard and Bowler, 1987, Buleon *et al.*, 1998). Cereal starches contain phosphorus, which is mainly in the form of phospholipids. Potato starch has a exceptionally

high content of ash because of the presence of phosphate monoesters. **Table** 2.3 shows the ash content of various native starches.

2.4.3.3 Lipids

Lipid content of different starches are presented in **Table** 2.3. Lipids associated with cereal starch granules have been found to occur on the surface as well as inside the granules (Morrison, 1981, Morrison *et al.*, 1993b). It has been suggested that all lipids found on the surface of starch granules have to be considered as surface lipids (Galliard and Bowler, 1987). However, the location of the lipids at the surface of starch granule is still unknown (Buleon *et al.*, 1998). The surface lipids are mainly triglycerides, with some amount of free fatty acids, glycolipids and phospholipids (Morrison, 1981, Buleon *et al.*, 1998). The internal lipids of cereal starches are predominantly monoacyl lipids, with the major components being lysophospholipids and mixtures of free fatty acids (Hargin and Morrison, 1980, Morrison, 1981, Morrison, 1988, Swinkles, 1985a). Lysophosphatidylcholine is the major phospholipid found in maize starch. The amount of free fatty acids varies from 1/3 to 1/2 of the total lipids in maize and rice starch (Morrison, 1988).

It is likely that both surface and internal lipids may be present in the free state as well as bound to starch components, either in the form of amylose inclusion complexes (Acker, 1977) or linked via ionic or hydrogen bonding to hydroxyl groups of the starch components (Hoover and Vasanthan, 1992).

Most cereal starches are of the A-type and have more lipid (around 1%) than starches of the B- and C-types (0.1%) (Hizukuri *et al.*,1980). Maize starch contains 0.8% of lipid (free fatty acid and lysolecithin, 0.62, 0.18 respectively) (Hizukuri *et al.*, 1980). Amylomaize starch has been found to contain more lipid than normal maize starch (Buleon *et al.*, 1998). The reason for the presence of starch lipids is not known. Hizukuri *et al.* (1980) reported the high amylomaize starch is capable of including more lipid and thus of lowering the lipid level inside the amyloplast membrane. Hizukuri *et al.*, (1980) suggested that lipid may play an effective role in the polymorph shift (towards the A-type from the B-type) under certain conditions. In most cereal starches there is a strong correlation between the amylose content and the lipid content. Most waxy starches have comparatively low amount of lipids (**Table** 2.3). Both lysophospholipid and amylose content increase with maturity of maize starches (Morrison and Gadan, 1987, Buleon *et al.*, 1998). The lipid content of oat starch has been found to be negatively correlated with average granule size and amylose content (Wang and White, 1994a). However, oat is unusual among cereals, in that most of the oil is located in the endosperm (Morrison, 1978). Swinkels (1985a) summarized the effects of starch lipids as: (i) reducing the water-binding capacity, swelling and solubilization of starches; (ii) giving rise to undesirable flavours by oxidation of unsaturated lipids (presumably this must be due to surface lipids, because internal, amylose-bound lipids are remarkably resistant to oxidation); (iii) forming 'inert' complexes with amylose in starch pastes and films, hence preventing part of the amylose from contributing to the thickening power of gelatinized starch; (iv)

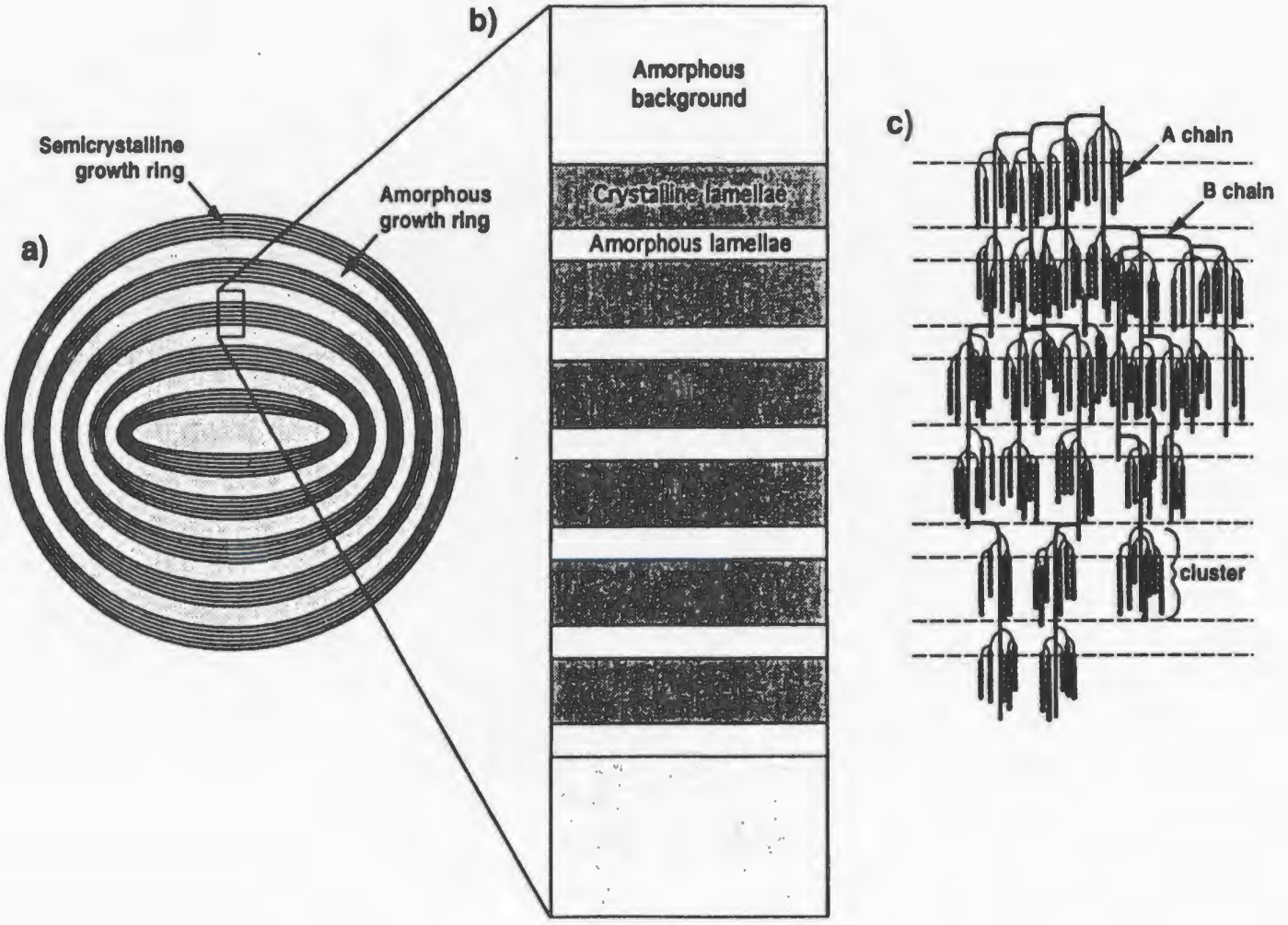
giving rise to cloudy or opaque starch paste and films, due to the presence of relatively insoluble starch-lipid complexes.

Starch damage has been reported to occur mainly during isolation of cereal starches (Evers *et al.*, 1984, Vasanthan and Bhatty, 1996). The type of damage may extend from cracks, pits, cuts and other abrasions on the surface to cleavage of amylose and amylopectin (Vasanthan and Hoover, 1992a). Therefore, there is a greater possibility that cross-contamination of surface (free and bound) with internal (free and bound) lipids, and *vice versa*, could occur respectively during cold and hot solvent extractions (Vasanthan and Hoover, 1992a).

2.4.4 Amorphous and crystalline regions of starch granules

A model of the arrangement of the amorphous and crystalline regions of starch granules is schematically shown in **Figure** 2-7a. Regions of amylopectin double helix formation fall within the crystalline lamellae, whilst the amylopectin branch points lie in the amorphous lamellae (Jenkins *et al.*, 1994, Jane *et al.*, 1997). These crystalline lamellae exist in the granule alternately with amorphous lamellae (**Figure** 2-7b). The combined thickness of crystalline plus amorphous lamellae is 9nm and 9.2nm for A-type starches and B-type starches respectively (Jenkins *et al.*, 1993, Jane, 1997). Jenkins *et al.*, (1994) postulated that most of the amylose is deposited in amorphous growth rings which represents the amorphous background. It has been shown that the clusters of amylopectin short chains are the crystalline domains of the molecule (Yamaguchi *et al.*, 1979).

Figure 2-7 Schematic diagram of starch granule structure. (a) a single granule, comprising concentric rings of alternating amorphous and semi-crystalline composition. (b) Expanded view of the internal structure. The semi-crystalline growth ring contains stacks of amorphous and crystalline lamellae. (c) The currently accepted cluster structure for amylopectin within the semi-crystalline growth ring. A-chain sections of amylopectin form double helices, which are regularly packed into crystalline lamellae. B-chains of amylopectin provide intercluster connections. Branching points for both A and B chains are predominantly located within the amorphous lamellae. (Jenkins *et al.*, 1994, reproduced with permission)


a)
Semicrystalline growth ring
Amorphous growth ring
b)
Amorphous background
Crystalline lamellae
Amorphous lamellae
c)
A chain
B chain
cluster

Furthermore, these clusters could well be the origin of the concentric layers of crystalline, relatively acid-resistant material, that have been observed in transmission electron microscopy (Coultate, 1984). However, the amorphous region of the starch granule has received scant attention, though it accounts for ~ 70% of the granule (Oostergetel and Van Bruggen, 1993). It has been pointed out that the amorphous regions are loosely packed and, thus, more susceptible to chemical and enzyme attack (Biliaderis, 1982).

2.4.5 Crystallinity

The fact that starch is a semicrystalline material has been clear since the classic work of Katz and Vanltallie (1930). The crystalline nature of the starch granule is mainly due to the branched amylopectin rather than the linear amylose (Meyer and Bernfeld, 1940, Montgomery and Senti, 1958, Jenkins and Donald, 1995). Native starch granules shows birefringence due to crystallinity within the molecule (Swinkels, 1985b, Kirby, 1987). The crystalline nature of amylopectin is remarkably important for efficient packing of the starch in the granule (Hancock and Tarbet, 2000). Different techniques have been used to determine the absolute crystallinity of native starches. However, the values obtained vary depending on the technique used for calculation of crystallinity (Khairy *et al.*, 1966) and the level of moisture content [Sterling, 1960, Cheetam and Tao, 1998] (Table 2.4).

Table 2.4 The relationship between amylose content, degree of crystallinity crystalline type, and hydration levels of starches from various botanical sources

Starch source	Amylose %	% crystallinity at moisture *			Crystalline type	Reference
		~10%	~20%	~30%		
Cereal						
Normal maize	-	39.0	-	-	A	Hizukuri, 1996
Normal maize	28.0	-	30.3	-	51.6	A	Cheetham and Tao, 1998
Normal maize	27.0	40.0	-	-	-	A	Zobel, 1988a
Normal maize	-	-	27.0	-	-	A	Cooke and Gidley, 1992
Normal maize	-	-	-	39.0	-	A	Nara *et al.*, 1978
Maize	40.0	-	21.8	-	47.9	C	Cheetham and Tao, 1998
Amylomaize V	56.0	-	19.5	-	43.7	B	Cheetham and Tao, 1998
Amylomaize V	65.0	-	17.6	-	42.7	B	Cheetham and Tao, 1998
Amylomaize VII	84.0	-	17.2	-	41.3	B	Cheetham and Tao, 1998
Amylomaize VII	55-75	15-22	-	-	-	B	Zobel, 1988a
Amylomaize VII	-	19-24	-	-	-	B	Hizukuri, 1996
Amylomaize VII	-	24.0	-	-	-	B	Khairy *et al.*, 1966
Waxy maize	0	-	41.8	-	74.6	A	Cheetham and Tao, 1998
Waxy maize	0	40.0	-	-	-	A	Zobel, 1988a
Waxy maize	0	-	28.0	-	-	A	Cooke and Gidley, 1992
Rice	17.0	38.0	-	38.0	-	A	Zobel, 1988a,
Rice	-	38.0	-		-	A	Hizukuri, 1996
Rice	-	-	-	38.0	-	A	Nara *et al.*, 1978
Rice (soft)	18.4-22.5	32.5-39.3	-	-	-	A	Ong and Blanshard, 1995
Rice (hard)	22.4-29.5	29.2-35.4	-	-	-	A	Ong and Blanshard, 1995

Cont.,							
Oat	23.0	33.0	-	-	-	A	Zobel, 1988a
Oat	-	28.2	-	-	-	A	Chun *et al.*, 1997
Wheat	23.0	36.0	-	-	-	A	Hizukuri1996,Zobel1988a
Root and tuber							
Potato	22.0	28.0	-	-		B	Zobel,1988a
Potato	-	-	-	25	-	B	Nara *et al.*, 1978
Sweet potato	20.0	38.0	-	-	-	C	Zobel, 1988a
Sweet potato	-	-	-	37	-	C	Nara *et al.*, 1978
Cassava	18.0	38.0	-	-	-	C	Zobel, 1988a
Cassava	28.8	39.5	-	-	-	-	Atichokudomchai *et al.*, 2000

* Moisture content not available

2.4.5.1 Degree of Crystallinity

Starch is biosynthesized as semi-crystalline granules with varying polymorphic types and degrees of crystallinity. However, determination of the degree of crystallinity in native starches is difficult because of small crystal size (Lineback, 1984) and the extent of hydration (Buleon *et al.*, 1998). Typically, native starch granules range in degree of crystallinity from about 15% to 45% (Zoble, 1988b). However, Lineback (1984) reported the degree of crystallinity ranges from 0 to 60%. Furthermore, in cereal starches, the sharpness of X-ray diffraction bands increases only between 9% and 25% moisture content [dry basis] (Guilbot *et al.*, 1961). High-amylomaize starches reveal poorer crystallinity and are less birefringent than normal or waxy maize starches (Banks *et al.*, 1974, Lineback, 1984, Zobel, 1988a).

There is considerable evidence that water molecules play an important role in maintaining the crystal structure. It has been reported that there is a significance increase in peak resolution and intensity upon hydration (Hizukuri *et al.*, 1964, Nara *et al.*, 1978, Buleon *et al.*, 1982, Veregin *et al.*, 1986, Buleon *et al.*, 1987, Hibi *et al.*, 1993). The positions of the water molecules do have a significant effect especially upon the 5.5° and 25.5° 2θ peaks (Blanshard, 1987). The crystallinity of starch granules can be destroyed by mechanical disruptions such as ball milling, washing or subjecting to high pressure starch at room temperature will eventually completely destroy both the optical birefringence and the X-ray patterns (Lineback, 1984, Baldwin *et al.*, 1994).

2.4.5.2 A-, B-, and C- type X-ray diffraction (polymorphic) patterns

Native starch granules contain crystalline regions as shown by their unique X-ray powder diffraction patterns (XRD). Therefore granular crystallinity can be studied with X-ray diffraction techniques. Four distinct X-ray patterns have been described; 1) The 'A'-pattern (**Figure** 2-8)[cereal starches, except high amylose varieties] (Zobel, 1988a, Cheetham and Tao, 1998); 2) The 'B'-pattern [tuber, root, stem (pith), banana (Lineback, 1984), high amylose varieties (Cheetham and Tao, 1998), and retrograded starches (Kodama *et al.*, 1978, Zobel, 1988a, Gidley, 1989, Biliaderis, 1991, Morrison *et al.*, 1993a); 3) The 'C'-pattern (a mixture of 'A' & 'B' pattern), characteristics to 40% amylose containing maize starches, legume, rhizome, sweet potato, tapioca starches (Zobel, 1988a, Gernat *et al.*, 1990, Hizukuri 1996, Cheetham and Tao, 1998); and 4) 'V'-pattern characteristic of crystalline amylose helical inclusion compounds (Eliasson and Gudmundsson, 1996, Blanshard, 1987). The A-and B-types polymorph differ clearly, but the C-type appears to be a mixture of the A- and B-types in various proportions (Sarko and Wu, 1978, Hizukuri *et al.*, 1980, Lineback, 1984). **Figure** 2-9 shows the X-ray diffractograms of maize starches of differing amylose content.

2.4.5.2.1 Factors that influence polymorpic patterns

Polymorphism of the α-glucans is one of the main characteristics of the crystalline parts in starch granules. Hizukuri *et al.*, (1980) reported that both the environmental temperature (growth and soil temperature) and the physiological

Figure 2-8 X-ray diffraction patterns of A-, B-, C-type starches with their characteristic *d*-spacing (adapted from Zobel, 1988b). A-type pattern, with strong peaks at 2θ 15.27° or with a intercrystalline spacing *d*=5.8 Angstrom (Å) and 23.40° (*d*=3.8Å), and an incomplete doublet at 2θ 17°.05 (*d*=5.2 Å)and, 18.1° (*d*=4.9 Å) (Zobel, 1988b). The *d*-spacing at 4.4Å is characteristic of amylose-lipid complex (Vasanthan and Bhatty, 1996). B-type shows a peak at 2θ 5.6-5.52 (*d*=15.8-16.0Å), a broad medium intensity peak at 2θ 15.01 (*d*=5.9Å), the strongest peak at 2θ 17.05 (*d*=5.2Å) and medium intensity peaks at 2θ 19.72 (*d*=4.5 Å) , 22.22 (*d*=4.0 Å) and 24.04 (*d*=3.7Å). There is a peak at 2θ 5° (*d*=17.7Å) which is characteristic of the B pattern. C-type is the same as A-type except for the addition of the medium to strong peak at about 2θ 5.52(*d*=16.0 Å)(Zobel, 1988b, Shi and Seib, 1992, Cheetham and Tao, 1998)

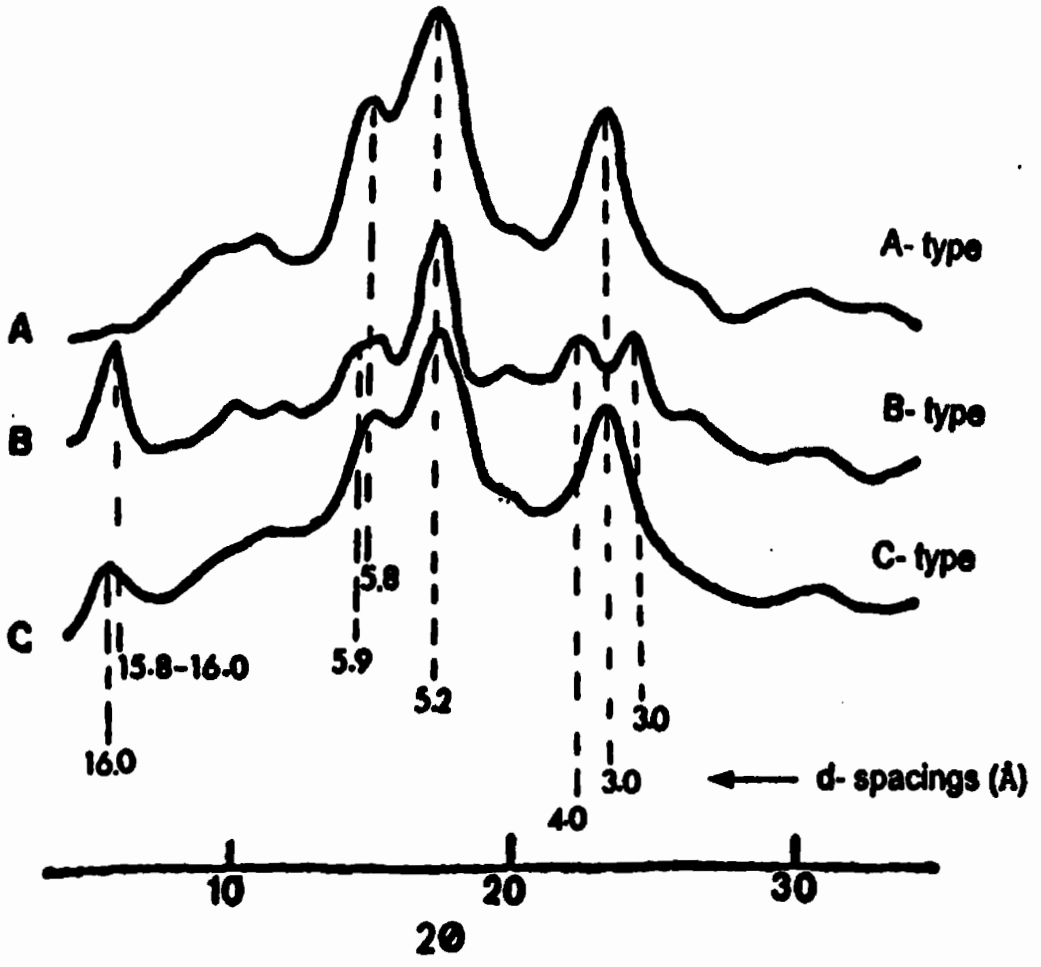
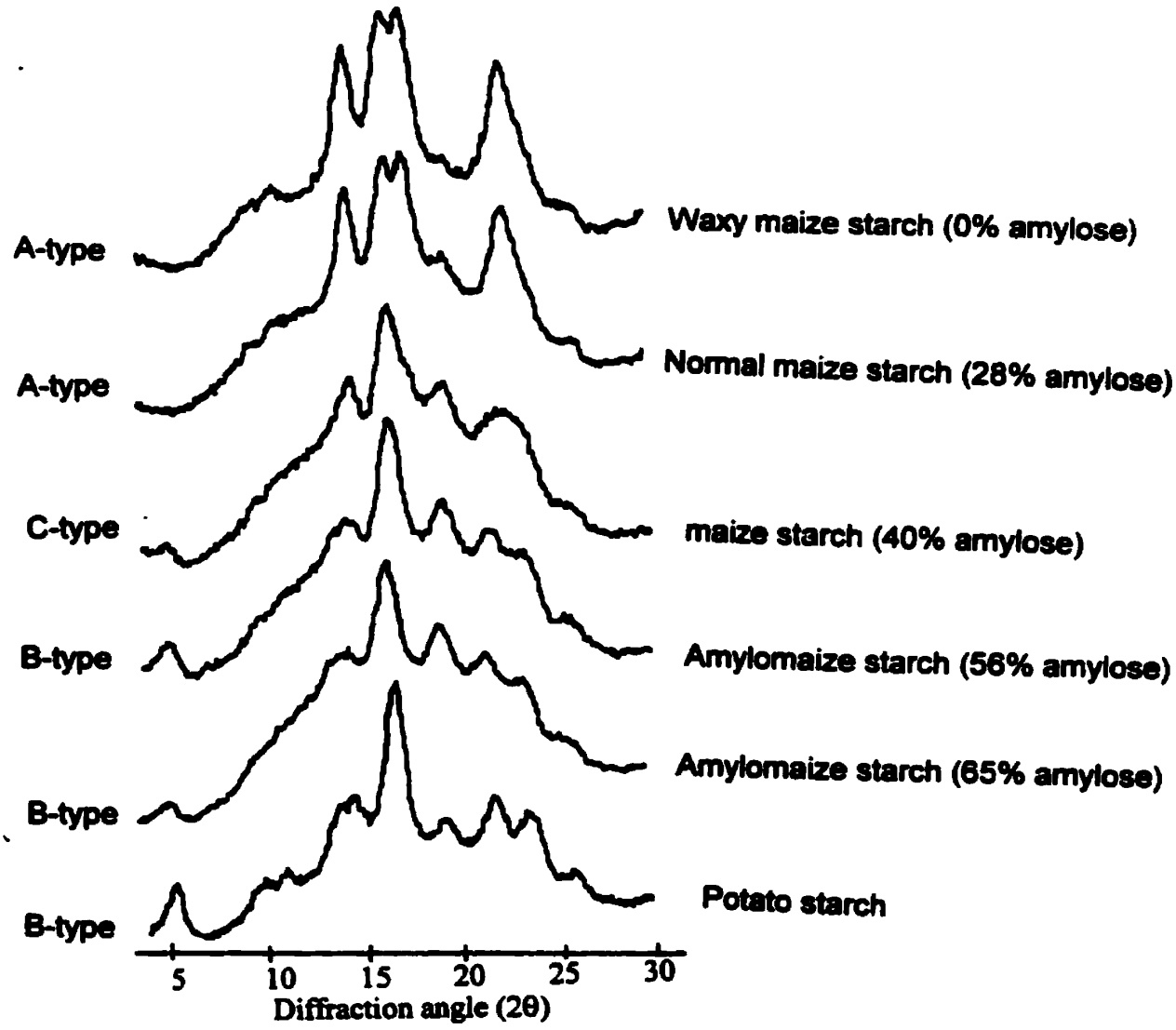

A
B
C
A- type
B- type
C- type
15.8-16.0
16.0
5.8
5.9
5.2
4.0
3.0
3.0
d- spacings (Å)
10
20
30
2Θ

Figure 2-9 Wide angle X-ray powder diffraction spectra for maize starches with different amylose contents (Cheetham and Tao, 1998, reproduced with permission)


A-type
Waxy maize starch (0% amylose)
A-type
Normal maize starch (28% amylose)
C-type
maize starch (40% amylose)
B-type
Amylomaize starch (56% amylose)
B-type
Amylomaize starch (65% amylose)
B-type
Potato starch
5
10
15
20
25
30
Diffraction angle (2θ)

conditons inside cells influence the type of polymorph. Studies have also shown that the polymorphic forms of starch crystalline structures depend on numerous factors such as α-D(1→4) glucan chain length (Robin *et al.*, 1974, Hizukuri *et al.*, 1983, Hizukuri, 1985, Hanashiro *et al.*, 1996, Pfannemuller, 1987, Gidley, 1987, Gidley and Bulpin, 1987), concentration of starch solution (Gidley, 1987, Gidley and Bulpin, 1987, Hizukuri, 1960) temperature (Hizukuri, 1961, Gidley 1987, Whittam *et al.*, 1990), and the presence of other solutes and solvents (Hizukuri *et al.*, 1960, Hizukuri *et al.*, 1980, Ring *et al.*, 1987b) and organic molecules (Gidley, 1987).

2.4.5.2.2 Chain length and polymorphic patterns

Starches with amylopectins of relatively short average branch chain lengths (DP 23-29), such as waxy maize, normal maize, and rice display the A-type X-ray diffraction pattern. The long amylopectin branch-chain lengths (DP 30-44), such as high amylomaize starches show the B-type X-ray pattern (Gernat *et al.*, 1993) and intermediate chain length is associated with C-type crystallinity (Hizukuri, 1985, Hizukuri *et al.*, 1983, Jenkins and Donald, 1995, Hanashiro and Hizukuri, 1996, Jane, *et al.*, 1997). The difference in the average chain length between the A-type and B-type starches can be as little as one glucose unit (Hanashiro *et al.* 1996). These are probably due to mixtures of A- and B-type crystallites, either within individual granules or as mixtures of A-and B-type granules (Lineback, 1984). The intermediate amylopectin branch-chain lengths (DP 26-36) display the C-type X-ray patterns (Hizukuri *et al.*,1983 and Hizukuri, 1985).

2.4.5.2.3 Arrangement of water molecules in A- and B- type polymorph

A-and B-amylose helices are right-handed (Singh *et al.*, 1993). The double helices of A-and B- structures differ in the crystalline packing of the helices and the water content (Wu and Sarko, 1978a,b). The unit cell of B-amylose has much more space available for water than the A-amylose unit cell (Gidley and Bociek, 1985). The A-amylose and B-amylose crystallizes in orthogonal 8 and hexagonal 36 water molecules per unit cell respectively (**Figure** 2-10). The lateral distance between the helices are nearly identical in both A-and B-amylose which suggest a possibility of interconversion of the two structures (Sarko and Wu, 1978, Wu and Sarko, 1978a,b). However, Kainuma and French (1972) reported that water is not a integral constituent of B-starch crystals.

2.5 Starch properties

2.5.1 Granular swelling

Granule swelling is an important physical parameter which has been extensively studied because of its influence on physicochemical properties of starches (Leach *et al.*, 1959). Most starches are insoluble in cold water and undergo a limited reversible (on drying) swelling due to diffusion and absorption of small amounts of water into the amorphous regions (an exothermic process) (Collison, 1968). When water is added to a starch granule water enters the amorphous domains of the starch more readily than the crystalline domains (Hancock and Tarbet, 2000). The sequence of events during swelling of potato starch is presented in **Figure** 2-11. Granular swelling has been shown to be influenced by granular size (Vasanthan and Bhatty, 1996), amylose content (Eliasson, 1985,

Figure 2-10 Double helix packing arrangement in A- and B-type unit cells (Adapted from Wu and Sarko, 1978a,b)

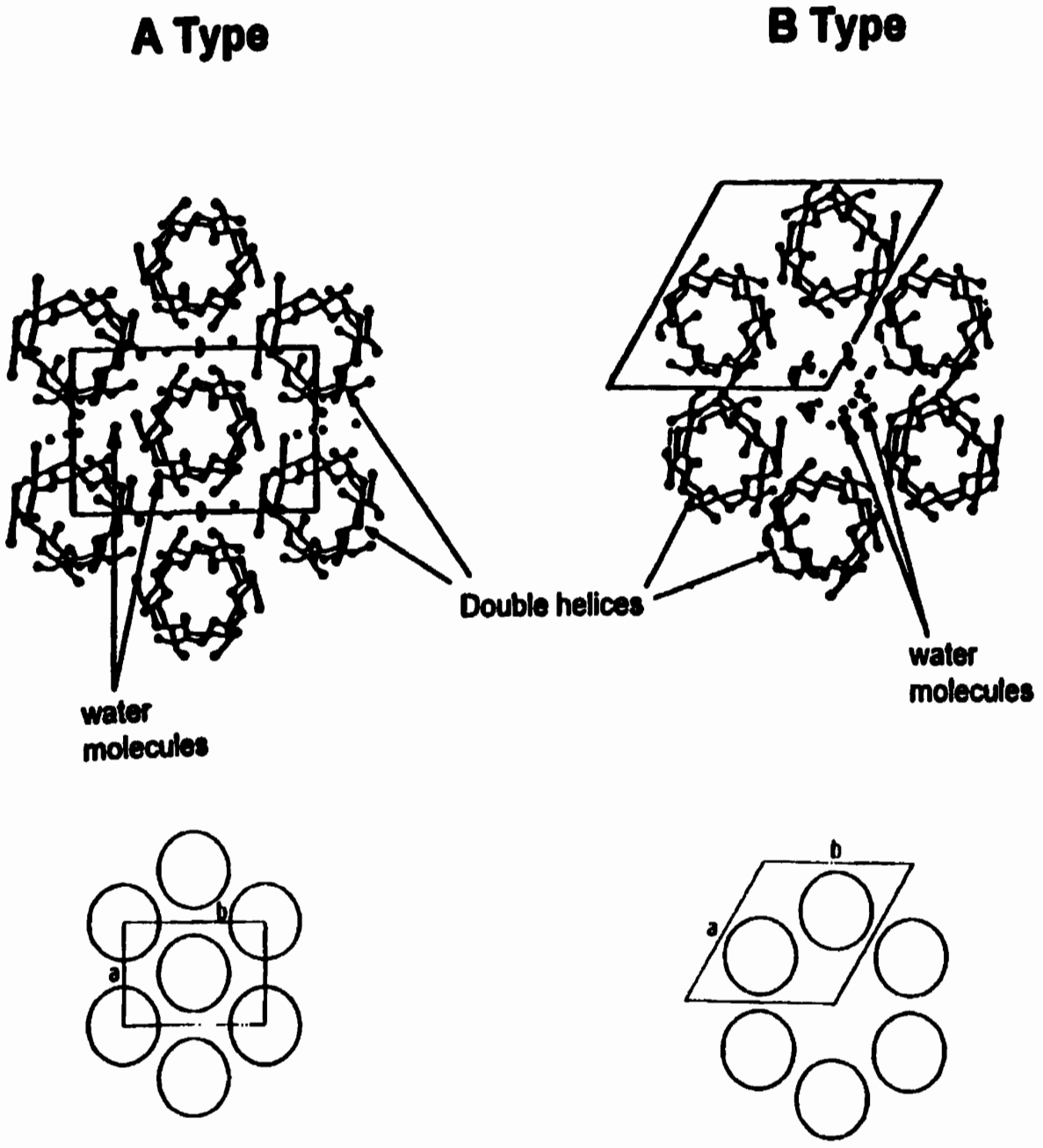
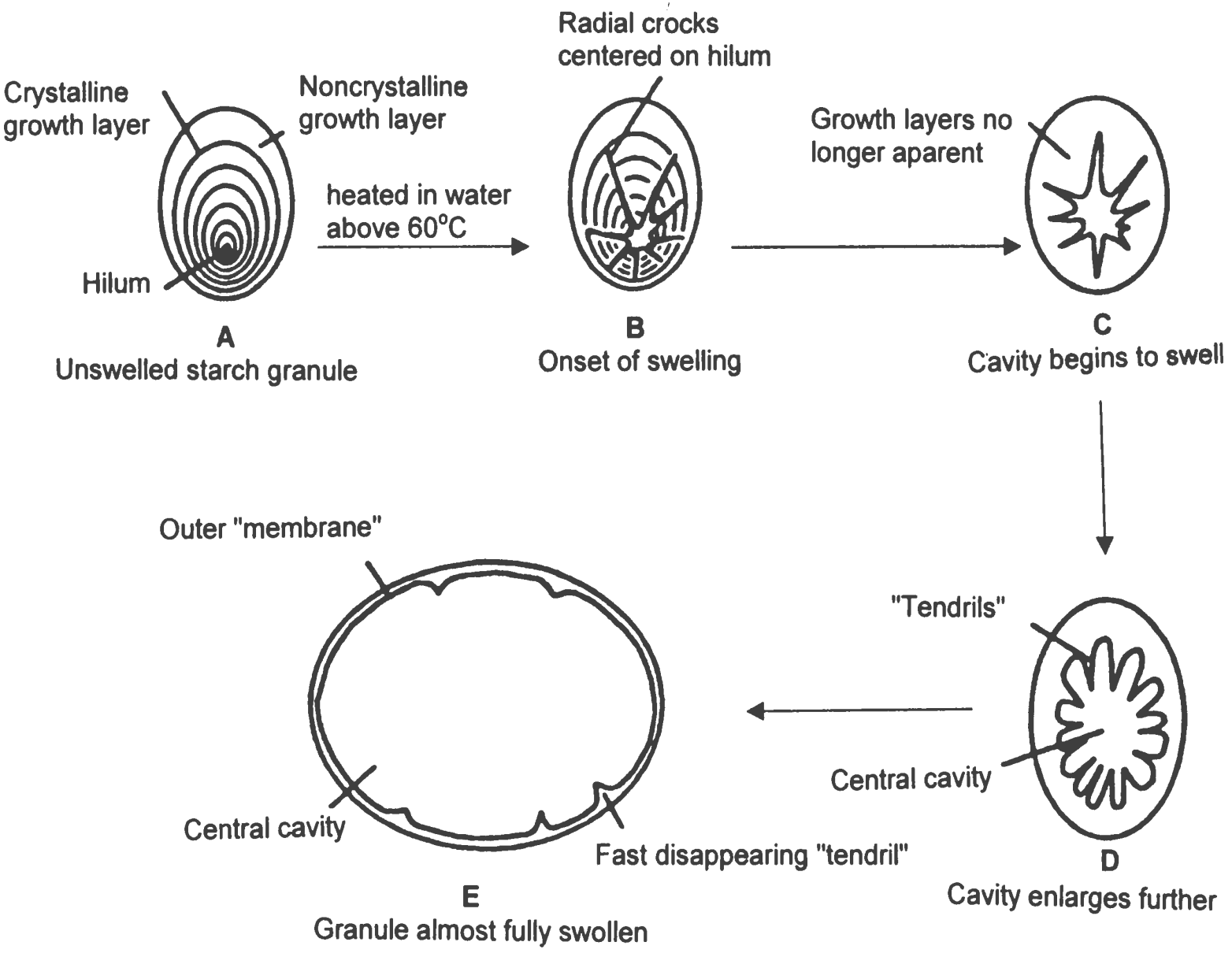

A Type
B Type
Double helices
water molecules
water molecules
b
a
b
a

Figure 2-11 Granular swelling of potato starch in hot water (Hancock and Tarbet 2000, reproduced with permission)


Crystalline growth layer
Noncrystalline growth layer
Hilum
A
Unswelled starch granule
heated in water above 60°C
Radial crocks centered on hilum
B
Onset of swelling
Growth layers no longer aparent
C
Cavity begins to swell
"Tendrils"
Central cavity
D
Cavity enlarges further
Outer "membrane"
Central cavity
Fast disappearing "tendril"
E
Granule almost fully swollen

Colonna and Mercier, 1985, Tester and Morrison, 1990a, 1992, Morrison *et al.*, 1993b, Wang and Seib, 1996), starch damage (Alsberg, 1938, Karkalas *et al.*, 1992, Tester *et al.*, 1994, Tester and Morrison, 1994, Morrison *et al.*, 1994, Morrison and Tester, 1994), temperature (Colonna and Mercier, 1985, Gudmundsson and Eliasson, 1989, Ellis *et al.*, 1989), bound lipid content (Gudmundsson and Eliasson, 1989, Tester, 1997a), and crystallinity (Robin *et al.*, 1975). The extent of swelling of some starches are presented in **Figure** 2-12. 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. Consequently, ordered amylopectin double helices within the semicrystalline growth ring are destabilized leading to a complete loss of crystallinity.

2.5.2 Gelatinization

Gelatinization has been defined as an irreversible change of granular swelling and melting of starch crystallites when native starch is heated in water under specific temperature ranges and certain moisture levels (Donovan 1979, French 1984, Biliaderis, 1990, Cooke and Gidley 1992, Colonna *et al.*, 1992, Eliasson and Gudmundsson 1996, Jacobs and Delcour 1998). There are actually two processes occurring during the gelatinization phase transition: first, the melting of the starch crystallites, which is an endothermic process, and second, the formation of the amylose-lipid complexes, which is an exothermic process (Biliaderis *et al.*, 1986b, Eliasson and Gudmundsson 1996). This phase transition is associated with the diffusion of water into the granule, water uptake

Figure 2-12 Swelling patterns of smooth pea (▲), wrinkled pea (■), normal maize (O), and high amylose maize (▽) starches [expressed as g of water per g of dry starch in the sediment] (Colonna and Mercier, 1985, reproduced with permission)

by the amorphous background region, hydration and radial swelling of the starch granules, leaching of amylose into the solution, increase in viscosity, loss of optical birefringence, loss of crystalline order, unraveling and dissociation of double helices (in the crystalline regions) and starch solubilization (Stevens and Elton, 1971, Lelievre and Mitchell, 1975, Donovan, 1979, Biliaderis *et al.*, 1980, Hoover and Hadziyev, 1981, Donovan *et al.*, 1983, Swinkels, 1985b, Atwell *et al.*, 1988, Biliaderis, 1991, Evans and Haismann, 1982, Jenkins, 1994, Biliaderis 1998). Jenkins (1994) showed by means of small angle neutron scattering studies, that the mechanisms proposed by Evans and Haismann (1982), Blanshard (1987), Biliaderis *et al.*, (1986b) were not compatible with his results, but were in broad agreement with gelatinization mechanism proposed by Donovan (1979). Jenkins (1994) has postulated that in excess water gelatinization is a primarily swelling driven process. Water uptake by the amorphous background regions is accompanied by swelling within these region. This swelling acts to destabilize the amylopectin crystallites within the crystalline lamellae, which are ripped apart. This process occurs rapidly for an individual crystallite, but over a wide range for the whole granule. Smaller crystallites are less soluble and destroyed first. In conditions of limiting water, initial crystallite disruption occurs by the same swelling driven mechanism. However, these is insufficient water for this process to proceed to completion. At higher temperatures the remaining crystallites slowly melt.

Many methods are presently available for the determination of starch gelatinization, such as Kofler hot-stage microscope (Watson, 1964), DSC

(Stevens and Elton, 1971), small angle X-ray scattering (Jenkins, 1994), pulsed nuclear magnetic resonance (Lelievre and Mitchell, 1975), and enzymic methods (Zobel, 1984). Because gelatinization is an endothermic process, thermal analysis methods, and differential scanning calorimetry in particular, have attracted most interest in studies of phase transitions of phenomena (Biliaderis *et al.*, 1986a). DSC measures the gelatinization transition temperatures [(onset [To], midpoint [Tp], conclusion [Tc] and the enthalpy (ΔH) of gelatinization (**Table** 2.5) Many factors influence the gelatinization temperature 1) water content, 2) starch source, 3) lipid, 4) damage starch, 5) environmental condition during growth, and 6) the presence of ions and solutes (Tester *et al.*, 1991, Shi *et al.*, 1994, Morrison 1995, Eliasson and Gudmundsson 1996). Perhaps the most fundamental influence is the influence of water. However, Noda *et al.*, (1996) have postulated that DSC parameters (To, Tp, Tc, ΔH) 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 crystalline region which corresponds to the amylose to amylopectin ratio. Tester (1997b) has postulated that the extent of crystalline perfection is reflected in the gelatinization temperature. Cooke and Gidley (1992) have shown (^{13}C.CP MAS-NMR and X-ray diffraction) that the enthalpic transition is primarily due to the loss of double helical order rather than the loss of crystallinity. However, Tester and Morrison (1990a) have postulated that ΔH reflects the overall crystallinity (quality and amount of starch crystallites) of amylopectin. Gernat *et al.*, (1993) have stated that the amount of double-helical order in native starches should be strongly

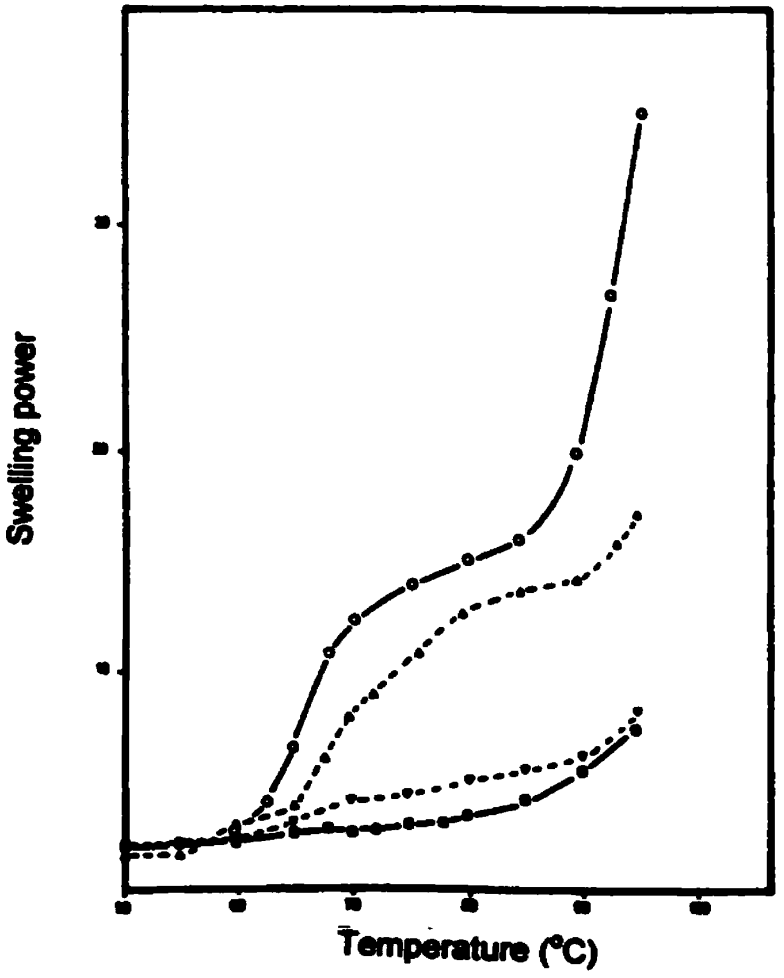

Swelling power
Temperature (°C)

Table 2.5. Gelatinization parameters of native cereal starches

Starch source	Temperature (°C) To	Tp	Tc	To-Tc	ΔH (J/g)	Reference
Normal maize	62.9	70.5	81.1	18.2	12.6	Tester *et al.*, 2000
Normal maize	66.1	70.7	75.2	9.1	13.2	McPherson *et al.*, 2000
Normal maize	61.0	66.0	72.0	11.0	15.1	Inouchi *et al.*, 1991
Normal maize	63.7	70.6	-	-	12.1	Campbell & Glover, 1996
Normal maize	70.0	78.0	89.0	19.0	18.0	Wootton & Bamunuarachchi 1979
Normal maize	60.0	70.0	82.0	12.0	11.4	Tester, 1997a
Normal maize	64.1	69.4	74.9	10.8	12.3	Jane *et al.*, 1999
Normal maize	59.0	70.5	85.0	26.0	12.2	Morrison *et al.*, 1994
Normal maize	64.7	69.9	79.2	10.4	11.3	Qian *et al.*, 1998
Normal maize	68.0	71.0	-	-	13.8	Inouchi & Glover 1984
Normal maize	60.0	67.0	89.0	29.0	13.8	Biliaderis, 1980
Normal maize	59.0	66.0	73.0	14.0	14.0	Hoover & Manual, 1996
Amylomaize V	63.0	76.0	82.0	19.0	6.3	Hoover & Manuel, 1996
Amylomaize V	67.7	91.0	104.8	37.1	17.7	Tester *et al.*, 2000
Amylomaize V	71.0	81.3	112.6	41.6	19.5	Jane *et al.*, 1999
Amylomaize V	70.1	75.6	-	-	8.6	Knutson, 1990
Amylomaize V	-	-	-	-	11.9	Fergason, 1994b
Amylomaize V	-	-	-	-	20.6	Klucinec &Thompson, 1999
Amylomaize V	71.0	82.0	114.0	43.0	17.6	Biliaderis, 1980
Amylomaize VII	50.0	-	133.0	83.0	31.8	Wootton & Bamunuarachchi 1979
Amylomaize VII	86.4	94.0	-	-	10.6	Knutson, 1990
Amylomaize VII	70.6	-	129.4	58.8	16.2	Jane *et al.*, 1999
Amylomaize VII	-	-	-	-	22.7	Klucinec & Thompson, 1999
Amylomaize VII	-	-	-	-	9.7	Fergason, 1994b

Cont.,

Waxy maize	62.6	72.6	84.4	21.8	15.6	Tester *et al.*, 2000
Waxy maize	66-70	73-74	71-82	5-12	15.5-16.3	Inouchi *et al.*, 1995
Waxy maize	62.0	69.0	81.0	19.0	19.3	Inouchi *et al.*, 1991
Waxy maize	-	-	-	-	15.0	Fergason, 1994b
Waxy maize	68.0	79.0	90.0	22.0	19.7	Wootton & Bamunuarachchi 1979
Waxy maize	60.0	72.5	87.0	17.0	15.2	Tester, 1997a
Waxy maize	64.2	69.2	74.6	10.4	15.4	Jane *et al.*, 1999
Waxy maize	-	73.0	-	-	16.0	Kugimiya & Donovan, 1981
Waxy maize	67.0	75.0	-	-	15.5	Inouchi & Glover, 1984
Waxy maize	67.2	72.8	-	-	15.6	Knutson, 1990
Waxy maize	64.0	71.0	97.0	33.0	16.6	Biliaderis, 1980
Waxy maize	65.0	73.0	82.0	17.0	16.0	Hoover & Manuel, 1986
Rice	59.7	67.8	82.6	22.9	13.0	Jacobs *et al.*, 1996
Rice	58.0	74.6	88.4	30.4	15.3	Chatakanonda *et al.*, 2000
Rice	-	73.2	-	-	13.8	Biliaderis & Tonogai, 1991
Rice	66.0	82.0	100.0	34.0	14.2	Stevens & Elton, 1971
Rice	68.0	79.0	108.0	40.0	16.3	Stevens & Elton, 1971
Rice	56.0	69.3	83.5	27.5	13.1	Tester, 1997a
Rice	70.3	76.2	80.2	9.9	13.2	Jane *et al.*, 1999
Rice (IR 480-5-9)	53.0	61.9	87.0	34.0	11.5	Paton, 1987
Rice (IR 5)	61.0	73.1	95.0	34.0	13.3	Paton, 1987
Rice (soft)	62.1-73.7	66.7-78.2	75.3-84.6	13.2-10.9	10.5-14.2	Ong and Blanshard, 1995
Rice (hard)	59.8-73.8	63.8-78	68.0-84.3	8.2-10.5	12.6-18.0	Ong and Blanshard, 1995
Oat	54.0	59.0	70.0	16.0	10.5	Shamekh, 1994
Oat	60.0	66.0	71.0	-	11.5	Hoover *et al.*, 1994
Oat	60.0	64.0	70.0	10.0	10.5	Hoover & Vasanthan, 1994b
Oat	61.0	66.0	73.0	12.0	10.4	Hoover & Vasanthan, 1992
Oat	61.1	66.8	82.0	20.9	9.1	Paton, 1987
Oat	60.4	64.1	70.0	9.6	10.1	Hoover & Vasanthan, 1994a

correlated to the amylopectin content, and granule crystallinity increases amylopectin content. Gelatinization enthalpies of native cereal starches are generally in the range of 9-23 J/g (**Table** 2.5).

2.5.3 Retrogradation

Retrogradation is generally defined as reassociation of gelatinized starch (gel) via chain aggregation by hydrogen bonding and recrystallization on storage (O'Dell, 1979, Swinkels, 1985b, Biliaderis, 1998). During this process squeezing of water out of the gel is called 'syneresis' (Hoseny, 1994, Biliaderis, 1998). Starch retrogradation is the main factor in staling of bread and other baked products (Eliasson and Gudmundsson, 1996). It has been recognized that retrogradation consists of two separable processes: (a) gelation of amylose solubilized during gelatinization, and (b) recrystallization of amylopectin within the gelatinized granules (Miles *et al.*, 1985a,b). Retrograded starch, as well as retrograded amylose, exhibits the B-type X-ray diffraction pattern (Young, 1984). Retrogradation is a complex phenomenon and it is favored by many factors, such as sources of starch, starch concentration, absence of branching, storage temperature, storage period, relatively low degrees of polymerization, neutral pH, low degrees of polydispersity, presence of solutes (lipids, electrolytes, sugars), presence of other dehydrating substances (Young, 1984, Swinkels, 1985b, Eliasson and Larsson, 1993). The water content together with the storage temperature are very important because they control the rate and the extent of retrogradation (Eliasson and Gudmundsson, 1996). The time-dependent increase in enthalpy of retrograded starch shows that starch crystallization

occurs at a faster rate and to a greater extent when a lower single storage temperature is employed within the range -1° and -43°C (Biliaderis, 1990). Retrogradation has a significant influence on texture, digestibility, and consumer acceptability of starch-based products (Ring *et al.*, 1987a, Biliaderis, 1998). During the storage of a starch gel the rigidity continues to increase with time. This increase is reversible to some extent: the part related to the crystallization of amylopectin can be reversed by heating (Eliasson and Gudmundsson, 1996).

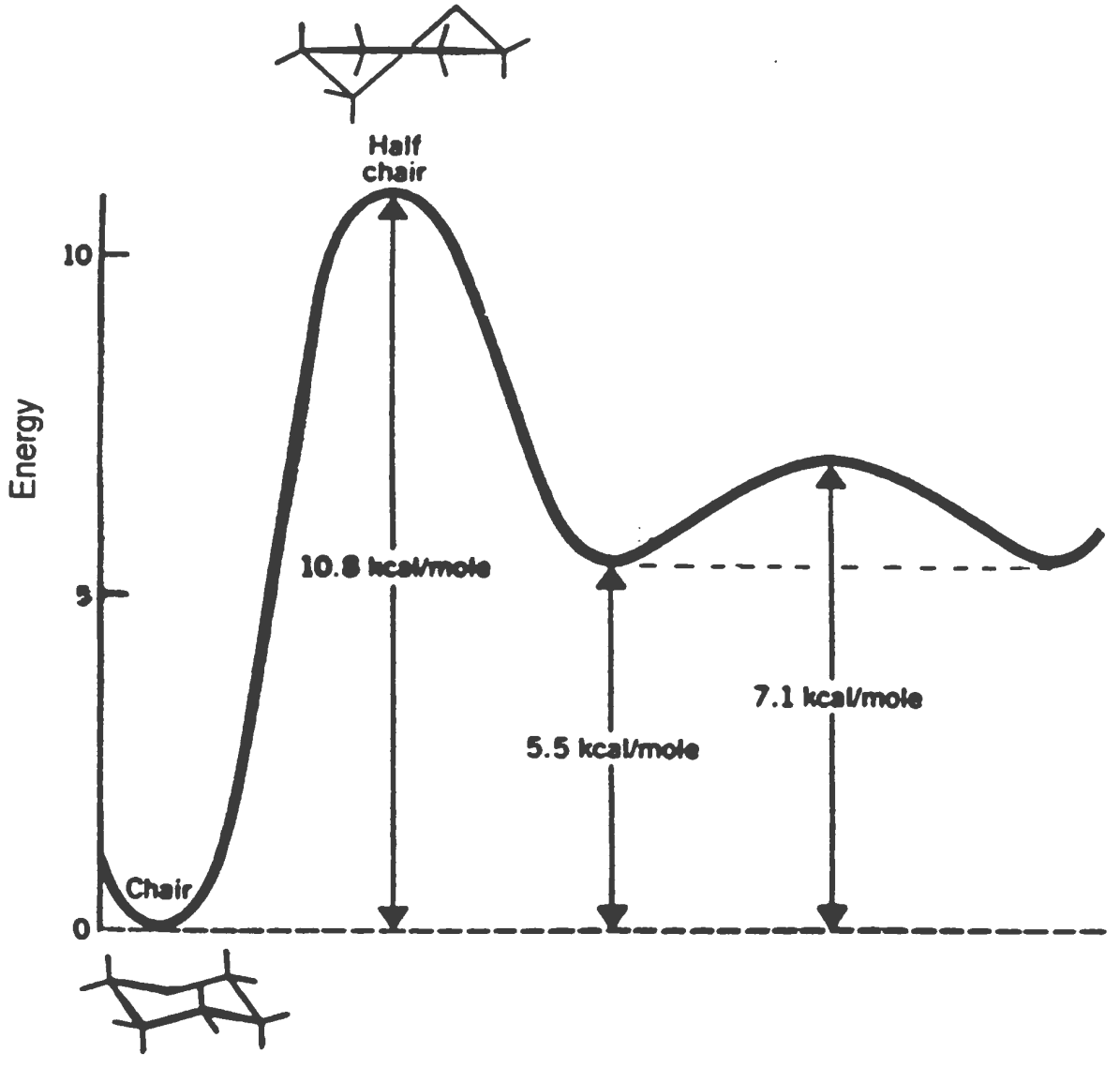
2.6 Acid hydrolysis

In general, enzymatic and acidic attack on starch are similar since they both involve hydrolysis of the bonds in the starch polymers. In more precise terms however, significant differences exist between the two types of attack with respect to bond specificity, mechanism of attack and extent of starch polymer degradation (Planchot, 1993, Zherebtsov *et al.*, 1995). Hydrolysis is a common processing approach which uses acids to etch away regions of the granule. This type of processing may be divided into two types, according to the acid used. Immersion in sulfuric acid (typically at a concentration of 15% (1.7M) at room temperature produces Nägeli amylodextrins (Nägeli, 1874). By contrast treatment with 2.2M HCl at elevated temperatures (typically 30-40°C) produces lintnerised starch (Lintner, 1886). When starch granules are subjected to controlled acid hydrolysis (2.2N HCl)(lintnerization), below the gelatinization temperature, the initial attack by acid is on the relatively unprotected amorphous regions between the clusters of short chains making up the crystalline areas (Lineback, 1984). The amorphous region is hydrolyzed much faster (whether it be at the surface or in

the interior) than the crystalline domains (Alsberg, 1938, Cowie and Grenwood, 1957, Buttrose, 1963, Kainuma and French, 1971, Robin *et al.*, 1974, Nara *et al.*, 1983, Biliaderis *et al.*, 1981, French, 1984, Blanshard, 1987, Pessa *et al.*, 1992, Jacobs *et al.*, 1998, Manelius *et al.*, 2000, Hoover, 2000). The far smaller size of the acid molecule (e.g. HCl compared to the enzyme) allows acidic attack to occur on a much greater scale than enzymic attack (Gallant *et al.*, 1997).

To account for the heterogeneous hydrolysis rate of the starch granule, three hypotheses have been proposed (Kainuma and French, 1971). First, the compact packing of starch chains within the starch crystallites does not readily permit the penetration of H_3O^+ into the regions. The amorphous domains of the starch molecule are easily accessible to penetration of hydrated protons and hydrolyze much faster. However, the crystalline domains would only be hydrolyzed at the exterior of the crystallites or at its junction with an amorphous domain. Second, acid hydrolysis of a glucosidic bond may require a change in conformation (chair→ half chair) of the D-glucopyranosyl unit (BeMiller, 1967). As long as the glucose units are held in a crystalline domain, chair→ half chair conformational change would require a very high energy of activation (**Figure** 2-13). Obviously, if the crystalline structure immobilizes the sugar conformation then chair→ half chair transition would be sterically impossible (Hoover, 2000). Third, although it has not been confirmed, there is a possibility that the starch chains may aggregate in some form that is intrinsically resistant to acidic hydrolysis. However, this aggregation would not induce a greater perfection of crystallinity (Kainuma and French, 1971).

Figure 2-13 Conformational change during the chair→half chair transformation


Half
chair
10
5
0
Energy
10.8 kcal/mole
5.5 kcal/mole
7.1 kcal/mole
Chair

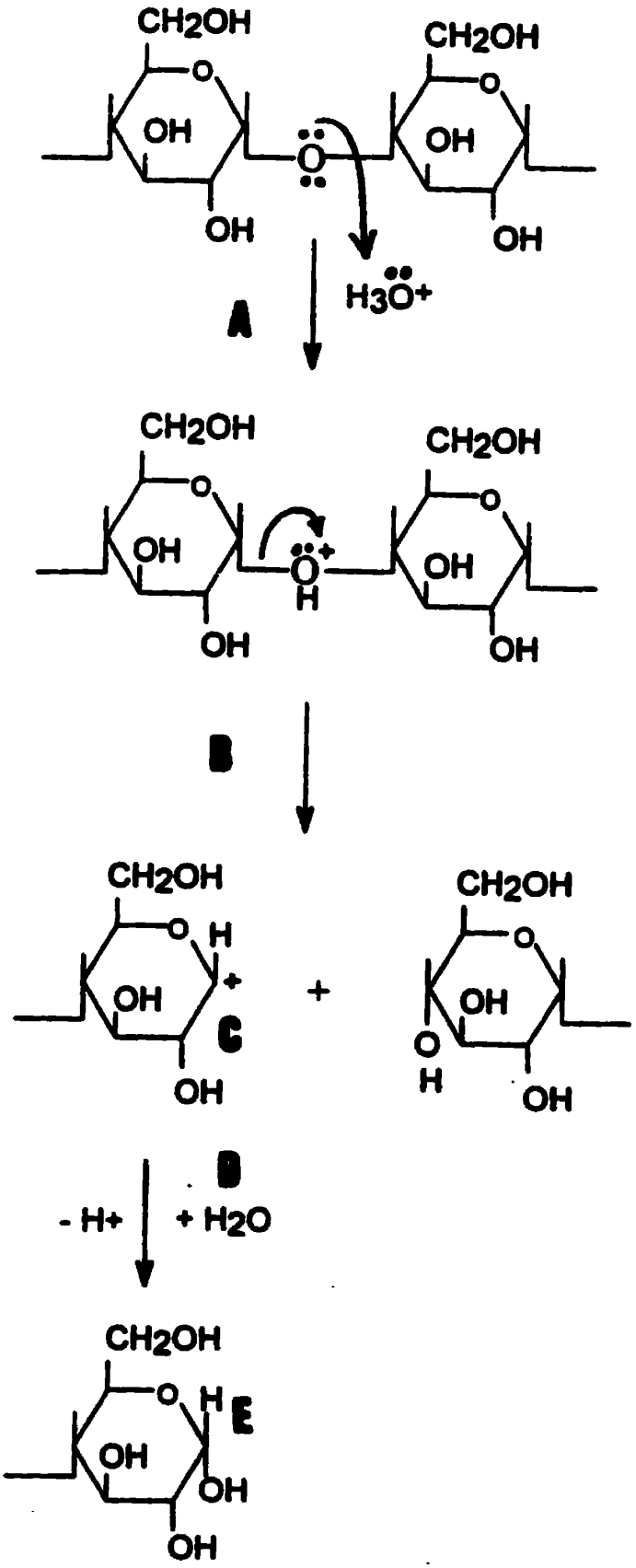
2.6.1 Mechanism of acid hydrolysis

In acid hydrolysis, hydronium ions (H_3O^+) carry out an electrophilic attack on the oxygen atoms of the α- (1→4) glucosidic bonds in starch molecules. The mechanism of cleavage of α- (1→4) glucosidic bonds is illustrated by **Figure** 2-14a. The scheme (**Figure** 2-14a) explains the electrophilic attack of hydronium ion on the oxygen atom (glucosidic oxygen) of the α- (1→4) glucosidic bond in starch molecule. In the next step (**Figure** 2-14b), because of a significantly greater negative induction effect, the glucosidic oxygen atom of the α- (1→4) glucosidic bond has a higher electron density than the anomeric carbon (C_1), as a result the electrons in one of the carbon-oxygen bonds move onto the glucosidic oxygen atom to generate an unstable, high-energy carbocation intermediate (**Figure** 2-14c). The reduced density of the electron cloud near anomeric carbon (C_1) is also caused by an induction effect of the glucose pyranose ring oxygen. This causes the electrophilic attack of a proton on the glucosidic oxygen atom and breaks the C_1-O bond. The carbocation intermediate is a Lewis acid, so it subsequently reacts with water (Lewis base) in the reaction mixture (**Figure** 2-14d), leading to regeneration of a hydroxyl group (OH^-) (**Figure** 2-14e) (Hoover, 2000). A similar mechanism works in the case of cleavage of the α- (1→6) glucosidic bond of amylopectin molecules (Zherebtsov *et al.*, 1995).

2.6.2 Susceptibility of α-(1→4), and α-(1→6) linkages

Wolfrom *et al.*, (1963) concluded that the α-D linkage is more prone to hydrolysis than the β-D linkage. The susceptibility of α-(1→4) and of α-(1→6) linkages to

Figure 2-14 Mechanism of acid hydrolysis of starch (Hoover 2000, reproduced with permission)


CH2OH
O
OH
OH
O
CH2OH
O
OH
OH
H3O+
A
CH2OH
O
OH
OH
O
H
CH2OH
O
OH
OH
B
CH2OH
O
H
OH
+
C
OH
+
CH2OH
O
OH
O
H
OH
D
- H+
+ H2O
CH2OH
O
H
E
OH
OH
OH

acid hydrolysis is an controversial issue. Levine *et al.*, (1942) postulated that the α-(1→6) glucosidic bond hydrolyzes at a rate at least as great as that of the α-(1→4) linkage. Swanson and Cori (1948), Jones *et al.*, (1955), Wolfrom *et al.*, (1963) have shown that α-(1→4) linkage has been found to be approximately four times more susceptible to acid hydrolysis than the α-(1→6) linkage. However, Robin *et al.*, (1974) and Singh and Ali (2000) have postulated that α-1,6 linkages are more acid susceptible.

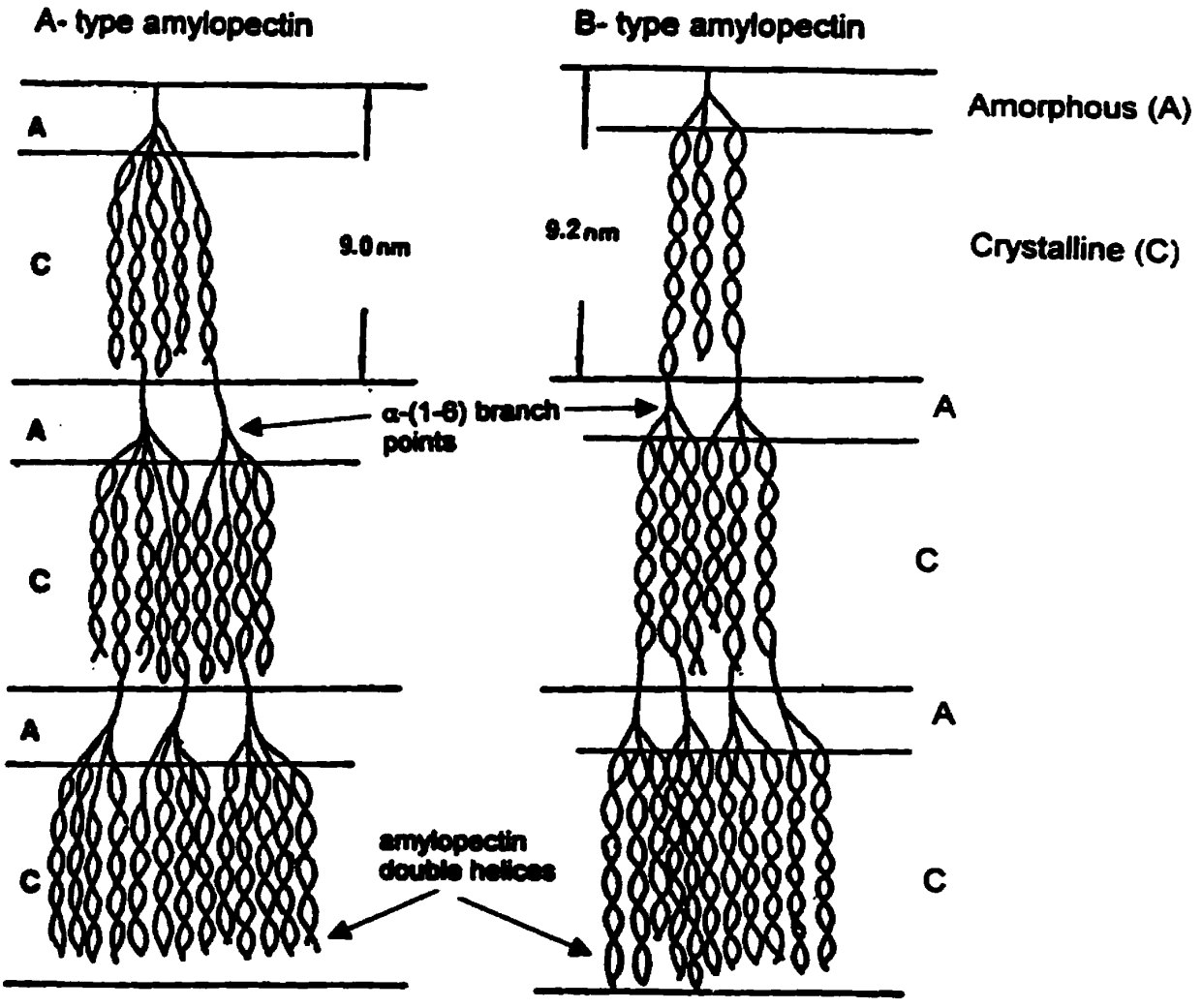
Jane *et al.*, (1997) have shown that the α-(1→6) branch linkages of A-type starches are scattered in between amorphous and crystalline domains (**Figure** 2-15). The above authors have postulated that some of the α-(1→6) linkages of the A-type starch are found within the crystalline domains, whereas others are in the amorphous domains. However, α-(1→6) branch linkages of the B-type starch present mainly in the amorphous lamellae and are thus more susceptible to H_3O^+ (Jane, 1997).

2.6.3 Structural studies on acid-treated starches

2.6.3.1 Effect of amylose-lipid complexes on acid hydrolysis

Morrison *et al.*, (1993a) reported from studies on barley starches that lipid-complexed segments of single chain V_6-amylose helices are resistant to acid hydrolysis. However, Inouchi *et al.*, (1987) and Vasanthan and Hoover (1992a) showed that lipid removal (*n*-propanol-water 3:1 v/v) from wheat, corn, and cassava starches does not cause any significant changes to the extent of acid hydrolysis.

Figure 2-15 Proposed models for A- (for waxy maize) and B- (from potato) type amylopectin branching patterns (Adapted from Jane, 1997)


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

2.6.3.2 Effect of acid hydrolysis on gelatinization parameters

Acid hydrolysis has been shown to have marked effect on gelatinization parameters. The gelatinization transition temperatures and the breadth of the gelatinization endotherm has been shown to increase on acid hydrolysis (**Figure** 2-16)(Shi and Seib, 1992). However, the influence of acid hydrolysis on gelatinization enthalpy has been reported to vary with botanical source, hydrolysis condition and duration (Biliaderis *et al.*, 1981, Muhr *et al.*, 1984, Komiya and Nara, 1986, Morrison *et al.*, 1993b, Jane *et al.*, 1997, Jenkins and Donald, 1997, Jacobs *et al.*, 1998) [**Table** 2.6]. Since acid hydrolysis preferentially attacks the amorphous domains in the granule (Kainuma and French, 1971, Robin *et al.*, 1974, Biliaderis *et al.*, 1981), the crystallites are decoupled from and no longer destabilized by the amorphous regions. Consequently, the starch crystallites of the acid-treated starches melt at a higher temperature than their native counterpart (Hoover, 2000). Morrison *et al.*, (1993a) suggested from studies on normal and waxy barley starch acid treated residues that the higher transition temperatures might be due to longer amylopectin double helices than in the unhydrolyzed amylopectin molecule, where the branch points might reduce the length of helix forming segments of the A and B chains.

2.6.3.3 Effect of acid hydrolysis on X-ray diffraction patterns and crystallinity

Several researchers have shown that starches retain their original X-ray diffraction pattern even after prolonged acid treatment. For instance waxy maize

Figure 2-16 DSC thermograms of native and acid treated (2.2M HCl at 30°C) waxy rice (IR 29) starch (Shi and Seib, 1992, reproduced with permission)

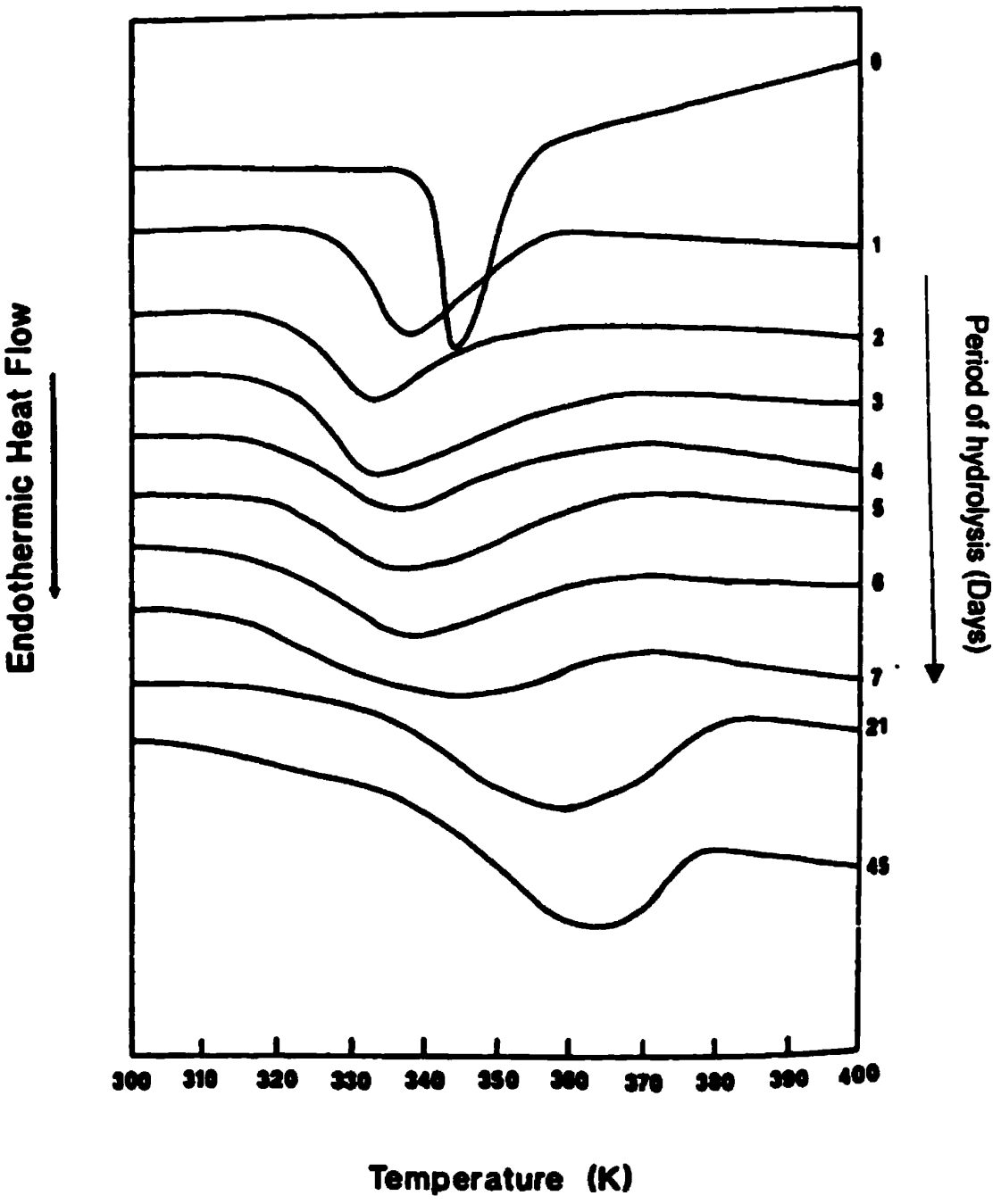

Endothermic Heat Flow
Period of hydrolysis (Days)
0
1
2
3
4
5
6
7
21
45
300
310
320
330
340
350
360
370
380
390
400
Temperature (K)

Table 2.6 Gelatinization enthalpy of native and acid treated starches

Starch source	ΔH (J/g)	Conditions	Reference
Cereal			
Wheat			Jacobs *et al.*, 1998
Native	11.8	-	
Lintnerized	11.5	2.2M HCl 35°C, 24h	
Barley (non-waxy)			Morrison *et al.*,1993b
Native	8.4-10.4	-	
Lintnerized	20-25	2M HCl 35°C, 140h	
Barley (waxy)			Morrison *et al.*, 1993b
Native	12-13.5	-	
Lintnerized	17-19	2M HCl 35°C, 140h	
Root and tuber			
Potato			Jacobs *et al.*, 1998
Native	18.7	-	
Lintnerized	23.0	2.2M HCl 35°C, 24h	

Cont.,			
Lintnerized	18.6	2.2M HCl 35°C, 7 days	
Lintnerized	13.7	2.2M HCl 35°C, 20 days	
Potato			Komiya and Nara 1986
Native	17.7	-	
Acid treated	17.54	1N H_2SO_4/ 30% ethanol 45°C, 2days	
Acid treated	17.38	1N H_2SO_4/ 30% ethanol 45°C, 6days	
Acid treated	13.82	1N H_2SO_4/ 30% ethanol 45°C, 10 days	
Legume			
Pea			Jacobs *et al.*, 1998
Native	12.6	-	
Lintnerized	13.0	2.2M HCl 35°C, 24h	

(Kainuma and French, 1971), potato (Kainuma and French 1971), rice (Maningat and Juliano, 1979), and legumes (Biliaderis *et al.*, 1981) and some cultivars of barley starches (Morrison *et al.*, 1993c) showed unchanged X-ray diffraction patterns after acid hydrolysis. Maningat and Juliano (1979), Muhr *et al.*, (1984), and Komiya *et al.*, (1987) showed that the X-ray diffraction intensities increase with progress of acid hydrolysis. Furthermore, several other researchers have also shown, by wide angle powder X-ray diffraction studies on acid treated starches, that the intensities of the major peaks centered in the region 18-23°, 2θ increases and becomes sharper on acid hydrolysis (Kainuma and French, 1971, French, 1972, Robin *et al.*, 1974, Robin *et al.*, 1975, Buleon *et al.*, 1987, Jane *et al.*, 1997, Jenkins and Donald, 1997). For instance crystallinity of potato (Muhr *et al.*,1984, Komiya and Nara, 1986), maize (Komiya *et al.*, 1987) and non waxy barley starches (Morrison *et al.*, 1993c) have been shown to increase on acid hydrolysis. This suggests preferential hydrolysis of the amorphous regions of the starch granule (Hoover, 2000). However, acid treated residues of waxy barley starches showed loss of crystallinity (Morrison *et al.*, 1993c). Furthermore, Jenkins and Donald (1997) have shown by small angle X-ray scattering that, as hydrolysis time increases, the small-angle peak loses definition. This result was in contradiction with the observation of increase in crystallinity with hydrolysis time (Muhr *et al.*,1984, Komiya *et al.*, 1987). However, using model-fitting techniques, Jenkins and Donald (1997) showed that, as starch is hydrolyzed, there is a decrease in the electron densities of the amorphous lamella and of the amorphous background. This decrease (due to etching away of the amorphous

region) was more pronounced in the latter. This was attributed to the greater accessibility of the amorphous background to the hydrated protons. However, a dearth of information still exists on the X-ray pattern and crystallinity of acid treated starch residues at different time intervals during acid hydrolysis.

Chapter 3

3. Materials and Methods

3.1 Materials

Normal maize (C*Gel 3420), amylomaize V (C*Gel 03001), amylomaize VII (C*Gel 03003), and waxy maize (C*Gel 4230) starches were obtained from Cerestar., Hammond, Ind., USA. Rice starch was obtained from Sigma Chemical Co., St. Louis, MO, USA. Oat grains (*Avena sativa* L.) was obtained from the Plant Research Center at Ottawa, Canada. Chemicals and solvents were analytical grade.

3.2 Methods

3.2.1 Oat starch isolation and purification

Oat grains were divided into two lots representing whole samples. Each lot was further subdivided into three equal sub samples and the starch was extracted and purified from each sub sample according to the following procedure:

Oat grains (300g) were cleaned and steeped in distilled water at room temperature over night. A ratio of 1 part soaked grains to 3 parts distilled water was mixed and ground at low and high speed, 35 and 5sec. respectively, in a Waring blender (Waring Model 33 BL 73, New Hartford, USA). The suspension was filtered under vacuum through a double layer cheese-cloth. The crude starch (filtrate) was collected and allowed to settle for 6h. The upper viscous layer was removed by siphoning and the sediment was suspended in excess 0.02% NaOH. Further purification was done by repeated suspension in distilled

water and 0.02% NaOH (alternatively) until disappearance of the amber colour of the supernatant. Then the sediment was filtered through a 70μ polypropylene screen (Spectra/Mesh. Macroporous filters, Spectrum Laboratory Products, California, USA). The filtrate was neutralized to pH 7.0 with dilute HCl. The final sediment was filtered through a 20μ polypropylene screen under vacuum and thoroughly washed on the filter with distilled water until no chloride reaction was observed with $AgNO_3$. The purified starch was dried for 24h at 30°C in a forced air oven (Fisher Scientific, Isotemp 615G, USA) to a moisture content of ~10%.

3.2.2 Acid hydrolysis

Acid-treated (lintnerized) starch samples were prepared by immersing granules (1.0g, dry basis/40mL of 2.2N HCl) in 250mL conical flask, in triplicate at 35°C. The starch slurries were shaken by hand daily to resuspend the starch granules. The amount of solubilized carbohydrate was measured after intervals of 0h, and 1, 2, 3, 4, 5, 6, 7, 8, 9, 11,13, and 15 days. The insoluble (acid treated) residues (1, 2, 3, 5, and 8 days) were recovered for subsequent analysis by centrifugation (IEC- Model HN-SII, Centrifuge, USA) at 2500 rpm for 10-15min. The residues were washed with deionized water until a pH of 4.0-5.0 was reached. Suspension were then neutralized with 0.1N NaOH. The residues were carefully washed repeatedly four times with deionized water to remove NaCl, the drained water was tested until no chloride reaction was observed with $AgNO_3$. The residues were freeze dried (LABCONCO® Freeze Dryer 5, Model 75050, Kansas City, USA) overnight and kept in airtight containers at room temperature for subsequent analysis. The extent of acid hydrolysis was determined by measuring

total soluble carbohydrates (Dubois *et al.*,1956) in the original supernatant before washing. The percentage hydrolysis was calculated from the following equation (Komiya and Nara,1986):

$$\text{Hydrolysis (\%)} = \frac{\text{The amount of total glucose formed} \times 0.9}{\text{The weight of original starch}} \times 100$$

The assay was performed by diluting an appropriate volume of the supernatant with distilled water. The mean values and the standard deviations were computed for each determination.

3.2.2.1 Determination of total soluble carbohydrates (Dubois *et al.*,1956)

1mL of 5% (w/v) phenol solution was added into appropriately diluted 2mL of carbohydrate solution. Then 5mL of concentrated sulfuric acid was added directly onto the solution surface and mixture was allowed to stand at ambient temperature for 10 min. The tubes were transferred into a 30°C water bath after mixing. After 15min, the absorbance was read against a reagent blank at 490nm. A standard series was prepared with known amounts of maltose [Fisher Scientific Fair Lawn, NJ. USA] (**Figure** I-1 in Appendix I).

3.2.3 Chemical composition of starch

3.2.3.1 Moisture content

The moisture contents of the native and acid treated starches were quantitatively determined according to AACC procedures (1984). The starch samples (3 ±0.01g) were weighed and heated in an air forced oven (Fisher Scientific, Isotemp 615G, USA) at 130 ± 1 °C for 1h. The samples were then removed and

cooled in a desiccator. The moisture content was calculated as the percentage weight loss of the sample.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Where: W_1 = weight of dish and sample before drying (g)
W_2 = weight of dish and sample after drying (g)
W_0 = weight of empty dish (g)

3.2.3.2 Nitrogen content

Nitrogen content was determined by the Micro Kjeldahl method. Samples (0.3 g dry basis) were weighed on nitrogen-free papers and placed in digestion tubes on a Buchi 430 digester (Buchi Laboratoriums - Technik AG, Flawil/Schweiz, Switzerland). The catalyst (two Kjeltabs M pellets) and 20mL of concentrated H_2SO_4 acid were 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 added, and the released ammonia was steam distilled into 50mL of 4% boric acid (H_3BO_3) containing 12 drops of end point indicator (N-point indicator, EM Science, NJ, USA) using a Buchi 321 distillation unit until 150mL of distillate was collected. The amount of ammonia in the distillate was determined by titrating it against 0.05N H_2SO_4 .The percentage nitrogen was calculated from the following equation (American Association of Cereal Chemists, 1984).

$$\text{\% Nitrogen} = \frac{(V_1 - V_2) \times N \times 14.0067}{W} \times 100$$

Where:

V_1 = volume (mL) H_2SO_4 to titrate sample
V_2 = volume (mL) H_2SO_4 to titrate blank
N = normality of H_2SO_4 acid
W = sample weight (mg ,dry basis)

3.2.3.3 Ash content

Pre-weighed (5.0±0.01g) samples were transferred into clean, dry porcelain ashing crucibles, and ignited over a flame until thoroughly carbonized. They were then placed in a pre-heated (525°C) muffle furnace (Lab Heat-Blue M model M30A-1C, Blue M Electric Co., Blue Island, IL, USA) and left until the samples was free from carbonaceous matter (~5h). The sample was cooled to room temperature in a desiccator and weighed. The percentage ash was calculated from the following equation (American Association of Cereal Chemists 1984).

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where:

W_1 = weight of empty crucible with cover (g)
W_2 = weight of crucible, cover and sample added (g)
W_3 = weight of crucible, cover and ash (g)

3.2.3.4 Starch lipids

3.2.3.4.1 Surface lipids

Surface lipids were extracted at ambient temperature (25-27°C) by mixing native starch (5g,dry basis) with 100mL of chloroform-methanol 2:1 v/v under vigorous agitation in a wrist action shaker for 1h. The solution was then carefully filtered (Whatmann No. 4 filter paper) into a 250mL round bottom flask and the residue was washed thoroughly with small amounts of chloroform-methanol solution.

The solution was then evaporated to dryness using a rotary evaporator (Rotavpor-R110,Buchi Laboratorimus-Technik AG, Flawill/Schweiz, Switzerland). The crude lipid extracts were purified by the method of Bligh & Dyer (1959) before quantification. The starch residue was saved for bound lipid extraction.

3.2.3.4.2 Bound lipids

Bound lipids were extracted using the residues left from surface lipid extraction. The residue was refluxed with *n*-propanol-water 3:1 v/v in a soxhlet apparatus at elevated temperatures (90-100°C) for 7h (Vasanthan and Hoover, 1992b). The solvent was evaporated to dryness using in a rotary evaporator (Rotavapor-R110, Buchi Laboratorimus-Technik AG, Flawill/Schweiz, Switzerland). The crude extract was purified by the Bligh and Dyer (1959) procedure before quantification.

3.2.3.4.3 Total lipids

Total starch lipid was determined by hydrolyzing native starch (2g, dry basis) with 25mL of 24% HCl at 70-80°C for 30 min, and extracting the hydrolyzate three times with *n*-hexane. The mixture was evaporated to dryness in a rotary evaporator. The crude total lipid extract were purified by Bligh and Dyer (1959) method before quantification.

3.2.3.4.4 Crude lipid purification (Bligh and Dyer, 1959, Vasanthan and Hoover, 1992a)

The crude lipids from the above extracts were purified by extractions with chloroform/ methanol/water (1:2:0.8v/v/v) and by forming a biphasic system

[chloroform/methanol/water (1:1:0.9v/v/v)] by the addition of chloroform and water at room temperature in a separatory funnel. The chloroform layer was then diluted with benzene and brought to dryness in a rotary evaporator followed by dryness at 60°C for 1h in a forced air oven. The dried lipids were cooled to room temperature in a desiccator. Then the heavy bottom layer was withdrawn into a pre-weighed 25mL round bottom flask and evaporated to dryness in the rotary evaporator. The samples were then removed and dried at 60°C in an air forced oven (Fisher Scientific, Isotemp 615G, USA), and cooled in a desiccator.

The lipids content was calculated using the following equation:

$$\% \text{ Lipid} = \frac{W_2 - W_1}{W_0} \times 100$$

Where: W_1 = weight of empty flask (g)
W_2 = weight of flask and lipid after drying (g)
W_0 = weight of the sample (g)

3.2.3.5 Amylose

Apparent and total amylose contents of cereal starches were determined as described by Hoover and Ratnayake (2001).

3.2.3.5.1 Apparent amylose content

Starch (20mg, dry basis) was weighed into a round bottom screw cap tube lined with a Teflon face rubber liner in the cap. This was followed by the addition of 8mL of 90% dimethylsulfoxide (Me_2SO). The contents were vigorously mixed for 2min using a vortex mixture followed by heating in a water bath (PolyScience, Model 2L-M PolyScience Niles, IL, USA) at 85°C for 15min with intermittent shaking. The tubes were then allowed to cool to room temperature (~45min) and

diluted to 25mL in a volumetric flask. 1mL of the diluted solution was mixed with water (40mL) 5mL of I_2/KI solution (0.0025M I_2 and 0.0065M KI mixture) was added and the final volume was adjusted to 50mL in a volumetric flask. After 15 min (for colour development), the absorbance was measured at 600nm using a UV-visible spectrophotometer (LKB Novospec Model 4029. LKB Biochrom, Ltd., Cambridge, UK). In order to correct for over-estimation of amylose content (due to complex formation between I_2 and long outer branches of amylopectin), amylose content was calculated from a standard curve prepared using mixtures of pure potato amylose and amylopectin (over the range 0-100% amylose and amylopectin 100-0% amylopectin) [**Figure** I-2 in Appendix I].

3.2.3.5.2 Total amylose content

Starch samples were defatted by extracting in a soxhlet extraction (85°C) with 3:1 (v/v) *n*-propanol-water for 7h prior to the determination of total amylose content by the above procedure.

3.2.3.6 Starch damage

Starch damage was estimated following the standard AACC (1984) procedures. Starch samples (1g, dry basis) were digested with fungal α-amylase from *Aspergillus oryzae* (12,500 Sigma units) having specific activity of 50-100 units/mg, in a water bath (30°C) for 15min. At the end of incubation, the enzyme action was terminated by adding 3.68N H_2SO_4 (3mL) and 12% (w/v) sodium tungstate (Na_2WO_4. $2H_2O$) (2mL), respectively. The mixtures were allowed to stand for 2 min and then filtered through Whatman No 4 filter paper. The amount

of reducing sugars in the filtrate was determined by the procedure outlined by Bruner (1964).

3.2.3.6.1 Bruner method for determination of reducing sugars

Aliquots (1mL) of the filtrate were mixed with 2mL chilled 3,5-dinitrosalycylic acid and diluted to 4mL with distilled water. The diluted samples were heated in a boiling water bath for 5min. The reaction mixture was chilled using an ice bath (10min), and diluted with 8mL distilled water. The absorbance was measured at 540 or 590nm against a reagent blank. Calibration curves (**Figures** I-3A and I-3B in **Appendix** I) were established with maltose (to calculate the maltose equivalents in the digest) and the percentage starch damage was calculated using the following equation:

% starch damage = [Mx1.64]/ [W x 1.05] x 100

Where:

M = maltose equivalents in the digest (mg)
W = starch weight (mg, dry basis)
1.64= the reciprocal of the mean percentage maltose yield from gelatinized starch (an empirical factor which assumes that under the experimental conditions, maximum hydrolysis is 61%)
1.05 = molecular weight conversion of starch to maltose

3.2.4 Degree of polymerization of native and acid treated starches

Native and lintnerized samples (0.09g dry basis) were mixed with 10mL of pure dimethylsulfoxide (Me_2SO). The solutions were heated (occasionally vortexed) to 60°C (in a water bath) until completely dissolved. The resulting solutions were cooled to room temperature. The solutions were divided into two equal parts and

total carbohydrate was determined by the method of Dubois *et al.* (1956) and the reducing sugar content was measured by the method of Bruner (1964) by diluting an appropriate volume of the sample with distilled water. A series of maltose (anhydrous) solutions were used to plot the standard curve for both analysis (**Figure** I-3A, I-3B and I-3C in **Appendix I**).

The average degree of polymerization (DP) values were calculated using the equation shown bellow (Jane and Robyt, 1984):

$$DP = \frac{\text{Total carbohydrate (as } \mu g \text{ maltose)}}{\text{Reducing sugar (as } \mu g \text{ maltose)}} \times 2$$

3.2.5 Granuler morphology of native and acid treated starches

Granule morphology of native and acid treated (0,1,5, and 8 day) starches were studied by scanning electron microscopy. Starch samples were mounted on circular aluminum stubs with double sided sticky tape and then coated with gold (20nm) and examined and photographed in a Hitachi, S 570 scanning electron microscope (Nissei Sangyo Inc., Rexdale, ON, Canada) at an accelerating voltage of 5kV.

3.2.6 X-ray diffractometry

X-ray diffractograms were obtained with a Rigaku powder diffractometer [horizontal goneometer system] (Rigaku ,RU200R Rigaku-Denki Co., Tokyo, Japan). The operating conditions were as follows: target voltage 40 kV, target current 100mA, step time, step interval 0.15°, Soller and divergence slit with 1°, receiving slit width 0.6°, scatter slit width 1mm and scanning speed 2.000°/min.

The moisture content of all samples used for X-ray studies was approximately 15%.

3.2.6.1 Relative crystallinity

Relative crystallinity was determined by the procedure outlined by *Nara et al.*, (1978) using the peak fitting software Origin-Version 6.0 (Microcal Inc., Northampton, MA, USA) **Figure II-1** in **Appendix II**. Amorphous starch was prepared by heating a 10% starch solution at 95°C for 30 min with continuos agitation and then drying it at 100°C for 24h. The dried sample was ground into a free flowing powder using a RP 202 Pulaerit communicator (Geoscience Instruments Corp., New York, NY, USA) with denatured alcohol as the solvent. The ground sample was air dried for 24h and passed through a 250μm sieve.

3.2.7 Determination of starch properties

3.2.7.1 Swelling factor (SF)

The swelling factor of native and lintner starches, heated at 80°C in excess water, was measured according to the method of Tester and Morrison (1990a). Starch samples (50mg, dry basis) were weighed into 10mL screw-capped tubes, 5mL of distilled water was added and heated at 80°C in 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, 0.5mL of blue dextran (Pharmacia, MW $2x10^6$, 5mg/mL) was added and mixed well. The tubes were then centrifuged at 2,000g for 5min and the absorbance of the supernatant (A_s) was measured at 620nm using a UV-visible spectrophotometer (LKB

Novospec Model 4029. LKB Biochrom, Cambridge, UK) against a reference without starch. The method measures only intragranular water and hence the true swelling factor at the given temperature. Results used for calculation were means of triplicate measurements.

The swelling factor (SF) is reported as the ratio of the volume of swollen starch granule to the volume of the dry starch (Tester and Morrison, 1990a). Calculation of swelling factor (SF) was based on starch weight corrected to 12% moisture, assuming a density of 1.4g/mL.

$$FW = 5.5\ (A_R / A_S) - 0.5 \qquad (1)$$

Where:

FW= free or interstitial (mL) + supernatant water (mL)
A_S = absorbance of supernatant of the sample (nm)
A_R = absorbance of reference sample (nm)

$$V_O = W/1{,}400 \qquad (2)$$

Where:

V_O= volume of the starch (mL)
W = starch weight (mg)

$$V_1 = 5.0 - FW \qquad (3)$$

Where:

V_1= volume of absorbed intragranular water (mL)

$$V_2 = V_O + V_1 \qquad (4)$$

Where:

V_2 = volume of the swollen starch granules (mL)

$$SF = V_2 / V_1 \text{ (according to definition)} \qquad (5)$$

This can also be expressed as follows:

$$SF = 1 + \{(7{,}700/W)[A_S - A_R] / A_S\} \qquad (6)$$

3.2.7.2 Amylose leaching

Native and acid treated starch (20mg, dry basis) in water (10mL) were heated (80°C) in volume calibrated sealed tubes for 30 min (tubes were shaken by hand every 5min to resuspend the starch slurry). The tubes were then cooled to ambient temperature (25°C) and centrifuged at 2000g for 10min. The supernatant liquid (1mL) was withdrawn and amylose content determined by the method of Hoover and Ratnayake (2001). Percentage amylose leaching was expressed as mg amylose leached per 100g of dry starch. Results used for calculation were means of triplicate measurements.

3.2.7.3 Differential scanning calorimetry (DSC)

DSC parameters were measured using a Seiko DSC 210 (Seiko Instruments Inc., Chiba, Japan) differential scanning calorimeter equipped with a thermal analysis data station. Heat flow and temperature calibrations were periodically performed using pure indium with a heat of fusion of 28.4J/g and a melting temperature of 156.66°C.

Native and lintner (0, 12, and 24h) starch samples (3.0mg, dry basis) of known moisture content, (weighed to an accuracy of ± 0.01mg) were added to aluminum DSC pan (PERKIN-ELMER, Kit. No. 0219-0062). Deionized water (11μL) was carefully added to the pan by a microsyringe (MICROLITER®, #702, Hamilton Co. Reno, Nevada, USA), and the pans were hermetically sealed. A starch-to-water ratio of 1:3 was used in all DSC runs. The pans were kept at ambient temperature for 24h, reweighed and then scanned from 20 to 130°C at a heating

rate of 10°/min. An empty aluminum pan was used as the reference (to balance the heat capacity of the sample pan). During the scans, the space surrounding the sample chamber was flushed with dry nitrogen at rate of 100mL/min to avoid condensation on the outside of the cells. For each thermogram, gelatinization transition temperatures [onset (T_o), mid-point (T_p), and conclusion (T_c) temperatures] and enthalpy of gelatinization (ΔH, J/g) were measured using a DSC software (SSC 5300 Work Station, version 2.71U, 1996). The gelatinization transition temperatures and areas taken for calculating ΔH are shown **Figure** II-2 in **Appendix II**. The enthalpy ΔH was calculated by drawing a base-line between T_o and T_c and integrating the area between the thermogram and the base-line under the peak and was expressed in terms of Joules per unit weight of dry starch (J/g). All DSC experiments were replicated at least thrice.

3.2.8 Statistical analysis

All determinations were replicated three times and mean values and standard deviations reported. Analyses of variance were performed and the mean separations were done by Tukey's HSD test at $p<0.05$ using SigmaStat (Version 2.0, 1995) (Jandel Scientific Inc., IL, USA).

Chapter 4

4. Results and Discussion

4.1 Proximate composition

The proximate analysis of the native cereal starches are presented in **Table. 4.1.** The purity of the starches was judged on the basis of composition (low nitrogen and low ash content) and microscopic observation (absence of any adhering protein). All starches were of high purity (<0.05% nitrogen). The low nitrogen content indicated the absence of non-starch lipids (lipids associated with endosperm protein). Therefore, the total lipid (obtained by acid hydrolysis with 24% HCl for 30 min at 70-80°C) in normal maize (0.86%), waxy (0.01%), amylomaize V (1.21%), amylomaize VII (1.49%), rice (1.01%), and oat (1.13%) starches mainly represent free and bound starch lipids [lipids complexed with amylose chains and/or lipids trapped between starch chains] (Hoover and Vasanthan, 1994b). The free (surface/unbound) lipids (obtained by extraction with chloroform-methanol 2:1v/v at 25-27°C) constituted 0.06%, 0.01%, 0.04%, 0.11%, 0.03% and 0.11% of the total lipid in normal maize, waxy maize, amylomaize V, amylomaize VII, rice and oat starches, respectively. The bound lipid content (obtained by extraction of the chloroform-methanol residue with hot *n*-propanol-water 3:1 v/v, for 7h at 90-100°C) in normal maize, amylomaize V, amylomaize VII, rice and oat starches was 0.76%, 1.16%, 1.33%, 0.98%, and 1.01%, respectively. However, waxy maize was devoid of bound lipid. The apparent amylose content (determined by I_2 binding before removal of bound lipids) was 23.7%, 1.1%, 49.0%, 66.9%, 15.2%, and 20.9% in normal maize,

Table 4.1 Chemical composition and some of the properties of normal maize, amylomaize V, amylomaize VII, waxy maize, oat, and rice starches

Characteristics	Percent[1]					
	Normal maize	Amylomaize V	Waxy maize	Amylomaize VII	Rice	Oat
Moisture	12.0 ± 0.03[a]	12.17 ± 0.03[b]	10.08 ± 0.05[c]	11.68 ± 0.09[d]	11.66 ± 0.03[d]	10.43 ± 0.13[e]
Ash	0.13 ± 0.00[a]	0.09 ± 0.00[b]	0.05 ± 0.01[c]	0.10 ± 0.02[b]	0.45 ± 0.02[d]	0.16 ± 0.01[e]
Nitrogen	0.01 ± 0.00[a]	0.02 ± 0.00[b]	0.02 ± 0.00[c]	0.03 ± 0.00[d]	0.05 ± 0.00[e]	0.01 ± 0.00[f]
Lipid						
Solvent extracted						
Chloroform-methanol[2]	0.06 ± 0.01[a]	0.043 ± 0.03[a,b]	0.01 ± 0.01[b]	0.11 ± 0.04[c]	0.03 ± 0.02[a,b]	0.11 ± 0.02[c,d]
n-propanol-water[3]	0.76 ± 0.01[a]	1.16 ± 0.00[b]	0.03 ± 0.02[c]	1.33 ± 0.01[d]	0.98 ± 0.00[e]	1.01 ± 0.01[f]
Acid hydrolyzed[4]	0.86 ± 0.04[a]	1.21 ± 0.14[b]	0.05 ± 0.01[c]	1.49 ± 0.01[d]	1.01 ± 0.12[e]	1.13 ± 0.03[b,e]
Amylose content						
Total[5]	26.52 ± 0.13[a]	61.95 ± 0.04[b]	0.08 ± 0.01[c]	78.37 ± 0.03[d]	20.63 ± 0.07[e]	29.29 ± 0.00[f]
Apparent[6]	23.70 ± 0.00[a]	49.02 ± 0.29[b]	0.01 ± 0.01[c]	66.95 ± 0.04[d]	15.20 ± 0.11[a]	20.92 ± 0.00[f]
Amylose complexed by lipids[7]	10.63 ± 0.04[a]	20.87 ± 0.11[b]	87.5 ± 0.01[c]	14.57 ± 0.02[d]	26.32 ± 0.02[e]	28.58 ± 0.00[f]
Starch damage	0.47± 0.11[a]	0.39 ± 0.24[a]	0.32 ± 0.12[a]	0.36 ± 0.14[a,b]	0.53 ± 0.32[a]	0.66 ± 0.13[a,c]
Range of granular sizes (diameters) μm	7-18	4-16	7-12	6-13	2-7	5-9

[1]All data reported on dry basis and represent the mean of three replicates and the values followed by the same superscript in each row are not significantly different (P<0.05) by Tukey's HSD test
[2]Lipid obtained from native starch by chloroform-methanol 2:1 (v/v) at 25°C (mainly unbound lipids).
[3]Lipid extracted by hot n-propanol water 3:1 (v/v) from the residue left after chloroform-methanol extraction (mainly bound lipids).
[4]Lipid obtained by acid hydrolysis (24% HCl) of native starch (total lipids).
[5]Total amylose determined by iodine binding after removal of free and bound lipids.
[6]Apparent amylose determined by iodine binding without removal of free and bound lipids.
[7]$\frac{\text{Total amylose - apparent amylose}}{\text{Total amylose}} \times 100$

waxy maize, amylomaize V, amylomaize VII, rice, and oat starches, respectively. The total amylose content (determined by I_2 binding after removal of bound lipids) was 26.5%, 1.1%, 61.9%, 78.4%, 20.6%, and 29.3%, respectively in normal maize, waxy maize, amylomaize V, amylomaize VII, rice and oat starches. The difference between total and apparent amylose reflects the amount of amylose complexed with native lipids. The results showed that in normal maize, amylomaize V, amylomaize VII, rice and oat starches, the percentage of total amylose complexed by native lipids was 10.6%, 20.8%, 14.7%, 26.2%, and 28.7%, respectively. The extent of starch damage during isolation was low in all starches (0.3-0.7%) (**Table** 4.1). This suggests that starch damage is unlikely to have any influence on the physicochemical properties of the cereal starches used in this study.

4.2 Granular morphology of native starches

The granule morphology and the specific surface features of native starch granules are presented in **Figures** 4-1 and 4-2. Oat starch granules tend to exist in clusters of individual granules (**Figure** 4-1a). The granules ranged from polygonal to irregular in shape with a range of granule sizes (diameters) of 5-12μm. The surface appeared to be smooth with no evidence of indentations, fissures or pores (**Figure** 4-1b). Rice starch granules also tend to exist in clusters (**Figure** 4-1e). The granules were small, irregular to polygonal in shape with a range of granule sizes (diameters) of 2-7μm. The surface of many rice starch granules were covered with pores of varying diameter (**Figure** 4-1f). Normal

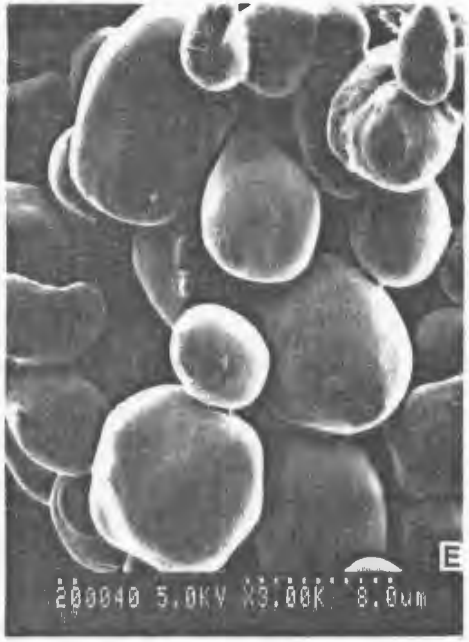
Figure 4-1 Scanning electron micrographs of native and acid treated (2.2N HCl, 35°C for 8 days) oat and rice starches: (A) native oat (3,000X); (B) native oat (10,000X); (C) acid treated oat (3.000X); (D) acid treated oat (10,000X); (E) native rice (3,000X); (F) native rice (10,000X); (G) acid treated rice (3,000X); (H) acid treated rice (10,000)


A
200027 5.0KV X3.00K 8.0um


B
200015 5.0KV X10.0K 2.40um


C
200030 5.0KV X3.00K 8.0um


D
200015 5.0KV X10.0K 2.40um


E
200040 5.0KV X3.00K 8.0um


F
200018 5.0KV X10.0K 2.40um

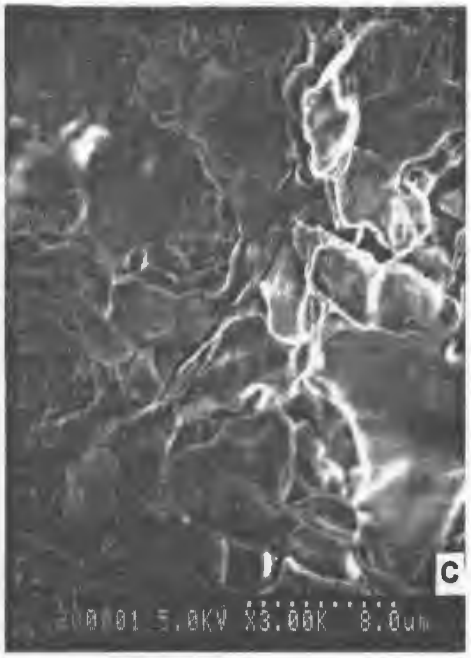

G
200025 5.0KV X3.00K 8.0um

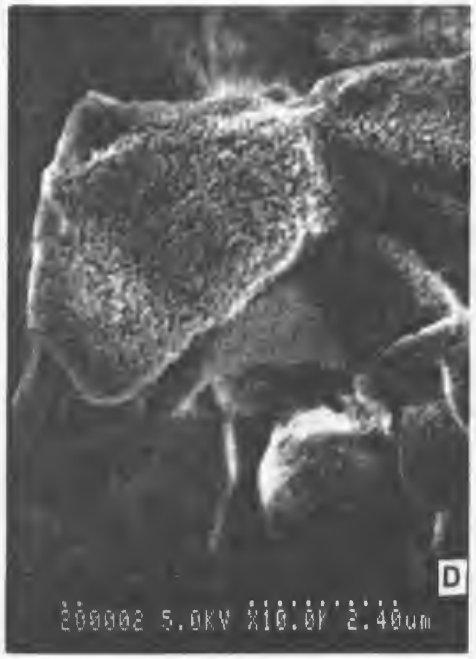

H
200023 5.0KV X10.0K 2.40um

Figure 4-2 Scanning electron micrographs of native maize starches: (A) normal maize (3,000X); (B) normal maize (10,000X); (C) waxy maize (3.000X); (D) waxy maize (10,000X); (E) amylomaize V (3,000X); (F) amylomaize V (10,000X); (G) amylomaize VII (3,000X); (H) amylomaize VII (10,000)


A
200018 5.0KV X3.00K 8.0um

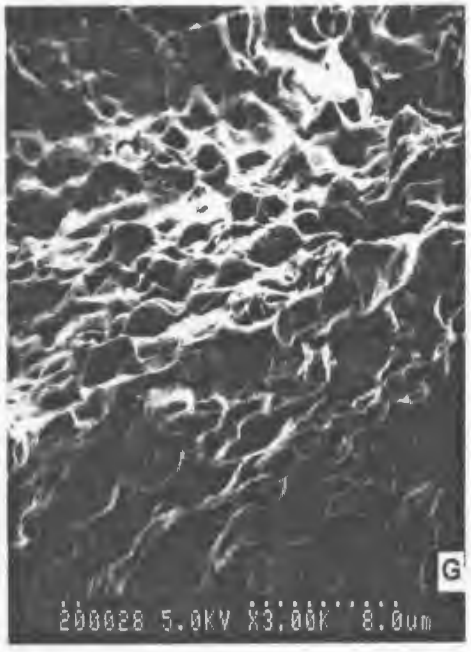

B

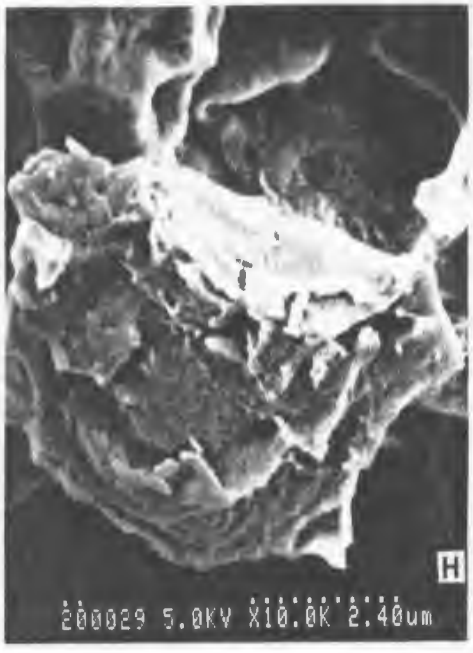

C


D
200002 5.0KV X10.0K 2.40um


E
200026 5.0KV X3.00K 8.0um


F


G
200028 5.0KV X3.00K 8.0um


H
200029 5.0KV X10.0K 2.40um

maize starch granules were angular, to spherical to irregular in shape with a range of granule sizes (diameters) of 7-18μm. Pores were present only on some granules (**Figures** 4-2a,b). Granules of waxy maize were irregular in shape (**Figures** 4-2c,d). Furthermore, many granules of waxy maize were smaller (range of granule sizes (diameters) 5-15μm) and contained less pores (**Figure**. 4-2c,d) than those present in normal maize (**Figures** 4-2a,b). Some granules of waxy maize were devoid of surface pores (**Figure** 4-2c). The granules (range of granule sizes (diameters) of 4-16μm) of amylomaize V ranged from oval, to spherical to irregular to rod shaped (**Figure** 4-2e). The surface of many of these granules were rough with pores and fissures (**Figure** 4-2f). The granules of amylomaize VII had the same shape (**Figure** 4-2g) as those of amylomaize V (**Figure** 4-2e). However, granules of amylomaize VII were smaller (6-13μm), and the granule surface was smooth with no evidence of fissures or indentations (**Figure** 4-2h). Pores on granule surfaces have been shown to be real anatomical features of the native granule structure and not artifacts of starch isolation, drying, specimen preparation or observation techniques (Fannon *et al.*, 1992). Surface pores on granules of maize, sorghum, and millet are openings to channels that penetrate in a radial direction through the granule (Fannon *et al.*, 1993, Baldwin *et al.*, 1994, Huber and Bemiller, 1997). Several researchers (Gallant *et al.*, 1973, Fuwa *et al.*, 1977) have postulated that pores on the granule surface increases the accessibility of α-amylase into the granule interior. This suggests, that the concentration of H_3O^+ inside the granule interior may also be influenced by granule porosity.

4.3 Solubility patterns

The solubilization patterns of native starches are presented in **Figure** 4-3. Similar curves have been reported using HCl (Robin *et al.*, 1975, Maningat and Juliano, 1979, Biliaderis *et al.*, 1981, Muhr *et al.*, 1984, Shi and Seib, 1992, Vasanthan and Bhatty, 1996, Jane *et al.*, 1997, Jacobs *et al.*, 1998, Shi *et al.*, 1998). A relatively high rate was observed during the first 6 days, followed by a slower rate there after. At the end of the 8th day of hydrolysis (corresponding mainly to the degradation of the amorphous region of the granule), normal maize, amylomaize V, amylomaize VII, waxy maize, oat, and rice starches were hydrolyzed to the extent of 61.1, 32.6, 28.5, 68.1, 64.4, and 62%, respectively (**Table** 4.2). Between the 9th and 15th day (corresponding mainly to the degradation of starch crystallites), the increase in the extent of hydrolysis was more pronounced in normal maize (9.3%) than in oat (8.3%), rice (8.1%), waxy maize (7.2%), amylomaize V (3.0%), and amylomaize VII (3.1%) starches. At the end of the 15th day, normal maize, amylomaize V, amylomaize VII, waxy maize, rice and oat starches were hydrolyzed to the extent of 73.4, 37.0 32.3, 77.3, 75.3, and 72.9%, respectively. Differences in the extent and rate of hydrolysis between the starches during the initial stages (1-8 days) of hydrolysis has been attributed mainly to differences in: (1). granule size, (smaller granules are hydrolyzed faster than larger granules (Vasanthan and Bhatty. 1996); (2) amount of lipid complexed chains (lipid complexed amylose chains have been shown to resist degradation by acids) [Inouchi *et al.*, 1987, Morrison *et al.*, 1993a]; and (3) extensive interaction between starch chains within the amorphous domains of the

Figure 4-3 Acid hydrolysis (2.2N HCl, 35°C) profiles of cereal starches (solubilized carbohydrates as a function of time of native cereal starches)

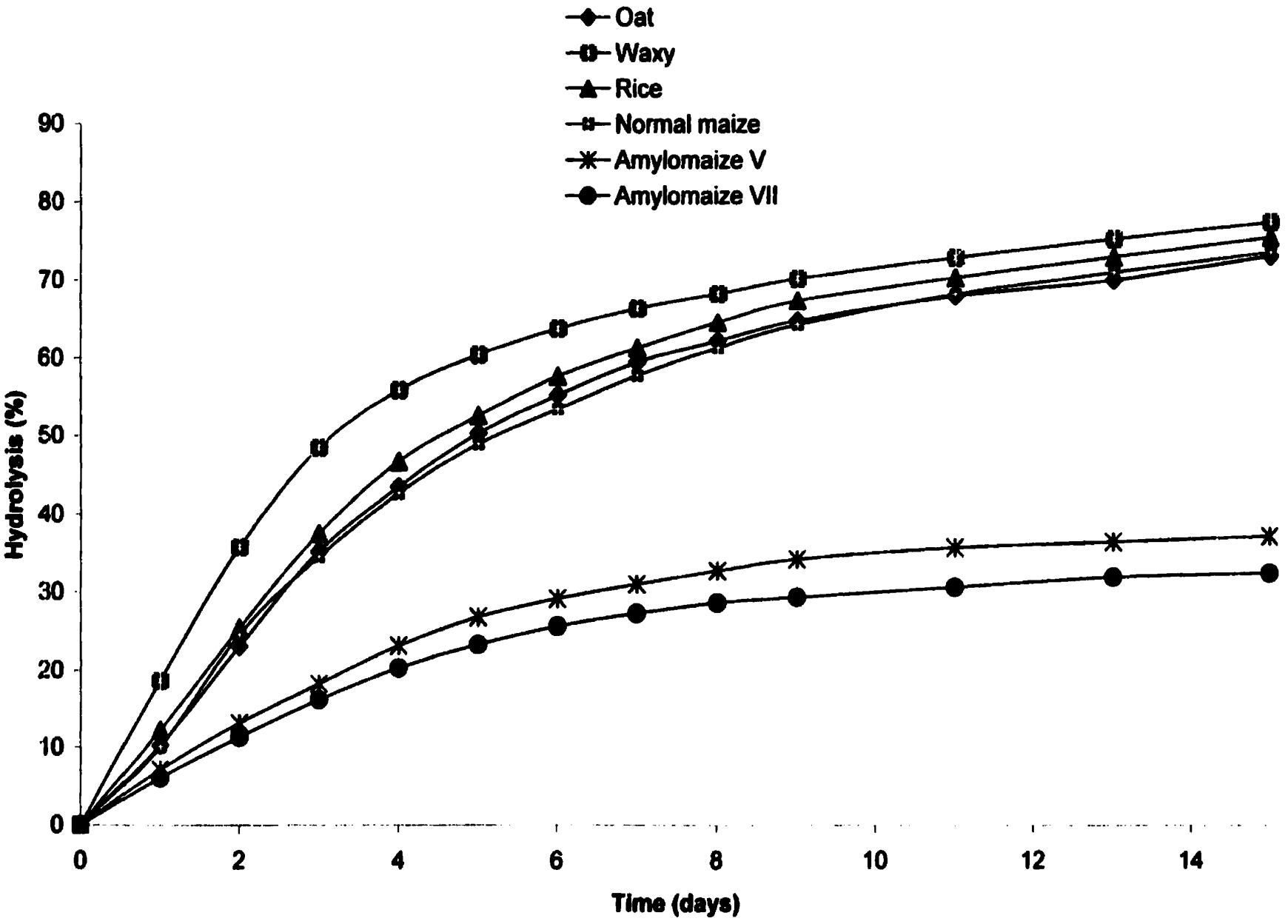

Oat
Waxy
Rice
Normal maize
Amylomaize V
Amylomaize VII
Hydrolysis (%)
0
10
20
30
40
50
60
70
80
90
0
2
4
6
8
10
12
14
Time (days)

Table 4.2 % Hydrolysis (2.2N HCl, 35°C) of native cereal starches*

	Number of days												
	0	1	2	3	4	5	6	7	8	9	11	13	15
Starch source	%												
Normal maize	0.0 ± 0.0	10.0±0.01^{a}	24.60 ±0.09^{a}	34.50± 0.14^{a}	42.60 ±0.01^{a}	48.80±0.11^{a}	53.30±0.03^{a}	57.60±0.10^{a}	61.10±0.19^{a}	64.08±0.07^{a}	68.06±0.10^{a}	70.85±0.00^{a}	73.40±0.14^{a}
Amylomaize V	0.0 ±0.0	7.1±0.11^{b}	13.13±0.13^{b}	18.25±0.01^{b}	23.18±0.03^{b}	26.80±0.06^{b}	29.10±0.02^{b}	30.90±0.01^{b}	32.60±0.01^{b}	34.01±0.02^{b}	35.60±0.03^{b}	36.32±0.06^{b}	37.02±0.01^{b}
Amylomaize VII	0.0 ± 0.0	6.0±0.01^{c}	11.30±0.02^{c}	16.20±0.00^{c}	20.30±0.15^{c}	23.30±0.01^{c}	25.60±0.11^{c}	27.20±0.06^{c}	28.50±0.03^{c}	29.20±0.13^{c}	30.50±0.06^{c}	31.80±0.20^{c}	32.30±0.04^{c}
Waxy maize	0.0± 0.0	18.6±0.02^{d}	35.76±0.06^{d}	48.40±0.02^{d}	56.80±0.01^{d}	60.30±0.00^{d}	63.60±0.06^{d}	66.20±0.12^{d}	68.10±0.11^{d}	70.07±0.04^{d}	72.70±0.16^{d}	75.10±0.00^{d}	77.30±0.09^{d}
Rice	0.0±0.0	12.2±0.13^{e}	25.40±0.17^{e}	37.50±0.05^{e}	46.60±0.03^{e}	52.50±0.16^{e}	57.52±0.01^{e}	61.10±0.00^{e}	64.40±0.19^{e}	67.20±0.13^{e}	70.20±0.10^{e}	72.80±0.01^{e}	75.30±0.11^{e}
Oat	0.0±0.0	10.2±0.05^{f}	23.10±0.00^{f}	35.20±0.12^{f}	43.40±0.00^{f}	50.20±0.10^{f}	55.10±0.15^{f}	59.30±0.01^{f}	62.00±0.01^{f}	64.60±0 02^{f}	67.81±0.01^{f}	69.90±0.21^{f}	72.92±0.00^{f}

*Results are the means of the at least four replicates. The values of % hydrolysis followed by the same superscript in the same column are not significantly different ($P<0.05$) by Tukey's HSD test.

granule (interaction between starch chains will hinder the conformational transformation (**Figure** 2-13) required for protonation of the glycosidic oxygens) [Wu and Sarko, 1978b, Hoover and Manuel, 1996, Jacobs *et al.*, 1998]. It is likely, that pores on the granule surface may allow direct access of H_3O^+ into the granule interior, and thus, should also be considered as a factor influencing the rate and extent of acid hydrolysis.

In this study, the observed extent of hydrolysis during the initial stages (1-8 days) of hydrolysis cannot be explained solely on the basis of the amount of lipid complexed amylose chains (oat> rice> amylomaize V> amylomaize VII> normal maize (**Table** 4.1), granule size (normal maize > amylomaize V > waxy maize > amylomaize VII > oat > rice (**Table** 4.1), presence of pores (**Figures** 4-1 and 4-2) on the granule surface (rice > amylomaize V > normal maize > waxy maize) or amylose content (amylomaize VII > amylomaize V > oat > normal maize > rice (**Table** 4.1). It is likely, that the interplay of the above factors must be considered when explaining the rate and extent of starch hydrolysis. For instance, if the amount of lipid complexed amylose chains was the sole factor influencing acid hydrolysis, then amylomaize V should have been hydrolyzed to a lower extent [due to its higher content of lipid complexed amylose chains (**Table** 4.1)] than amylomaize VII. However, the observed trend in hydrolysis (**Figure** 4-3) suggest that the presence of surface pores (**Figure** 4-2f) and the lower amylose content of amylomaize V (**Table** 4.1), may have negated the influence of lipid complexed amylose chains on acid hydrolysis. The difference in acid hydrolysis between waxy maize and the other starches (during the first 9 days) reflects the absence

of lipid complexed amylose chains in waxy maize and the greater degree of accessibility of H_3O^+ [due to the presence of pores (**Figure** 4-2d) and absence of amylose chains] into the amorphous regions of waxy maize starch. The difference in hydrolysis between rice and normal maize starches (**Figure** 4-1) can be attributed to differences in granular size (**Table** 4.1) [normal maize> rice] and to the amount of pores on the granule surface (**Figures** 4-1f and 4-2b) [rice> normal maize]. The results indicate that the combination of the above two factors negate the influence of lipid complexed amylose chains (rice> normal maize) [**Table** 4.1] on hydrolysis. Difference in hydrolysis between the starches beyond the 9^{th} day can be attributed to the interplay of the following factors: (1) the extent of distribution of the α (1→6) branches between the crystalline and amorphous regions of amylopectin; (2) amylopectin content and (3) degree of packing of the double helices within the crystalline region.

Recently it was shown (Jane *et al.*, 1997) that in A-type starches, the branch α(1→6) linkages are located within the crystalline and amorphous areas, whereas in B-type starches, the branches are located solely within the amorphous area (**Figure** 2-15). The above authors have also shown that branch linkages inside the crystalline area are protected from acid hydrolysis. On this basis, the B-type starches (amylomaize V and amylomaize VII) should have been hydrolyzed to a greater extent than the A-type (normal maize, waxy maize, rice and oat) starches. However, the difference in hydrolysis (after the 9^{th} day) [**Figure** 4-1] between and among A- and B-types starches, suggests that the susceptibility of the crystalline regions of these starches towards H_3O^+ is more

likely influenced by differences in the packing arrangement of the double helices within the crystallite, rather than by the mode of distribution of the α (1→6) branches between the amorphous and crystalline regions. It is likely, than in B-type starches, double helices in crystallites are formed to a larger extent, by the interwining of linear amylose chains (due to their high amylose content) [**Table** 4.1]. Whereas, in A-type starches (lower amylose content [**Table** 4.1] double helices in crystallites are formed mainly by the interwining of the outer branches of amylopectin. Thus, double helices in the B-type starches would be more tightly packed due to strong interactions (*via* hydrogen bonding and Van der Waals forces) between linear amylose chains than those present in A-type starches. Strong interactions within and between double helices could reduce chain flexibility, thereby rendering the conformational transformation (chair→half chair) extremely difficult. This could then explain the difference in hydrolysis (beyond the 8^{th} day) between the A and B type starches and between the B-type starches (**Figure** 4-1). The difference in hydrolysis between the A-type starches (**Figure** 4-1) during the above time period, can be attributed to differences in their amylopectin content (waxy maize> rice> normal maize> oat [**Table** 4.1]. The greater susceptibility of waxy maize starch to hydrolysis, suggests the presence of loosely packed double helices (which are mostly formed by interwining of the outer branches of amylopectin) within the crystallites.

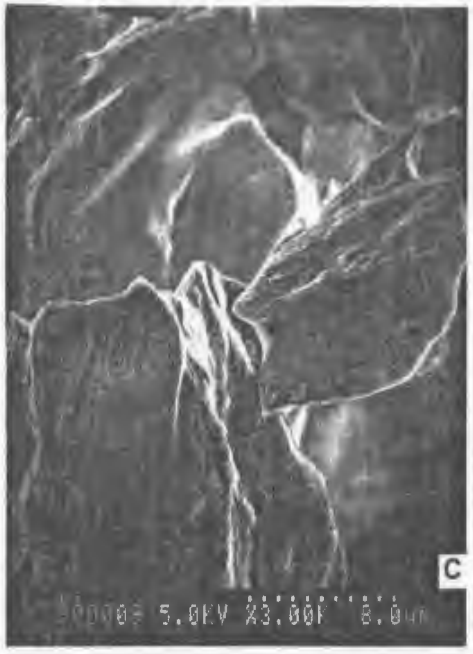
4.4 Granular morphology of acid treated starches

The external morphology of the starch granules after 8 days of hydrolysis are presented in **Figures** 4-2 and 4-4. In oat starch, many granules were deformed

Figure 4-4 Scanning electron micrographs of acid treated (2.2N HCl, 35°C for 8 days) maize starches: (A) normal maize (3,000X); (B) normal maize (10,000X); (C) waxy maize (3.000X); (D) waxy maize (10,000X); (E) amylomaize V (3,000X); (F) amylomaize V (10,000X); (G) amylomaize VII (3,000X); (H) amylomaize VII (10,000)

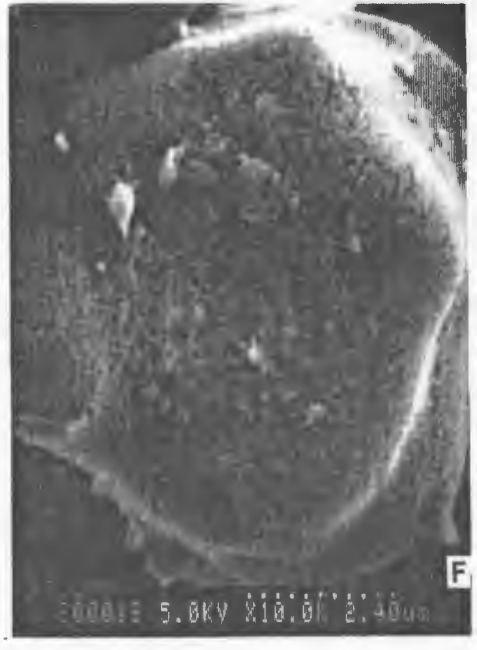

A
200005 5.0KV X3.00K 8.0um

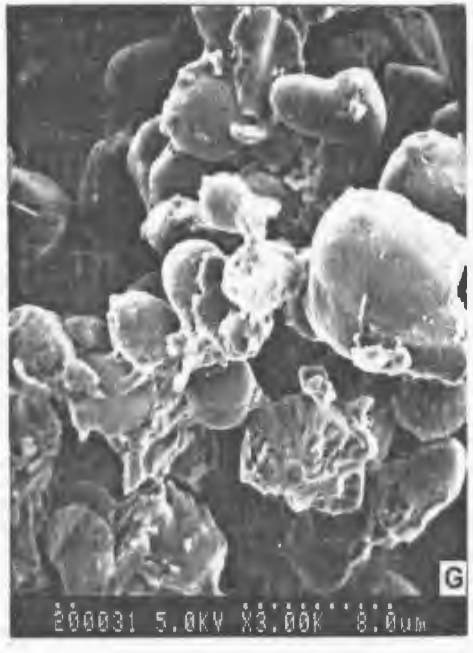

B

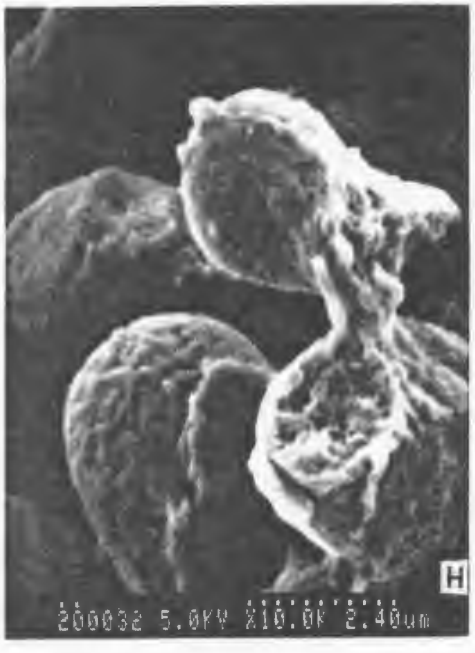

C


D


E
200011 5.0KV X3.00K 8.0um


F


G
200031 5.0KV X3.00K 8.0um


H
200032 5.0KV X10.0K 2.40um

and their surfaces were completely covered with numerous small pores (**Figures** 4-2c, d). In contrast, rice starch was devoid of granular form (**Figure** 4-2g) and contained many fragmented granules (**Figure** 4-2h). Pores were absent on fragmented granules. In normal maize starch (**Figure** 4-4a), granular form was still discernible in many granules. Furthermore, granule deformation was less than in rice (**Figure** 4-2g) and oat (**Figure** 4-2c) starches. The surface of normal maize starch was completely eroded with numerous craters and pores of varying dimensions (**Figure** 4-4b). In contrast, waxy maize starch exhibited a total loss of granular shape (**Figure** 4-4c), and the whole mass was covered with numerous pores and cracks (**Figure** 4-4d). However, granule shape was clearly discernible in both amylomaize V (**Figure** 4-4e) and amylomaize VII (**Figure** 4-4f) starches. However, their external appearance was entirely different. The surface of some amylomaize V granules was extensively corroded with numerous pores and cracks (**Figures** 4-4e,f). Whereas, the surface of amylomaize VII granules were wrinkled and devoid of cracks (**Figures** 4-4g,h). Very few pores were present on the granules surface of amylomaize VII (**Figures** 4-4g,h) starch.

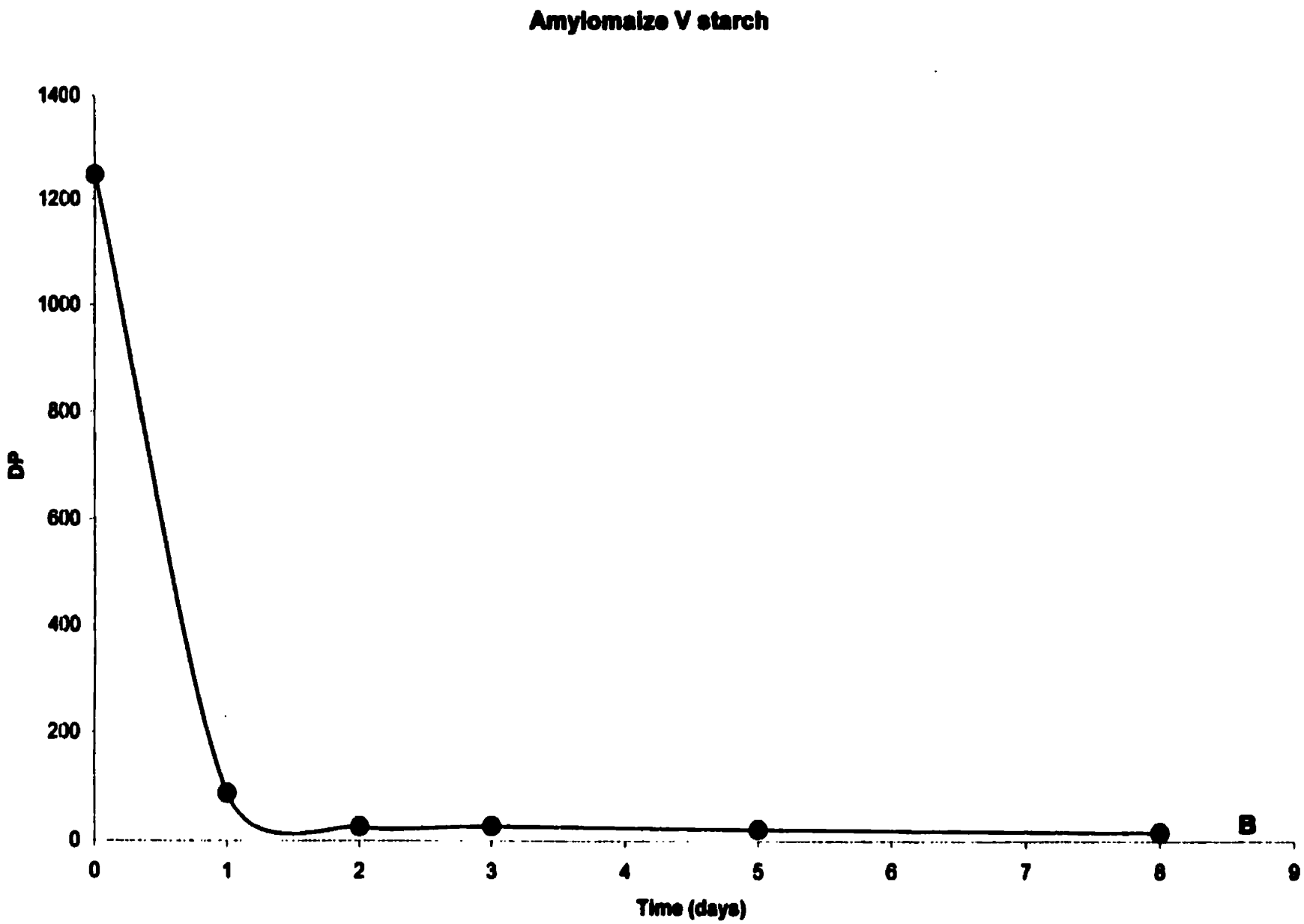
4.5 Starch structure

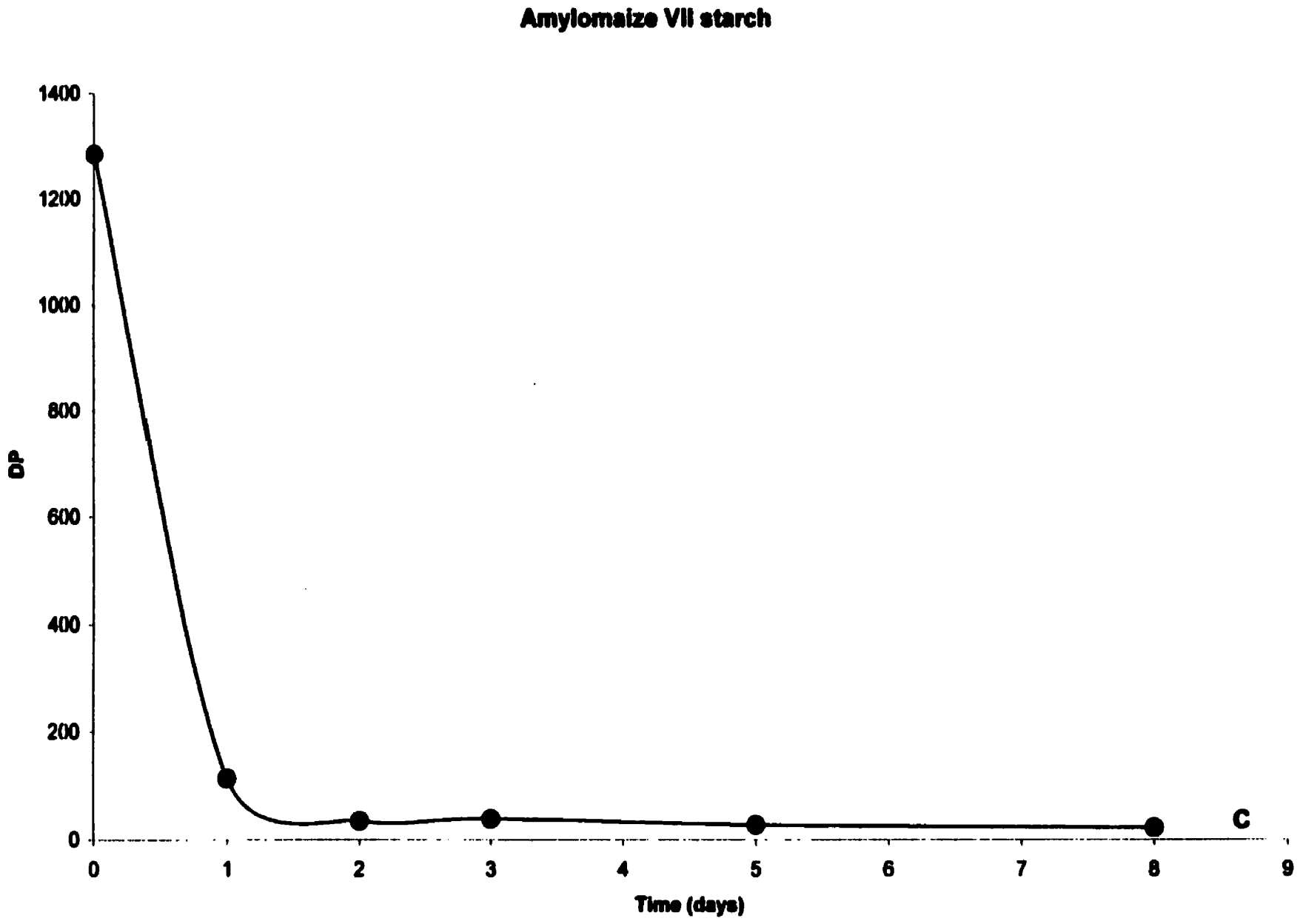
4.5.1 Molecular structure

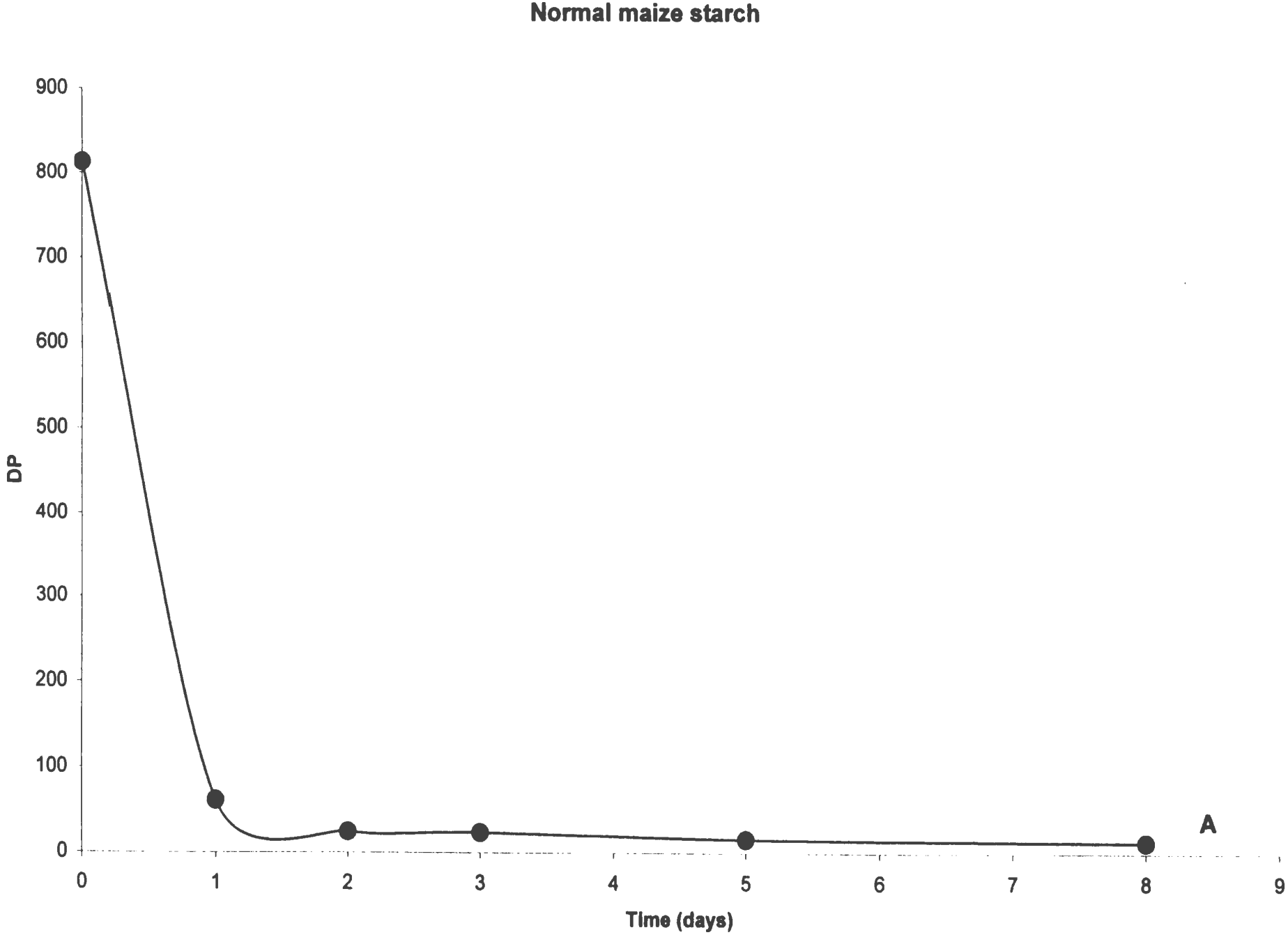
4.5.1.1 Average degree of polymerization (DP)

The change in the average degree of polymerization (DP) of the native starches during the first 8 days of hydrolysis is presented in **Figure** 4-5 and **Table** 4.3. In all starches, DP decreased rapidly during the first 48h, followed by a slower

Figure 4-5 A-H Changes to the average degree of polymerization (DP) of cereal starches with time course of hydrolysis


Amylomaize V starch
1400
1200
1000
800
600
400
200
0
DP
0
1
2
3
4
5
6
7
8
9
Time (days)
B


Amylomaize VII starch
1400
1200
1000
800
600
400
200
0
DP
0
1
2
3
4
5
6
7
8
9
Time (days)
C


Normal maize starch
900
800
700
600
500
400
300
200
100
0
DP
0
1
2
3
4
5
6
7
8
9
Time (days)
A

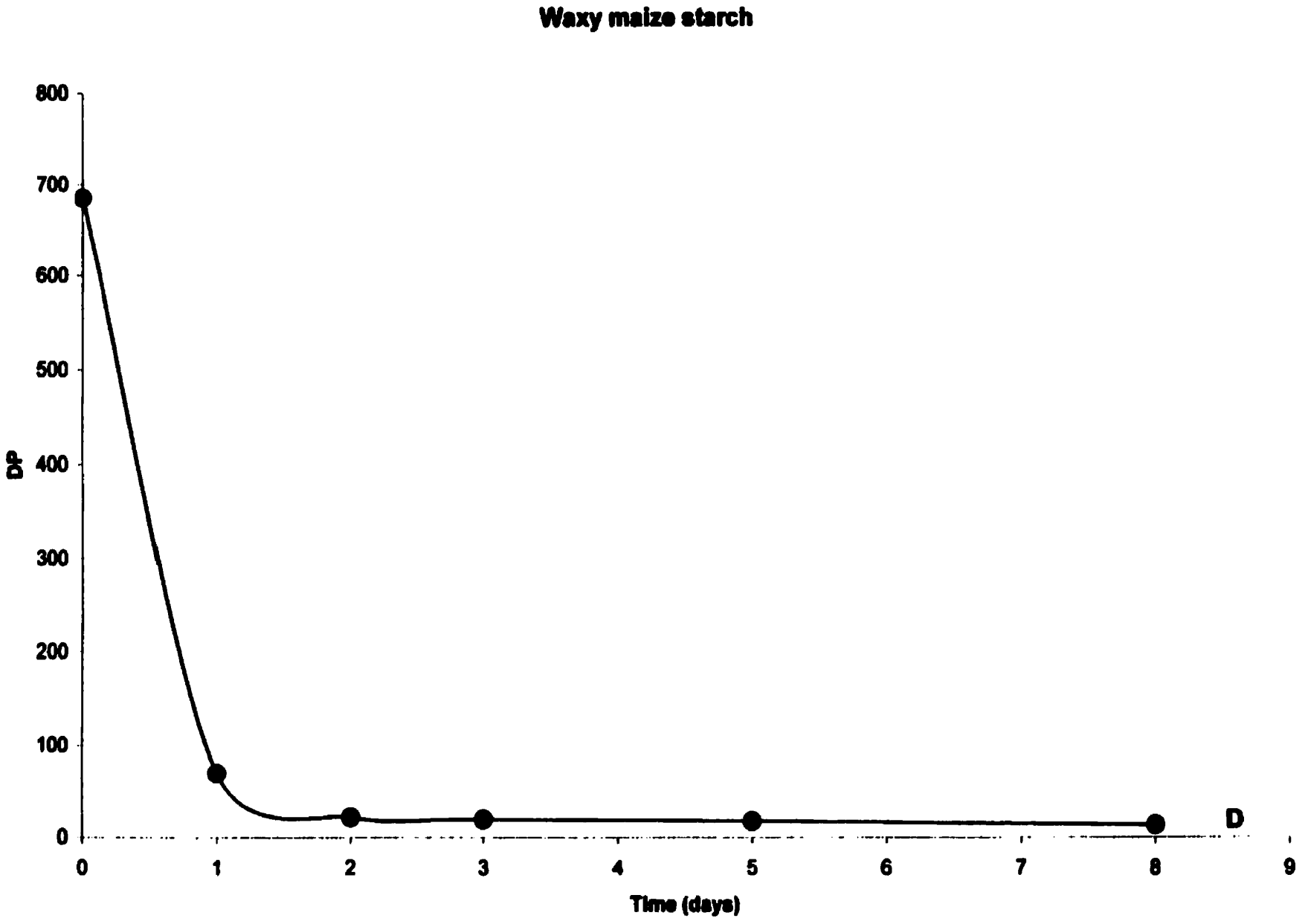
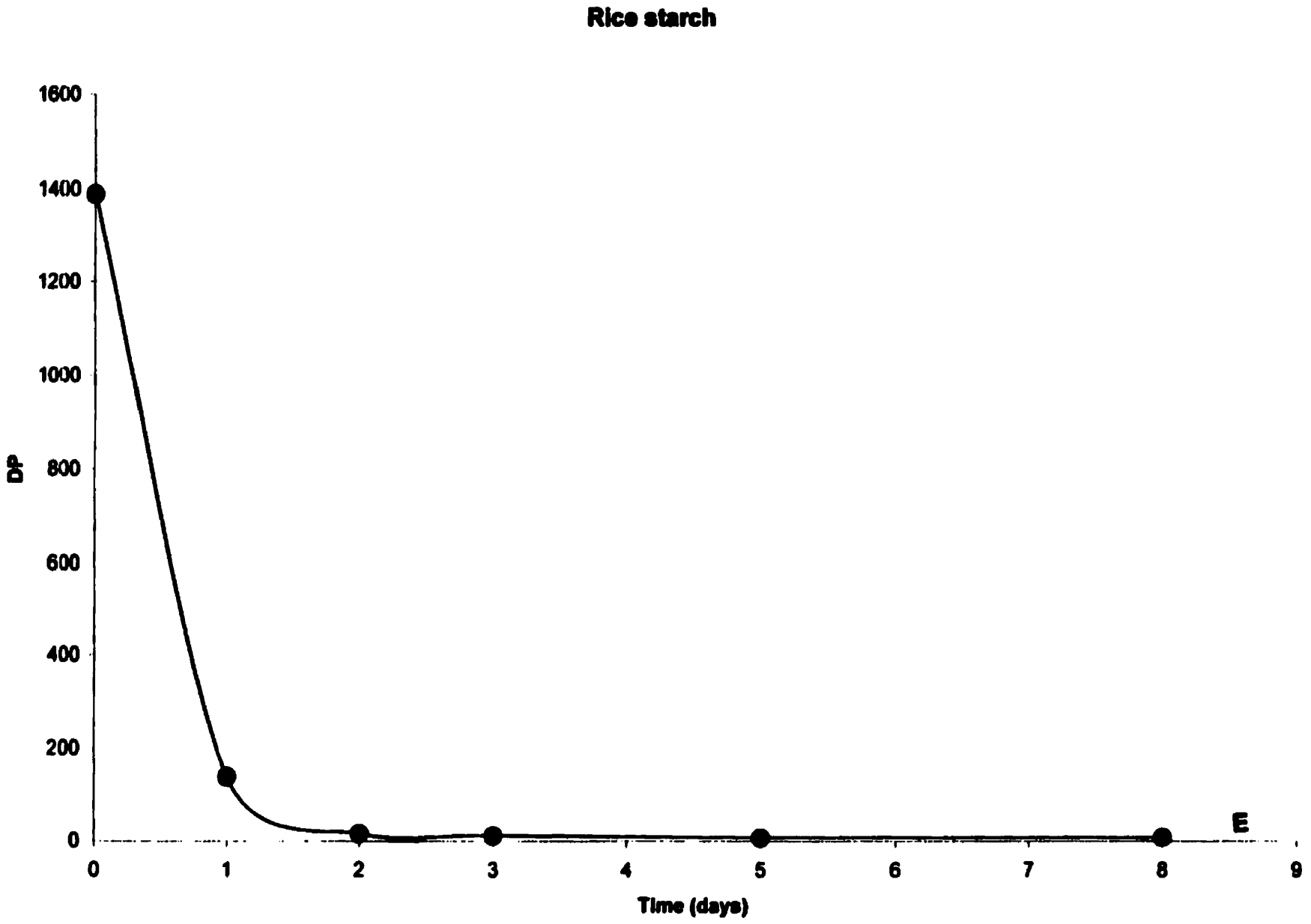

Waxy maize starch
800
700
600
500
400
300
200
100
0
DP
0
1
2
3
4
5
6
7
8
9
Time (days)
D

Table 4.3 Average degree of polymerization (DP) of native and acid treated starches with time course of hydrolysis

Type	Oat starch	Rice starch	Normal maize	Amylomaize V	Amylomaize VII	Waxy maize
Native starch	708 ± 11^{a}	1389 ± 16^{b}	813 ± 17^{c}	1247 ± 14^{d}	1285 ± 10^{e}	685 ± 18^{a}
Day 1	51 ± 4^{a}	139 ± 9^{c}	49 ± 6^{a}	$58 \pm 9^{a,b}$	88 ± 7^{d}	69 ± 0^{b}
Day 2	17 ± 3^{a}	16 ± 2^{a}	$27 \pm 3^{b,c}$	31 ± 6^{c}	54 ± 0^{e}	$21 \pm 4^{a,b}$
Day 3	16 ± 2^{a}	12 ± 3^{a}	$24 \pm 5^{b,c}$	27 ± 4^{c}	39 ± 2^{e}	$19 \pm 7^{a,b}$
Day 5	$12 \pm 2^{a,b}$	8 ± 1^{b}	$16 \pm 3^{a,c}$	$21 \pm 2^{c,d}$	28 ± 6^{e}	$17 \pm 4^{f,c}$
Day 8	$11 \pm 1^{a,b,g}$	8 ± 2^{b}	$13 \pm 4^{a,c}$	$16 \pm 0^{c,d}$	22 ± 1^{e}	$13 \pm 3^{c,d,f,g}$

Results are the means of the replicates. The values of DP followed by the same superscript in the same row are not significantly different ($P<0.05$) by Tukey's HSD test.


Rice starch
1600
1400
1200
1000
800
600
400
200
0
DP
0
1
2
3
4
5
6
7
8
9
Time (days)
E

decrease thereafter (**Figure** 4-5). The extent of this decrease was fairly similar for all starches. The extent of decrease in DP beyond 48h followed the order: amylomaize VII> amylomaize V> normal maize> rice ~ waxy maize> oat (**Figure** 4-5). In oat (**Figure** 4-5f) and rice (**Figure** 4-5e) starches, DP reached a constant limiting value after 72h. However, DP continued to decrease even beyond 72h in normal maize (**Figure** 4-5a) waxy maize, (**Figure** 4-5d), amylomaize V (**Figure** 4-5b) and amylomaize VII (**Figure** 4-5c) starches. The limiting DP observed in rice (**Figure** 4-5e) and oat starches (**Figure** 4-5f) suggests the presence of insoluble double helical structures formed by interaction between fragmented chains of DP 8-12, which are released after 72h hydrolysis. The absence of a limit DP during the time course of hydrolysis for normal maize (**Figure** 4-5a), amylomaize V (**Figure** 4-5b), and amylomaize VII (**Figure** 4-5c) and waxy maize (**Figure** 4-5d) starches suggests that the fragmented chains are too long for rapid formation of double helices. This seems plausible, since the extent of decrease in DP beyond 72h (amylomaize VII> amylomaize V> normal maize> waxy maize) paralleled the DP of their fragmented chains (**Figure** 4-5).

4.5.2 Crystalline structure

4.5.2.1 X-ray diffraction patterns of native starches

The X-ray diffractograms of native and acid treated normal maize, amylomaize V, VII, waxy maize, rice and oat starches are presented in **Figure** 4-6. Native normal maize, waxy maize, rice, and oat starches, exhibited the typical A-type X-ray spectrum of cereal starches (**Figures** 4-6a,d,e,f). Whereas, the amylomaize

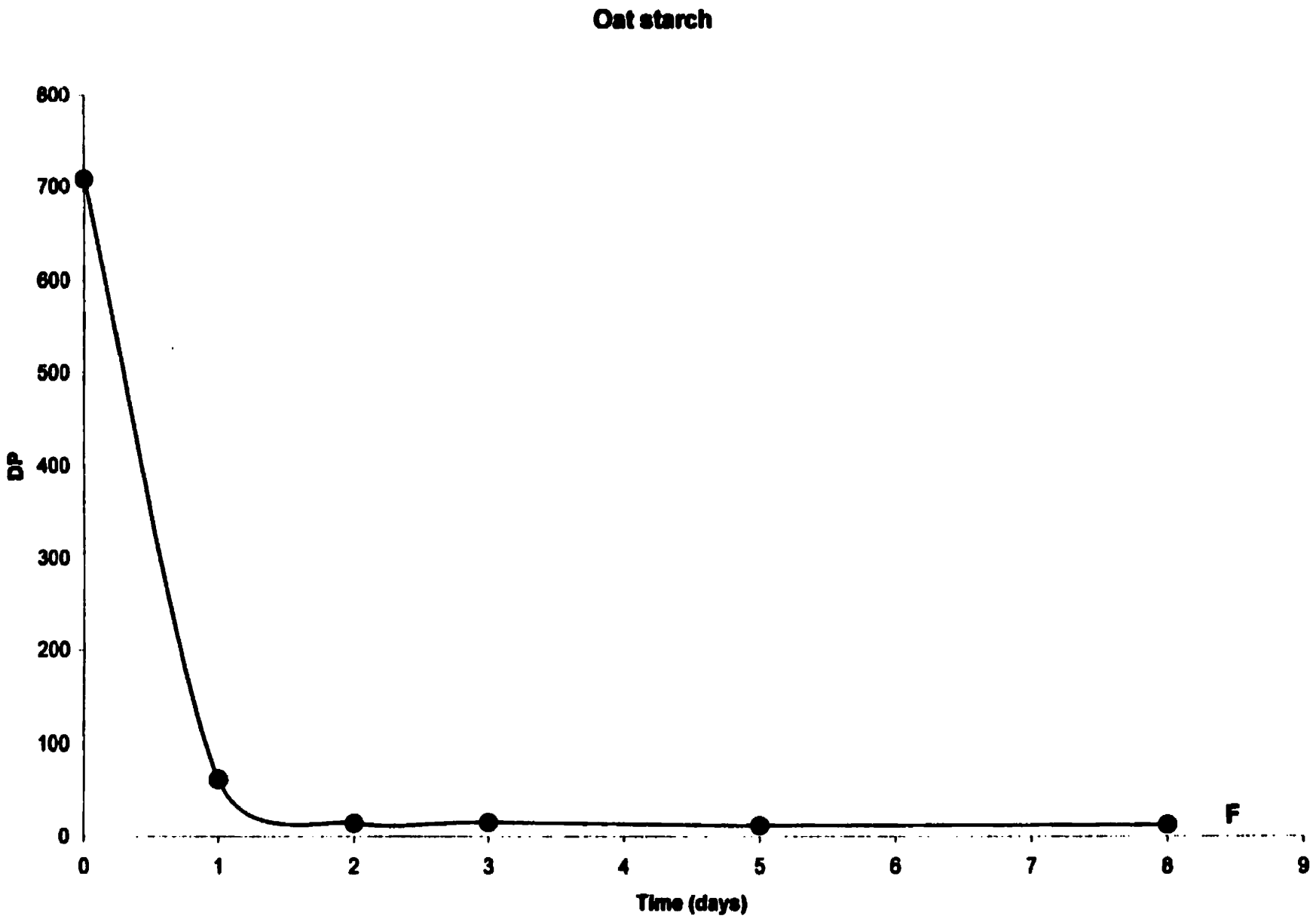
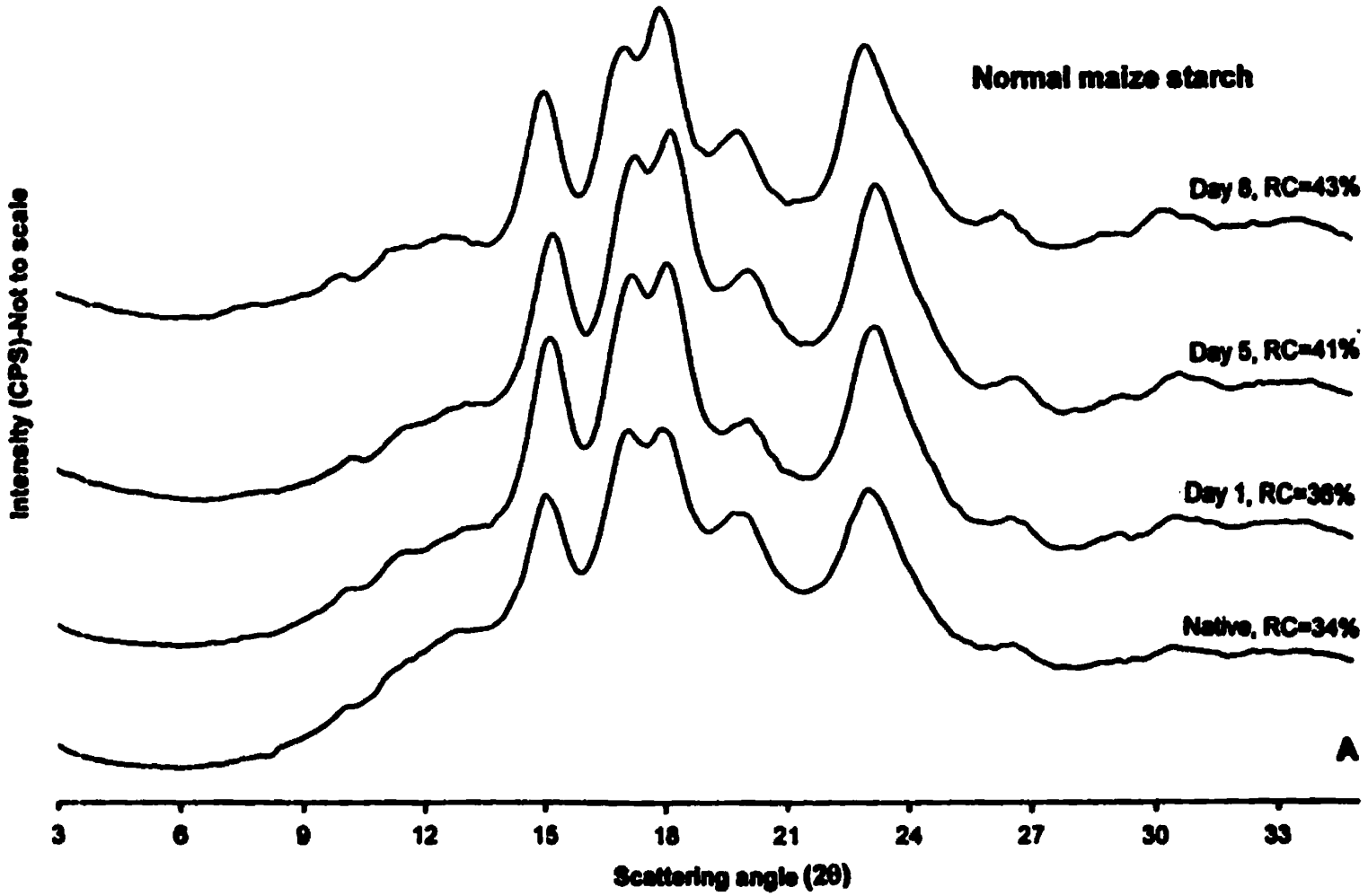
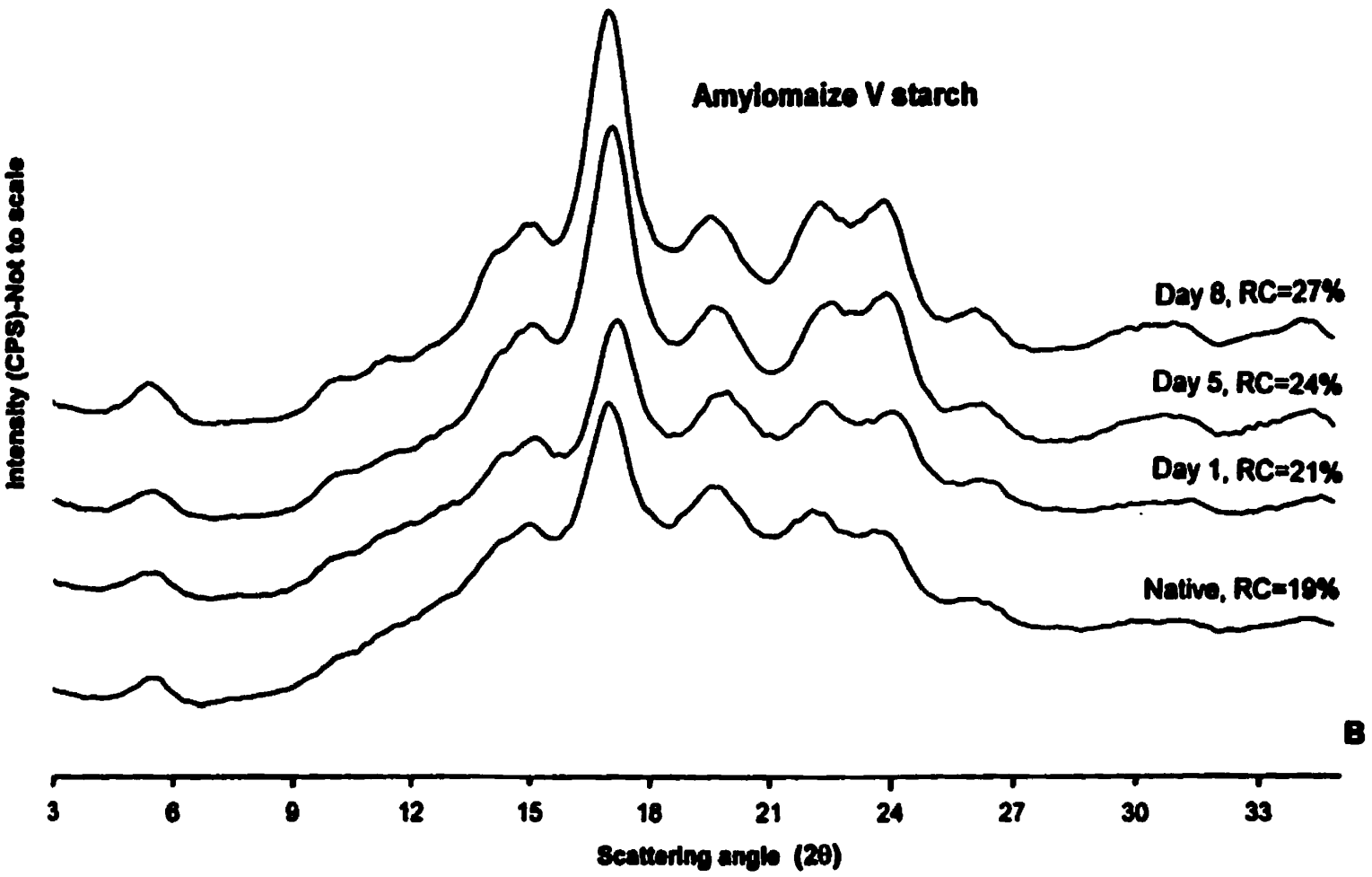
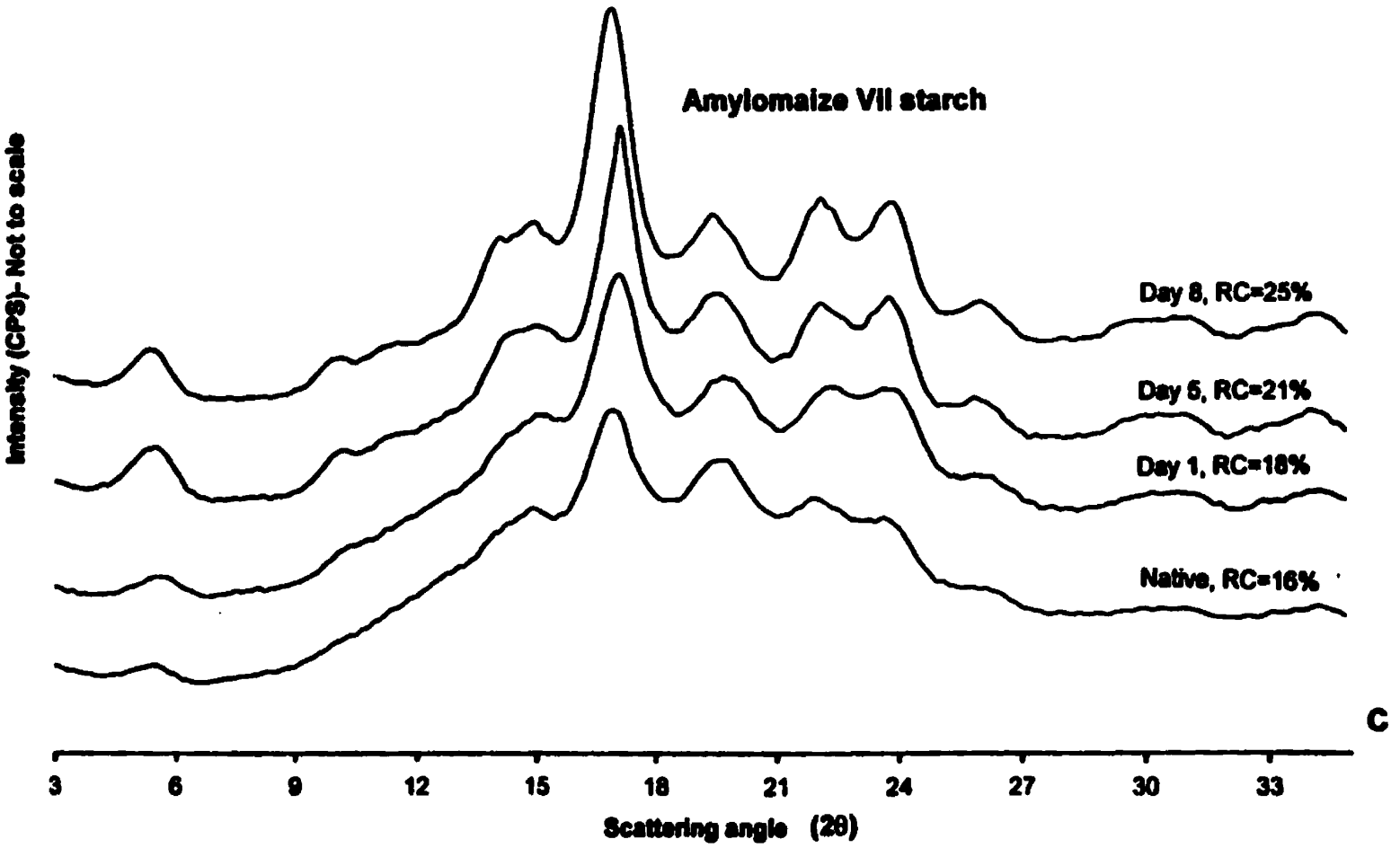
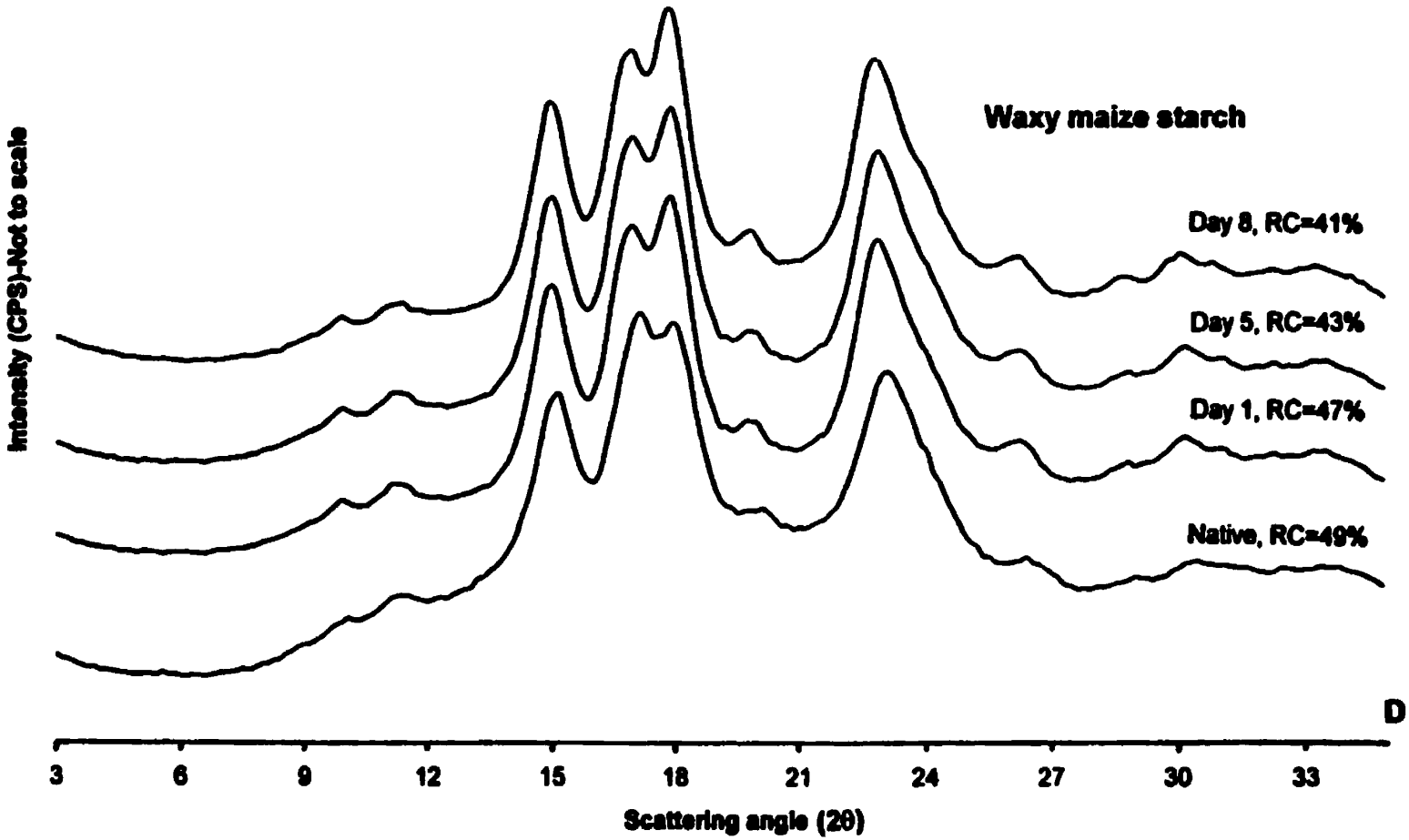

Oat starch
800
700
600
500
400
300
200
100
0
DP
0
1
2
3
4
5
6
7
8
9
Time (days)
F

Figure 4-6 A-H X-ray diffraction spectrum and relative crystallinity (RC) of native and acid treated cereal starches (Intensity: counts per second = CPS)


Normal maize starch
Day 8, RC=43%
Day 5, RC=41%
Day 1, RC=38%
Native, RC=34%
A
Intensity (CPS)-Not to scale
3
6
9
12
15
18
21
24
27
30
33
Scattering angle (2θ)

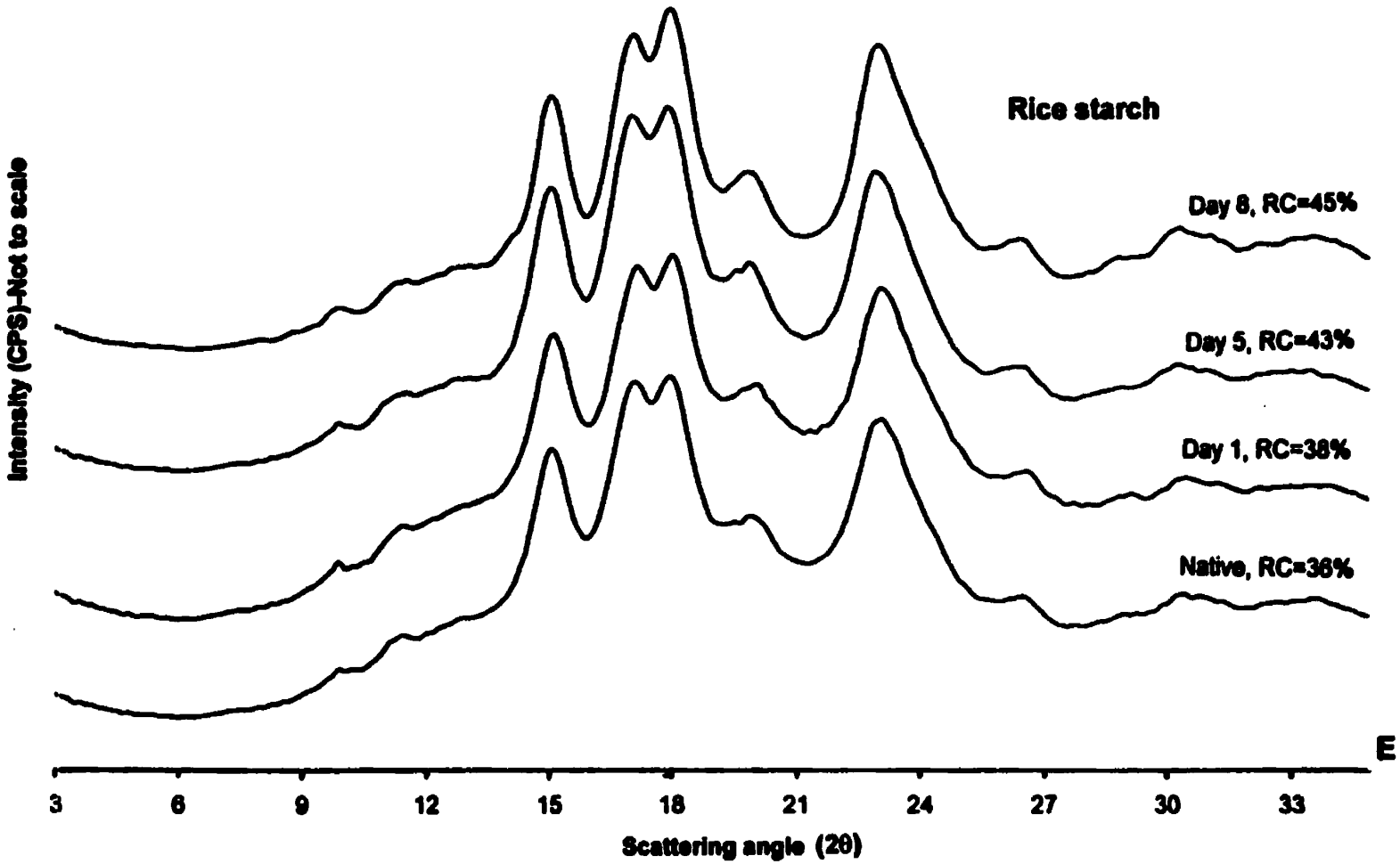

Amylomaize V starch
Intensity (CPS)-Not to scale
Day 8, RC=27%
Day 5, RC=24%
Day 1, RC=21%
Native, RC=19%
B
3
6
9
12
15
18
21
24
27
30
33
Scattering angle (2θ)


Amylomaize VII starch
Intensity (CPS)- Not to scale
Day 8, RC=25%
Day 5, RC=21%
Day 1, RC=18%
Native, RC=16%
C
3
6
9
12
15
18
21
24
27
30
33
Scattering angle (2θ)


Waxy maize starch
Day 8, RC=41%
Day 5, RC=43%
Day 1, RC=47%
Native, RC=49%
Intensity (CPS)-Not to scale
D
3
6
9
12
15
18
21
24
27
30
33
Scattering angle (2θ)

(-V and -VII) starches exhibited a B-type X-ray spectrum (**Figures** 4-6b,c). For the A-type spectrum, the most intense peaks corresponded to Bragg angles (2θ): 9.9° , 15°,17°, 18.1°, and 23.5°. The B-type spectrum was marked by an additional peak at 5.6° (2θ) Bragg angle, a decrease in the intensity of the peak at 15°, a single peak at 17° (instead of the doublet at 17° and 18° as in the A-type starches), and a splitting of the peak at 23.5° into two individual reflections at 22° and 23° (2θ) (**Figure** 4-6b,c). The relative crystallinity of the native starches followed the order: waxy maize (49%) > rice (36%) > normal maize (34%) > oat (32)> amylomaize (19%)> amylomaize VII (16%) (**Figures** 4-6a, f).

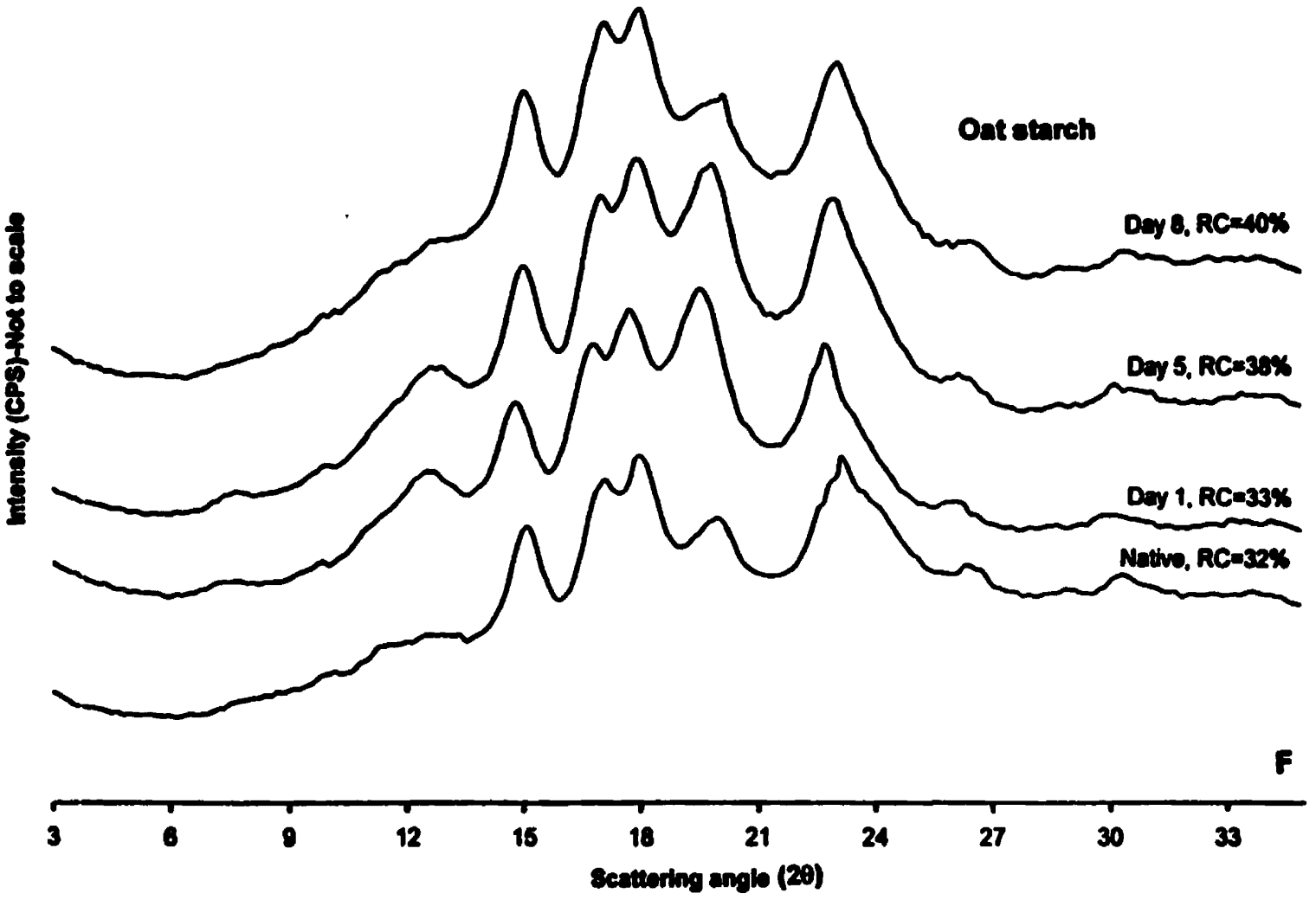
Crystallinity arises from ordered linear segments of amylopectin that are present in the form of double helices with a length of approximately 5nm. These double helices are crystallized into their (~5 nm) lamellar regions. In A-type starches, the amylopectin has a closer packing arrangement than that of B-type starches, and is characterized by amylopectin chains (DP 23-29) which are shorter than in B-type starches (Hizukuri, 1985, 1986) (DP 30-44). The extent of crystallinity is influenced by: (1) the amount of double helices that are organized into a crystalline array, (2) crystallites size and (3) amylose content. Small angle X-ray scattering studies on normal maize, waxy-maize and high amylose maize starches, have shown that amylose acts to disrupt the packing of the amylopectin double helices within the crystalline lamella (Jenkins and Donald, 1995). A reduction in crystalline lamella was observed with increase in amylose content (Jenkins and Donald 1995). It was suggested (Jenkins and Donald, 1995), that the disrupting effect of amylose on amylopectin could be due to co-crystallization


Rice starch
Day 8, RC=45%
Day 5, RC=43%
Day 1, RC=38%
Native, RC=36%
Intensity (CPS)-Not to scale
3
6
9
12
15
18
21
24
27
30
33
Scattering angle (2θ)
E

between amylose and amylopectin or to penetration of amylose into the amorphous region (where the α (1→6) branch points are located). It was interesting to observe that the relative crystallinity of native starches decreased with an increase in their amylose content (amylomaize VII> amylomaize V> oat> normal maize> rice> waxy maize [**Table** 4.1].

4.5.2.2 X-ray diffraction patterns of acid treated starches

The X-ray pattern of all starches remained unchanged on acid hydrolysis (**Figures** 4-6a-f). However, relative crystallinity decreased (7.4%) in waxy maize starch (**Figure** 4-6d) but increased (7.7-8.7%) in all other starches (**Figures** 4-6a-c,e,f). The extent of increase in relative crystallinity on hydrolysis followed the order: normal maize ~ rice ~ amylomaize VII> oat> amylomaize V. In amylomaize starches (V and VII), the intensity of the peak at 5.6 (2θ), increased with hydrolysis (**Figures** 4-6b,c). The increase in X-ray crystallinity on acid hydrolysis has been attributed to preferential hydrolysis of the amorphous lamella and of the amorphous background (Jenkins, 1994). The extent of hydrolysis being greater in the latter. In waxy maize starch, crystallites would be readily accessible to H_3O^+ due to the presence of only trace quantities of amylose. Thus, starch chains within crystallites would be hydrolyzed faster and to a greater extent in waxy maize than in the other starches. This would then explain the decrease in relative crystallinity (**Figure** 4-6d) and the high initial extent of hydrolysis (**Figure** 4-1) for waxy maize starch.


Oat starch
Day 8, RC=40%
Day 5, RC=38%
Day 1, RC=33%
Native, RC=32%
Intensity (CPS)-Not to scale
F
3
6
9
12
15
18
21
24
27
30
33
Scattering angle (2θ)

4.6 Starch properties

4.6.1 Swelling factor (SF)

4.6.1.1 Swelling factor of native starches

The swelling factor [which is an index of the swelling power of starches (Tester *et al.*, 1993] of native and acid treated starches at 80°C are illustrated in **Figure** 4-7 and **Table** 4.4. The SF of native starches followed the order: waxy maize> normal maize> oat> rice> amylomaize V> amylomaize VII. The above difference in SF can be attributed to the interplay of the following factors: (1) the level of lipid complexed amylose chains (**Table** 4.1) [lipid complexed amylose chains have been shown to inhibit granular swelling (Lorenz, 1976, Maningat and Juliano, 1980, Goshima *et al.*, 1985, Tester Morrison, 1990a, Vasanthan and Hoover, 1992, Morrison *et al.*, 1993b)] ; (2) molar proportion of amylopectin unit chains of DP 6-24 (oat> rice> waxy maize> normal maize> amylomaize V> amylomaize VII) [increased proportion of unit chains with DP 6-24 in single clusters corresponds to an increased number of branched points as well as number of chains, and results in a high molecular weight of interconnected non-crystalline branched regions next to crystallites. The increased effective molecular weight would decrease the mobility of chains in the amorphous phase and thereby restrict granular swelling (Shi and Seib, 1992)]; (3) amylose content (**Table** 4.1) [swelling power has been shown to decrease with increase in amylose content (Sasaki and Matsuki, 1998)] ; (4) extent of interaction between starch chains within the amorphous and crystalline domains (Hoover and Manuel, 1996) [hydrogen bonding between starch chains could decrease the

Figure 4-7 Swelling factor of native and acid treated cereal starches at 80°C

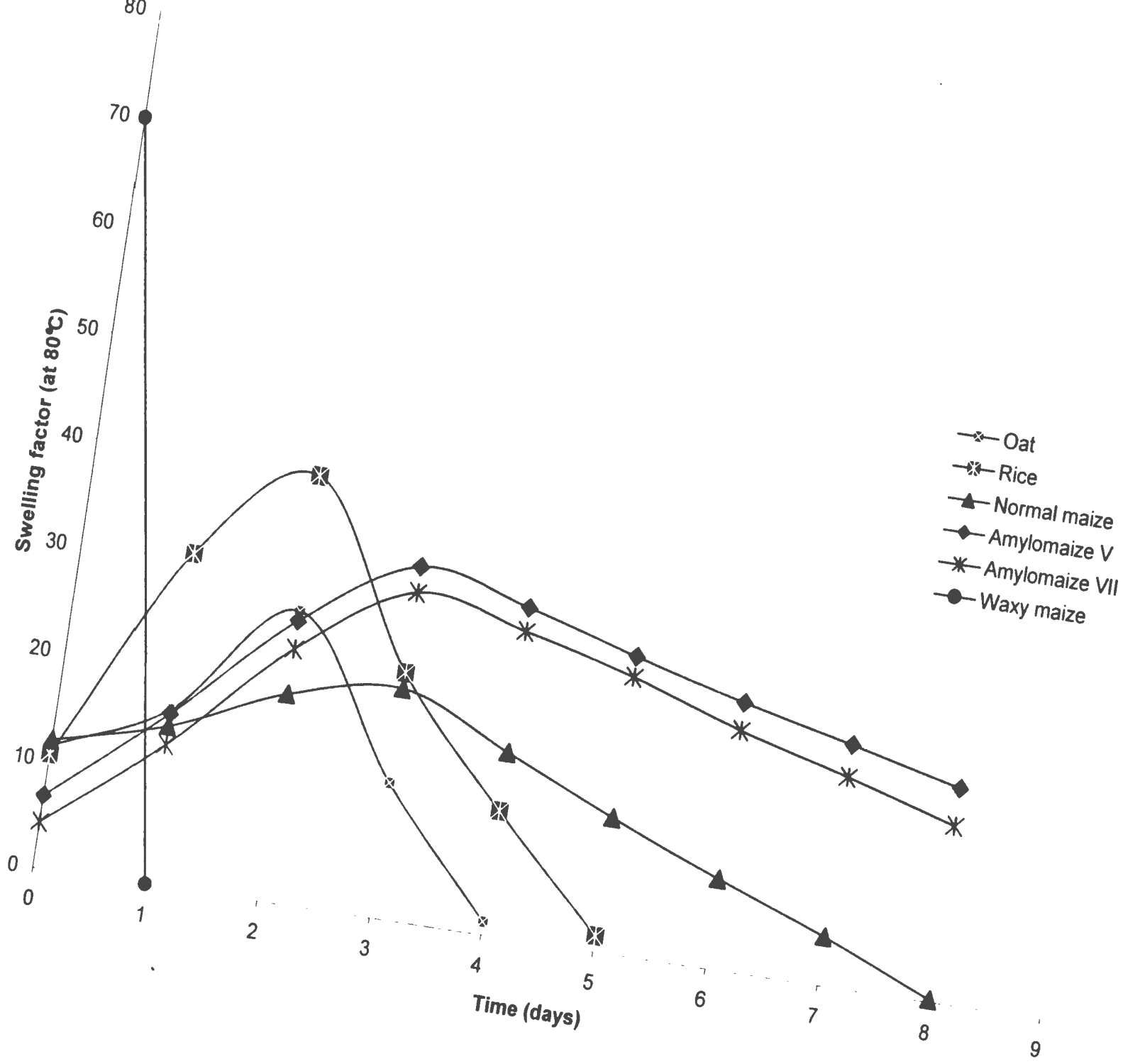

Swelling factor (at 80°C)
0
10
20
30
40
50
60
70
80
0
1
2
3
4
5
6
7
8
9
Time (days)
Oat
Rice
Normal maize
Amylomaize V
Amylomaize VII
Waxy maize

Table 4.4 Swelling factor of native and acid treated starches as a function of the time of hydrolysis by 2.2N HCl at 80°C

Starch source	Oat starch	Rice starch	Normal maize	Amylomaize V	Amylomaize VII	Waxy maize
Native starch	11.4 ± 0.0^{a}	10.6 ± 0.0^{b}	11.9 ± 0.0^{c}	6.7 ± 0.0^{d}	4.2 ± 0.2^{e}	70.0 ± 0.5^{f}
Day 1	16.3 ± 0.2^{a}	30.8 ± 0.0^{b}	14.7 ± 0.0^{c}	15.9 ± 0.1^{d}	13.0 ± 0.0^{e}	-
Day 2	27.0 ± 0.1^{a}	39.6 ± 0.0^{b}	19.2 ± 0.0^{c}	26.1 ± 0.2^{d}	23.5 ± 0.1^{e}	-
Day 3	12.6 ± 0.1^{a}	22.8 ± 0.0^{b}	21.3 ± 0.2^{c}	32.7 ± 0.2^{d}	30.3 ± 0.2^{e}	-
Day 4	1.2 ± 0.0^{a}	11.5 ± 0.1^{b}	-	-	-	-
Day 5	-	1.5 ± 0.0^{a}	12.4 ± 0.1^{b}	27.4 ± 0.1^{c}	25.5 ± 0.1^{d}	-
Day 6	-	-	8.3 ± 0.2	-	-	-
Day 7	-	-	4.5 ± 0.0	-	-	-
Day 8	-	-	0.2 ± 0.09^{a}	20.3 ± 0.3^{b}	16.3 ± 0.1^{c}	-

Results are the means of replicates. The values of SF followed by the same superscript in the same row are not significantly different ($P<0.05$) by Tukey's HSD test.

number of hydroxyl groups available for interaction with water; (5) crystallinity (Robin *et al.*, 1975) [very high or very low crystallinity could inhibit granular swelling].

4.6.1.2 Swelling factor of acid treated starches

After 24h hydrolysis, SF was completely lost in waxy maize starch, but increased in the other starches (rice> amylomaize V> amylomaize VII> oat> normal maize) [**Figure** 4-7]. A decrease in SF occurred after 2 days hydrolysis in oat and rice starches, but after 3 days for normal maize and amylomaize starches (V and VII) (**Figure** 4-7). SF was not detectable in oat, rice and normal maize starches after the 4^{th}, 5^{th}, and 8^{th} day of hydrolysis, respectively. However, the amylomaize starches (V and VII) showed measurable SF even after the 8^{th} day of hydrolysis (**Figure** 4-7). The complete loss of granular swelling in waxy maize starch 24h hydrolysis, suggests that both the α (1→6) branches within the amorphous region and the double helical chains forming the starch crystallites are extensively hydrolyzed during this time period. The extent of SF increase during the initial stages of hydrolysis of rice, oat, normal maize and amylomaize starches (V and VII) reflects interaction of hydrolyzed amylose chains (remaining within the granule) with water molecules. (These chains were originally associated with each other in the native granule, and with each other in the native granule, and thus unable to interact with water). The progressive decrease in SF with time of hydrolysis, reflects the continued erosion of the amorphous regions.

4.6.2 Amylose leaching (AML)

The extent of amylose leaching of native and acid treated starches at 80°C is presented in **Figure** 4-8 and **Table** 4.5. The AML of native starches followed the order: rice> normal maize> oat> amylomaize VII> amylomaize V. Thereafter, AML decreased gradually (oat> normal maize> rice> amylomaize VII). However, in amylomaize V, this decrease occurred only after 72h (**Figure** 4-8). The initial increase in AML could be attributed to leaching of hydrolyzed amylose chains. As discussed earlier, starch chains released by hydrolysis rapidly associate forming double helical structures. The extent of this association would be influenced by the; 1) chain length of hydrolyzed amylose chains; 2) extent of packing of the amylose chains within the amorphous regions; and by 3) the amount of lipid complexed amylose chains (lipid complexed amylose chains will inhibit starch chain association). This would then explain the decrease in AML beyond 24h (**Figure** 4-8).

4.6.3 Gelatinization parameters

4.6.3.1 Gelatinization of native starches

The gelatinization transition temperatures [onset (T_O), mid-point (T_P), conclusion (T_C), and gelatinization enthalpy (ΔH) of native and acid treated starches are presented in **Table** 4.6. The gelatinization transition temperatures range (T_C-T_O) for native starches followed the order; amylomaize VII> amylomaize V> rice> waxy maize> normal maize> oat. The enthalpy of gelatinization (ΔH) of the native starches followed the order: waxy maize> rice> normal maize> amylomaize VII> amylomaize V> oat. In all starches, T_O decreased, but T_P, T_C,

Figure 4-8 Extent of amylose leaching of native and acid treated starches at 80°C

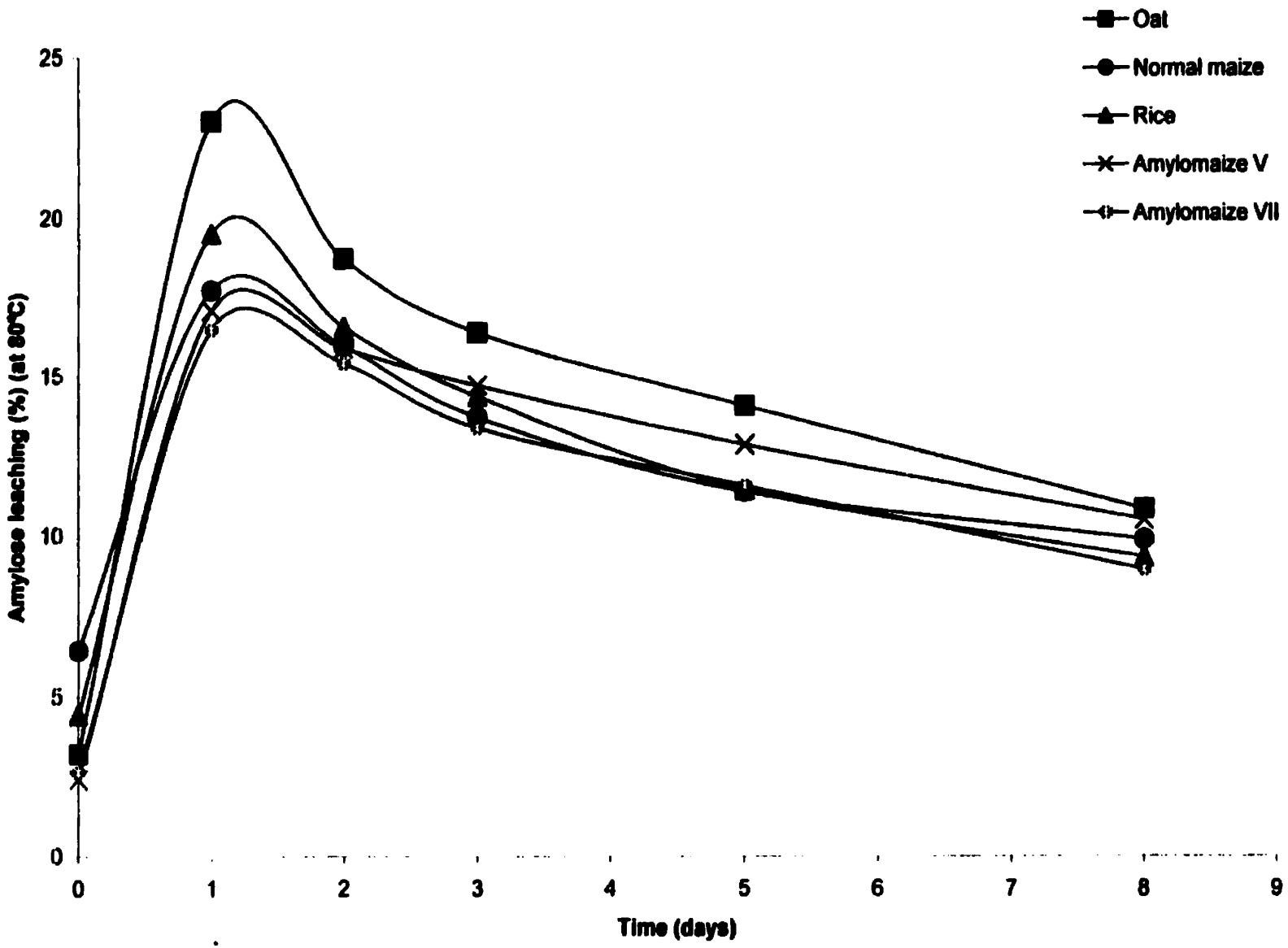

Oat
Normal maize
Rice
Amylomaize V
Amylomaize VII
Amylose leaching (%) (at 80°C)
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
Time (days)

Table 4.5 Amylose leaching (at 80°C) of native and acid treated starches

Type	Oat starch	Rice starch	Normal maize	Amylomaize V	Amylomaize VII
Native starch	3.2 ± 0.1^{a}	6.4 ± 0.1^{b}	4.4 ± 0.1^{c}	2.4 ± 0.1^{d}	2.6 ± 0.1^{e}
Day 1	23.0 ± 0.1^{a}	17.7 ± 0.2^{b}	19.5 ± 0.0^{c}	17.1 ± 0.0^{d}	16.5 ± 0.3^{e}
Day 2	18.7 ± 0.5^{a}	16.0 ± 0.1^{b}	16.6 ± 0.1^{c}	15.9 ± 0.1^{d}	15.4 ± 0.2^{d}
Day 3	16.4 ± 0.5^{a}	$13.7 \pm 0.3^{b,d}$	$14.3 \pm 0.1^{c,d}$	14.7 ± 0.1^{c}	13.3 ± 0.1^{b}
Day 5	14.0 ± 0.1^{a}	11.4 ± 0.2^{b}	11.4 ± 0.1^{d}	12.8 ± 0.3^{e}	$11.5 \pm 0.1^{b,c,d}$
Day 8	10.8 ± 0.4^{a}	9.9 ± 0.1^{b}	9.3 ± 0.1^{c}	10.5 ± 0.2^{a}	8.9 ± 0.2^{c}

Results are the means of replicates. The values of AML followed by the same superscript in the same row are not significantly different ($P<0.05$) by Tukey's HSD test.

Table 4.6 Gelatinization parameters[1] of native and acid treated starches[2]

Starch source	Hydrolysis time (h)	% Hydrolysis	Transition temperatures (°C)[3] T_o	T_p	T_c	T_c-T_o[4]	ΔH_G[5] (J/g)
Normal maize							
Native	0	0.0	64.8 ± 0.1[a]	72.6 ± 0.1[a]	79.7 ± 0.0[a]	14.9[a]	12.7 ± 0.0[a]
Acid treated	12	4.8	58.2 ± 0.3[b]	72.9 ± 0.2[b]	81.1 ± 0.1[b]	22.9[b]	13.3 ± 0.4[a]
Acid treated	24	10.0	56.9 ± 0.4[c]	74.6 ± 0.1[c]	94.4 ± 0.2[c]	37.5[c]	14.1 ± 0.3[b]
Amylomaize V							
Native	0	0.0	65.1 ± 0.9[a]	86.9 ± 0.7	103.4 ± 0.9[a]	38.3[a]	10.5 ± 0.4[a]
Acid treated	12	3.3	56.1 ± 0.3[b]	-	107.3 ± 0.5[b]	51.2[b]	14.4 ± 0.5[b]
Acid treated	24	7.1	51.6 ± 0.5[c]	-	115.0 ± 0.7[c]	63.4[c]	16.5 ± 0.2[c]
Amylomaize VII							
Native	0	0.0	67.1 + 0.5	96.5 ± 0.6	106.9 ± 0.3	39.8	12.5 ± 0.2
Acid treated	12	2.6	-[6]	-[6]	-[6]	-[6]	-[6]
Acid treated	24	6.0	-[6]	-[6]	-[6]	-[6]	-[6]
Waxy maize							
Native	0	0.0	65.8 ± 0.2[a]	71.1 ± 0.1[a]	82.3 ± 0.2[a]	16.5[a]	15.8 ± 0.0[a]
Acid treated	12	9.1	52.7 ± 0.3[b]	74.3 ± 0.2[b]	91.9 ± 0.5[b]	39.2[b]	16.2 ± 0.1[b]
Acid treated	24	18.6	49.9 ± 0.3[c]	78.4 ± 0.6[c]	96.2 ± 0.1[c]	46.3[c]	17.0 ± 0.2[c]
Rice							
Native	0	0.0	58.2 ± 0.1[a]	78.0 ± 0.3[a]	88.1 ± 0.1[a]	29.9[a]	13.0 ± 0.1[a]
Acid treated	12	5.9	56.7 ± 0.3[b]	79.1 ± 0.1[b]	90.0 ± 0.3[b]	33.3[b]	14.3 ± 0.2[b]
Acid treated	24	12.2	55.3 ± 0.7[c]	81.9 ± 0.6[c]	92.3 ± 0.2[c]	37.0[c]	15.7 ± 0.4[c]
Oat							
Native	0	0.0	58.1 ± 0.1[a]	62.9 ± 0.1[a]	71.5 ± 0.4[a]	13.4[a]	10.2 ± 0.0[a]
Acid treated	12	5.0	56.4 ± 0.3[b]	65.3 ± 0.2[b]	74.2 ± 0.3[b]	17.8[b]	11.0 ± 0.3[b]
Acid treated	24	10.2	52.2 ± 0.2[c]	67.5 ± 0.5[c]	77.7 ± 0.1[c]	25.5[c]	12.6 ± 0.0[c]

[1] Starch:water (1:3 w/w, dry basis)
[2] The starch were hydrolyzed with 2.2N HCl at 35°C .
[3] T_o, T_p, and T_c indicate the temperatures of the onset, midpoint and end of gelatinization respectively.
[4] T_c-T_o indicates the gelatinization temperature range.
[5] ΔH_G indicates the enthalpy of gelatinization
[6] The endotherms were too broad for an accurate estimation of T_o, T_p and T_c

and T_C-T_O increased with hydrolysis (**Table** 4.6). This result is in agreement with earlier studies (Chun *et al.*, 1997, Donovan and Mapes, 1980). The increase in T_C-T_O (after 24h hydrolysis) followed the order: waxy maize> amylomaize V> normal maize> oat> rice.

In all starches, ΔH increased with hydrolysis (**Table** 4-6). The extent of increase in ΔH (after 24h hydrolysis) followed the order: amylomaize V> rice> oat> normal maize> waxy maize. This was in agreement with those reported for barley (Morrison *et al.*, 1993c), potato (Jacobs *et al.*, 1998, Muhr and Blanshard, 1984), and pea (Jacobs *et al.*, 1998) starches.

Gelatinization is primarily a swelling driven process (Donovan and Mapes, 1980, Cooke and Gidley, 1992). Water uptake by the amorphous growth ring is accompanied by swelling within this region (Donovan, 1979). This swelling imposes a stress upon the amylopectin crystallites which causes the amylopectin double helices within the crystallites to dissociate. This process occurs rapidly for an individual crystallite, but over a wide temperature range for the whole granule. Smaller crystallites are less stable and are destroyed first (Jenkins 1994). This swelling driven crystallite disruption is associated with the low temperature narrow endotherm for the native starches (**Table** 4.6). The difference in T_C-T_O among native starches (**Table** 4.6) reflect variations in crystallite shape, crystallite size, degree of crystal perfection and on the type of starch chain interwining [amylose-amylose (AM-AM), amylose-amylopectin (AM-AMP), amylopectin-amylopectin (AMP-AMP), that produce the double helical chains of starch crystallites. In starches containing a very high amylose content

(amylomaize V and VII), crystallites would originate not only from AMP-AMP interactions; but also from interaction between AM-AM and AM-AMP (co-crystallization between amylose and amylopectin). Consequently, variations in crystallites stability in the amylomaize starches (V and VII) would be much greater than in the other starches. This would then explain the broader endotherm (**Table** 4.6) of native amylomaize starches (V and VII).

Gelatinization enthalpy (ΔH) has been shown to reflect the loss of molecular (double helical) order rather than the loss of crystalline register (Cooke and Gidley, 1992). The differences in ΔH among native starches (**Table** 4.6) reflect the interplay of: (1) differences in long branch chain lengths of amylopectin (amylomaize VII> amylomaize V> normal maize> waxy maize> rice> oat) [large amount of energy (ΔH) would be required to gelatinize crystallites of long chain lengths]; (2) amount of free lipids (amylomaize VII ~ oat> normal maize> amylomaize V> rice> waxy maize), [interaction of free lipids with amylose during gelatinization is an exothermic process (Biliaderis *et al.*, 1986b). A net endothermic process is determined by DSC, and the net energy (ΔH) required to form this endotherm is less when free lipids are present in the starch-water system] ; and (3) amount of lipid complexed amylose chains (**Table** 4.1) [melting of the V-single helix amylose-lipid complexes (~104°C) has been shown to decrease ΔH (Billiaderis *et al.*, 1985).

4.6.3.2 Gelatinization of acid treated starches

In all starches, To decreased, but Tp, and Tc-To increased with hydrolysis (Table 4.6). This result is in agreement with earlier studies (Robin *et al.*, 1975, Biliaderis *et al.*, 1981). The increase in extent of Tc-To (after 24h hydrolysis) followed the order: waxy maize > amylomaize V > normal maize > oat > rice. In all starches, ΔH increased with hydrolysis (Table 4.6). The extent of increase in ΔH (after 24h hydrolysis) followed the order: amylomaize V > rice > oat > normal maize > waxy maize. This was in agreement with those reported for barley (Morrison *et al.*, 1993a), potato (Jacobs *et al.*, 1998, Muhr *et al.*,1984), and pea (Jacobs *et al.*, 1998), starches.

As discussed earlier, crystallite disorganization during gelatinization of native starches in excess water results in a narrow endotherm, due to swelling of the amorphous regions (bulk and intercrystalline) which exert a destabilizing effect on the crystallites. This is made possible due to close interaction between the amorphous regions and crystallites. However, the destruction of the amorphous regions on acid hydrolysis, will decrease their destabilizing effect on crystallites. Consequently, in all starches, crystallinity is lost over a wider temperature range, and Tc-To is observed to broaden (Table 4.6). Studies on normal and waxy barley starch acid residues (Morrison *et al.*, 1993a), led to the suggestion, that higher transition temperatures might be due to longer amylopectin double helices than in the unhydrolyzed amylopectin molecule, where the branch points α (1→6) linkages might reduce the length of helix forming segments of the A and B

chains. This would then explain the increase in Tc-To observed for normal maize, waxy maize, rice and oat starches (**Table** 4.6). However, the high Tc-To observed for hydrolyzed amylomaize V starches (**Table** 4.6) cannot be satisfactorily explained on this basis, (due to their lower content of α (1→6) linkages). It is likely, that in amylomaize V, the broad Tc-To mainly represents the disordering of those double helices that are formed by interwining of hydrolyzed lipid free amylose chains.

The increase in ΔH on acid hydrolysis (**Table** 4.6) reflects the increase in thermal energy required to unravel and melt long amylopectin double helices, and double helices formed by interaction between amylose-amylose and amylose-amylopectin chains during acid hydrolysis. It is highly unlikely, that ΔH represents changes within the crystalline domain, since the DSC endotherms represent hydrolysis within a 24h period. The pronounced increase in ΔH on hydrolysis of waxy maize starch represents the dissociation and melting of those double helices that were formed within the amorphous regions (resulting from hydrolysis of α (1→6) linkages).

Chapter 5

Most significant findings

The susceptibility of cereal starches (normal maize, waxy maize, amylomaize V and VII, rice and oat) towards hydrolysis by 2.2N HCl at 35°C for 15 days, and the physicochemical characteristics (granule morphology, degree of swelling, amylose leaching, x-ray pattern, crystallinity, average degree of polymerization and gelatinization parameters) of the lintnerized residues (at different time periods of hydrolysis) were examined. The difference in the extent of hydrolysis at the end of the 8th day (waxy maize > oat > rice > normal maize > amylomaizeV > amylomaizeVII) was influenced by the interplay between: 1) mode of distribution of α (1-6) branches between the amorphous and crystalline regions; 2) amylopectin content, and 3) degree of packing of the double helices within the crystallites. Granule morphology, x-ray crystallinity, average degree of polymerization, swelling factor, amylose leaching, enthalpy of gelatinization and the gelatinization temperature range were altered on acid hydrolysis. The extent of these changes differed among the starch sources. However, the x-ray pattern remained unchanged in all starches.

Chapter 6

Directions for future research

1) There is a dearth of information on the influence of hydrothermal treatment (at different time-temperature-moisture combinations) on the rate and extent of acid hydrolysis of starches with different crystalline polymorphic forms (A,B and C). Hydrothermal treatment such as heat-moisture treatment and annealing have been shown to cause structural rearrangement of starch chains and /or crystallite disruption. Thus, comparative studies on: (a) the rate and extent of acid hydrolysis of native, heat-moisture treated and annealed starches and b) the structure and properties of the acid hydrolyzed residues (obtained at different times of acid hydrolysis) from native and hydrothermally treated starches may provide a deeper insight into how starch chain organization within the amorphous and crystalline domains influence the accessibility of the hydronium ion into the granule interior.

2) A much-debated issue, in need of further research, concerns the influence of lipids on acid hydrolysis. A systematic study on the influence of potato starch-monoglyceride (of different chain length) complexes on the kinetics of acid hydrolysis may resolve this issue.

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Appendix I

Figure I-1 Standard curve for determination of total carbohydrate as glucose (Dubois *et al.*, 1956)

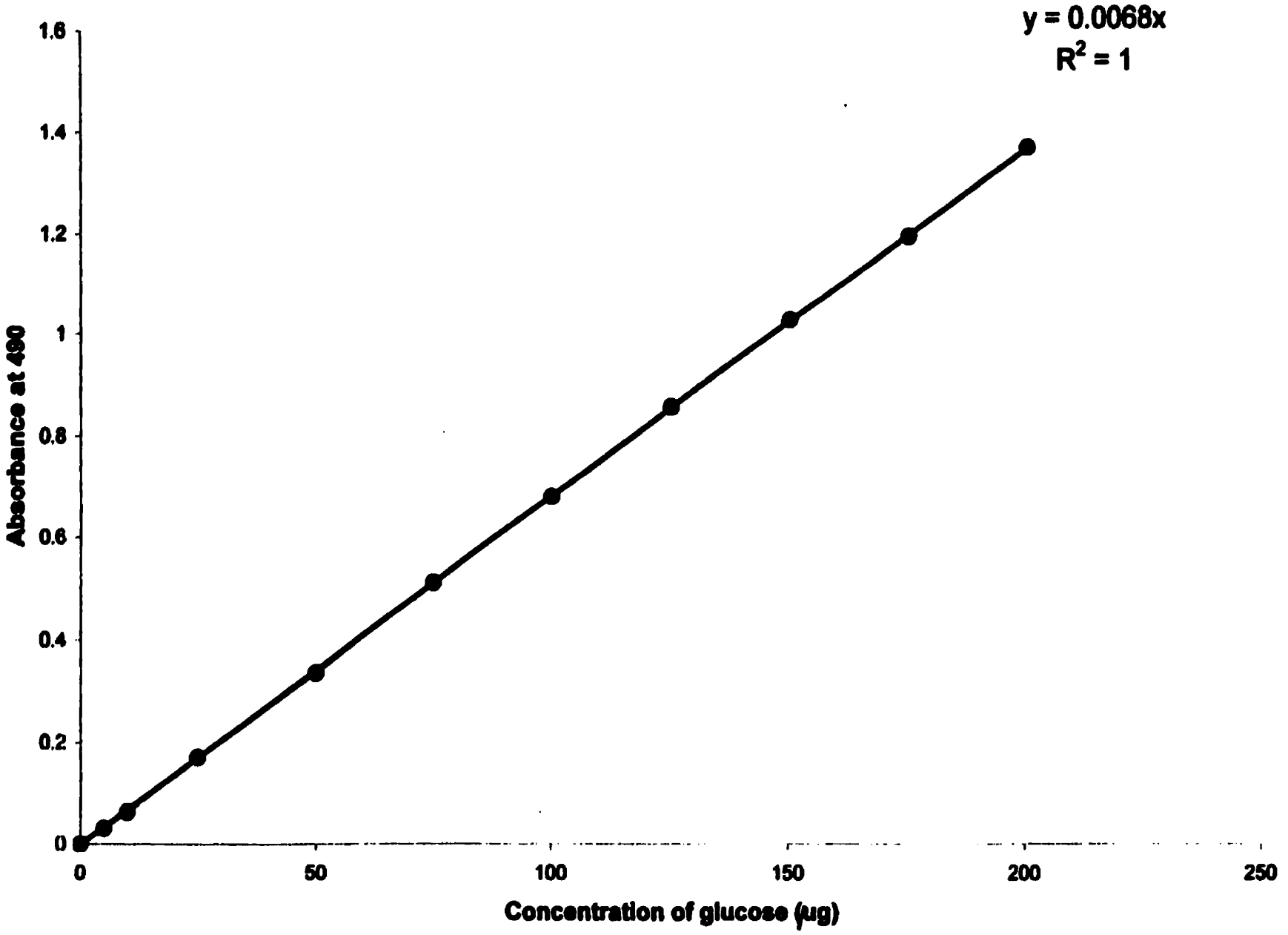

y = 0.0068x
R² = 1
Absorbance at 490
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
Concentration of glucose (µg)

Figure I-2 Standard curve for determination of amylose (Hoover and Ratnayake 2001)

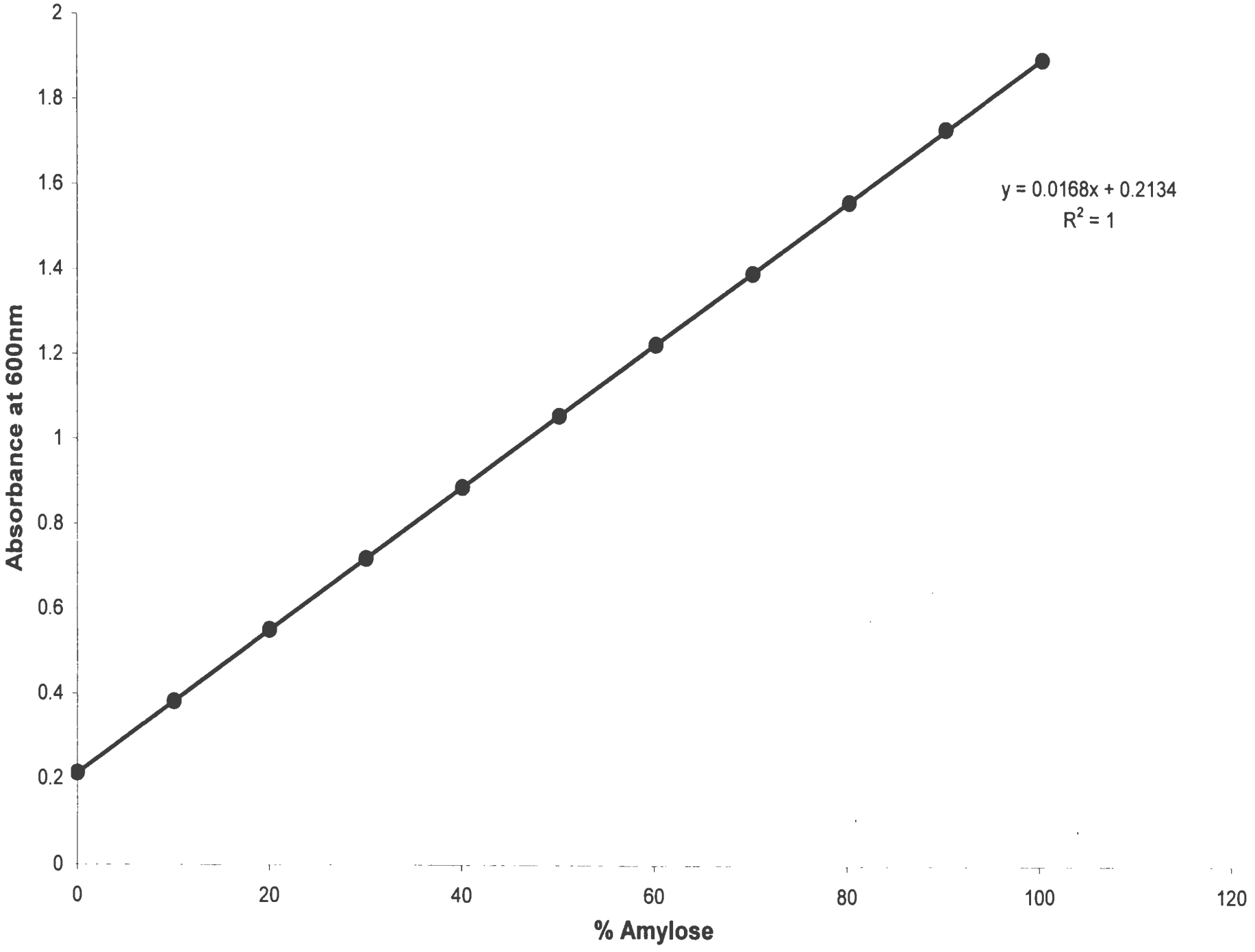

y = 0.0168x + 0.2134
R^2 = 1
Absorbance at 600nm
% Amylose
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
20
40
60
80
100
120

Figure I-3 A&B Standard curves for determination of reducing sugars as maltose (Bruner, 1964)

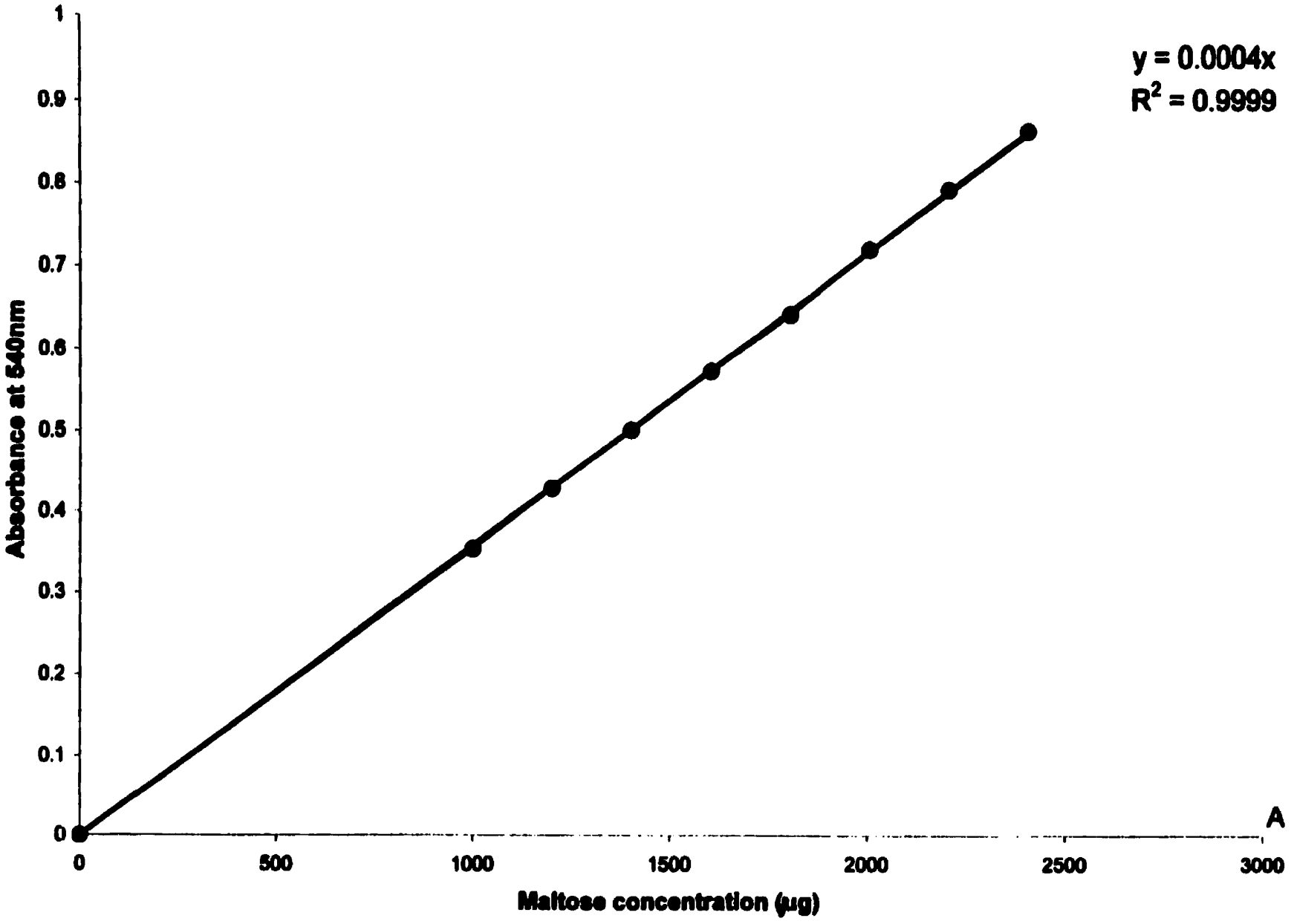
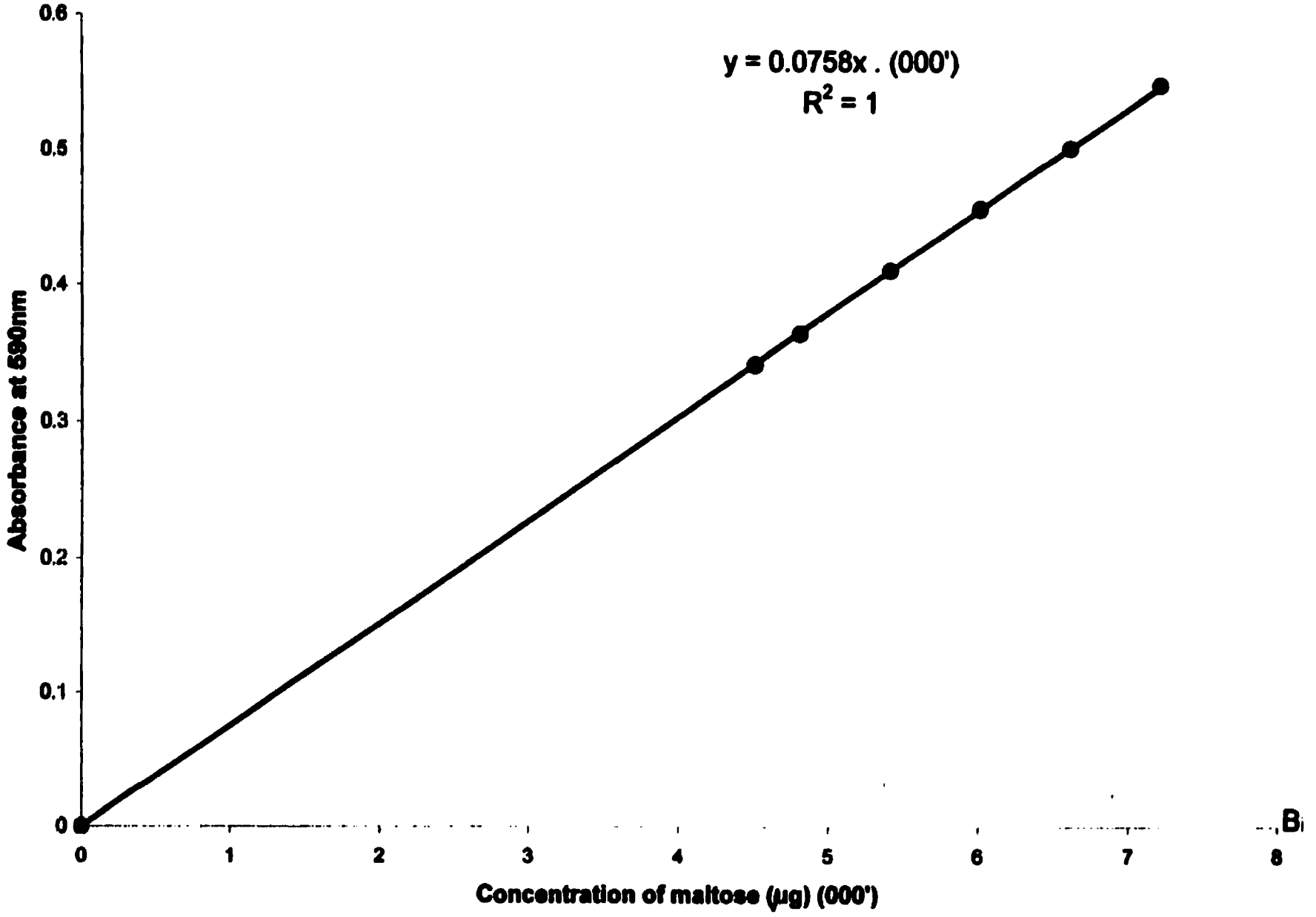

y = 0.0004x
R2 = 0.9999
Absorbance at 540nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
500
1000
1500
2000
2500
3000
Maltose concentration (µg)
A

Figure I-3C Standard curves for determination of total carbohydrate as maltose (Dubois *et al.*, 1956)

Appendix II


y = 0.0758x . (000')
R2 = 1
Absorbance at 590nm
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
Concentration of maltose (µg) (000')

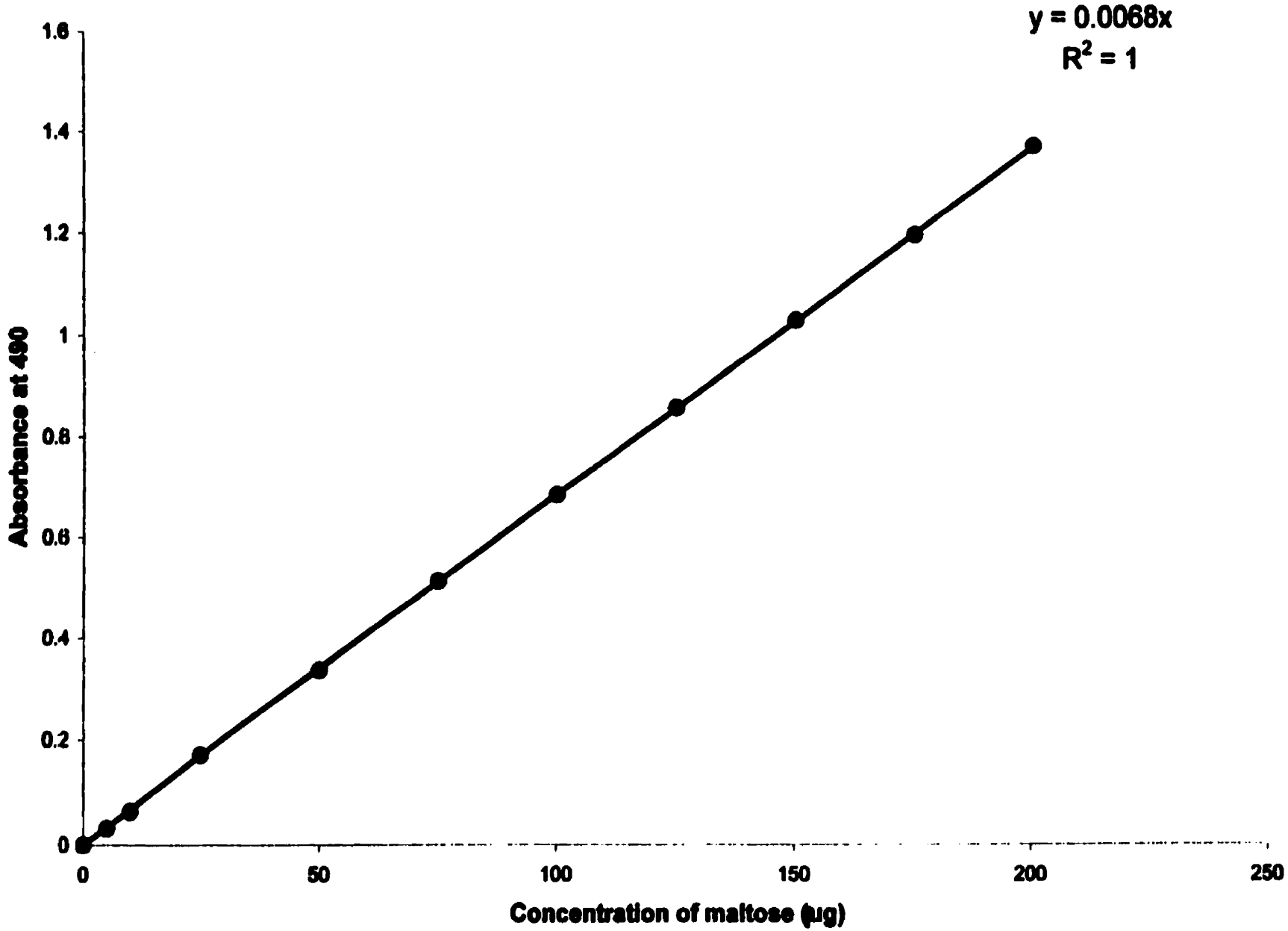
II. Determination of physicochemical properties of native and cereal starches

II.1 Determination of relative crystallinity

Because both background and peak intensities vary with hydration (Hizukuri *et al.*, 1964, Nara *et al.*, 1978, Buleon *et al.*, 1982, Veregin *et al.*, 1986, Buleon *et al.*, 1987, Hibi *et al.*, 1993), the usual method of Wakelin *et al.*, (1959) is not appropriate calculation of starch crystallinity. However, the method of Nara *et al.*, (1978) is an appropriate method to measure intensities, where the amorphous background curve is drawn through the minima of all peaks. Thus, apparent crystallinities are judged by reference (quartz) to intensities and sharpness of peaks. Therefore, the degree of crystallinity of samples was quantitatively estimated following the method of Nara *et al.*, (1978).

To measure the relative crystallinity of starch in the X-ray diffractogram, the baseline (C) joining background scattering points was drawn (**Figure** II-1). A smooth curve was then computer-plotted between the low-and high-angle points of intensity at 2θ of 3° and 35° for all starches. The upper region, above this curve (A), represented X-ray scattering of the crystalline fraction, and the lower part (B) represented that of the amorphous fraction. The upper diffraction peak area and total diffraction area over the diffraction angle 3°-35° 2θ were integrated on Origin 6.0 software (Microcal Inc., Northampton, MA, USA). The amorphous fraction was subtracted form the total area above the base line in order to obtain the crystalline fraction. A percent relative crystallinity of native and lintnerized starch was then determined by the ratio of the total upper integrated peak area (A) observed in the X-ray diffraction pattern of starches to that of quarts, over the total angular range from 3-35°C [2θ] (Nara *el al.*, 1978).

Figure II-1 Determination of relative crystallinity of starch in the X-ray diffractogram. A: crystalline area, B: amorphous area, C: baseline


y = 0.0068x
R² = 1
Absorbance at 490
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
Concentration of maltose (µg)

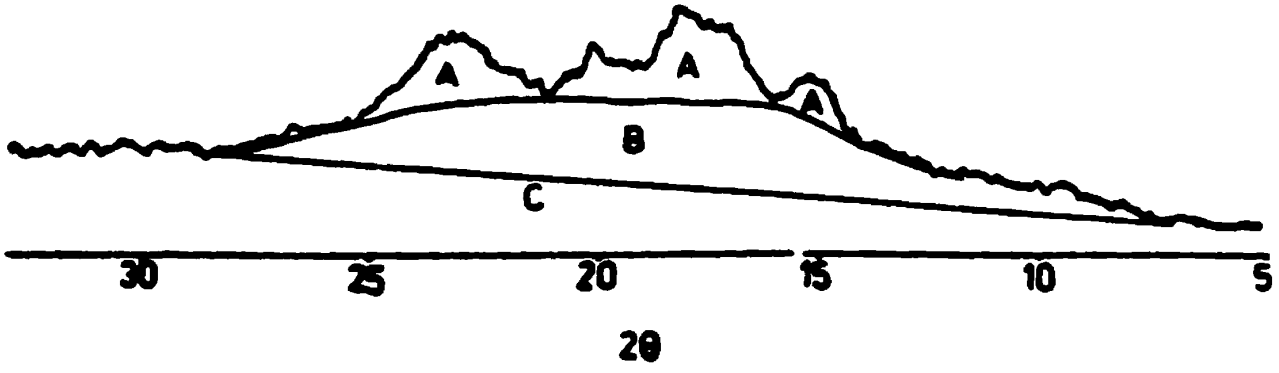
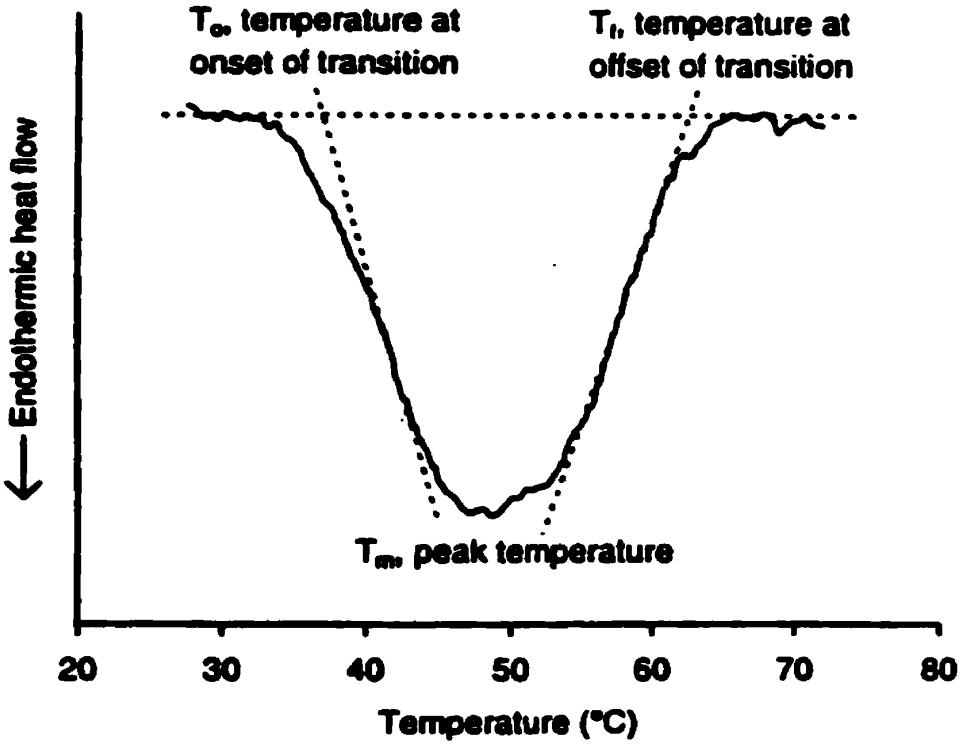

A
A
A
B
C
30
25
20
15
10
5
2θ

Figure II-2 Schematic representation of a DSC thermogram. The gelatinization enthalpy (ΔH)is evaluated as the area under the peak.


T_o, temperature at onset of transition
T_f, temperature at offset of transition
Endothermic heat flow
T_m, peak temperature
20
30
40
50
60
70
80
Temperature (°C)

Publications (in 2001)

Jayakody, J.A.L.P and Hoover, R., (2001). The effect of lintnerization on cereal starch granules. (Starch/Starke-in review).

Scholarships and Awards

1985-1990 Scholarship for Undergraduate studies, University of Peradeniya, Sri Lanka

1991 Professor R.R. Appadurai Memorial Award for the highest average (B.Sc. Honors), University of Peradeniya, Sri Lanka.

1998-2000 Graduate Fellowship, Dept. of Biochemistry, Memorial University of Newfoundland, Canada

1998-2000 Graduate Assistantship, (Dr. R. Hoover), Memorial University of Newfoundland, Canada