EFFECT OF HYDROXYPROPYLATION ON THE STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF NATIVE, DEFATTED AND HEAT-MOISTURE TREATED POTATO STARCHES

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EFFECT OF HYDROXYPROPYLATION ON THE STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF NATIVE, DEFATTED AND HEAT-MOISTURE TREATED POTATO STARCHES

BY

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ABSTRACT

Native potato starch was physically modified by heat-moisture treatment (100°C, 16h, 30% moisture) and defatting (75%, n-propanol water, 7h). The changes in structure and physicochemical properties on heat-moisture treatment and defatting were monitored by scanning electron microscopy (SEM), X-ray diffraction, differential scanning calorimetry (DSC), Brabender viscosities, swelling factor (SF) and amylose leaching (AML). SEM showed that neither defatting nor heat-moisture treatment altered the size, shape or the surface appearance of the native starch granule. Heat-moisture treatment decreased X-ray diffraction intensities and altered the 'B' type X-ray diffraction pattern to 'A+B'. The decrease in X-ray intensities on heat-moisture treatment is indicative of crystallite disruption and/or rearrangement of double helices. The gelatinization enthalpy (ΔH), Brabender viscosity (at 95°C), SF and AML decreased on heat-moisture treatment, whereas gelatinization transition temperatures (GTT), and thermal stability increased. Defatting increased the X-ray diffraction intensities and altered the X-ray pattern from 'B' to 'A+B'. The increased X-ray intensities on defatting is indicative of interactions between amylose - amylose (AM-AM), amylopectin - amylopectin (AMP-AMP) and amylose - amylopectin (AM-AMP) chains. These interactions in turn, increased GTT, ΔH and thermal stability. However, SF, AML and Brabender viscosity (at 95°C) decreased on defatting.

The reagents (NaOH and Na_2SO_4) used during hydroxypropylation did not alter granule morphology and AML in native, defatted and heat-moisture treated starches. X- ray diffraction patterns of native and defatted starches changed on alkaline treatment, whereas that of heat-moisture treated starch remained unaltered. These changes reflected double helical disruption (within the amorphous regions), and altered crystallite orientation. In all three starches, alkaline conditions decreased ΔH and Brabender viscosity (at 95°C), and increased SF. The extent of the above changes followed the order : native > defatted > heat-moisture treated. Gelatinization transition temperatures remained unchanged on alkaline treatment.

Native, defatted and heat-moisture treated starches were converted to a range of hydroxypropyl derivatives using propylene oxide (at concentrations ranging from 2-25%v/w). At 2%(v/w) propylene oxide, all three starches showed similar molar substitution (MS 0.05). However, at 5-25%(v/w) propylene oxide, the accessibility of hydroxypropyl groups into the starch granule followed the order : heat-moisture treated > native > defatted. This showed that the degree of accessibility of hydroxypropyl groups into the granule interior is dependent upon granule crystallinity. In all three starches, an increase in MS progressively decreased GTT, ΔH and AML . The influence of MS on SF of hydroxypropylated native, defatted and heat-moisture treated starches was due to the interplay that occurs between hydrogen bond disruption (due to hydroxypropyl groups) within the amorphous regions, and the increased interactions that occur between starch

chains during defatting and heat-moisture treatment. Pasting temperatures of all starches decreased with increased MS. In defatted starch, Brabender viscosity (at 95°C) progressively increased with increase in MS. However, in native and heat-moisture treated starches, Brabender viscosity (at 95°C) began to decrease (due to granule disruption) at MS levels beyond 0.18 and 0.20, respectively.

Enzyme digestibility studies showed that both defatting (hot 75% n-propanol, 0-7 h) and heat-moisture treatment (100°C, 30% moisture, 0-16 h) increased the susceptibility of potato starch granules towards hydrolysis by porcine pancreatic α- amylase. These differences were attributed to structural changes that occurred within the amorphous and crystalline regions of the starch granule during defatting and heat-moisture treatment. However, hydrolysis decreased (due to formation of new crystallites) when heat-moisture treatment and defatting were continued beyond 8 h and 9 h, respectively.

Native, defatted (7 h) and heat-moisture treated (16 h) potato starches were hydroxypropylated (to different levels of MS) with propylene oxide (2 - 20%). The results showed that the reagents (NaOH and Na_2SO_4) used during hydroxypropylation increased the susceptibility of the above starches (native > defatted > heat-moisture treated) towards hydrolysis by α-amylase. Addition of propylene oxide (hydroxypropylation) to alkali treated starches, further enhanced their susceptibility towards α-amylase. However, granule susceptibility towards

α-amylase did not increase exponentially with increase in MS. The extent of hydrolysis began to decrease at MS levels of 0.29 (native), 0.28 (heat-moisture treated) and 0.26 (defatted). The decrease in hydrolysis at higher MS levels is indicative of the steric effect imposed by bulky hydroxypropyl groups on the accessibility of α-amylase towards the glycosidic bonds of amylose and amylopectin.

The retrogradation properties of potato starch gels (stored for 24 h at 4°C and then for 34 days at 40°C) before and after physical (defatting and heat-moisture treatment), and chemical (hydroxypropylation) modification were monitored using turbidity measurements, SEM, DSC, X-ray diffraction and enzyme susceptibility. Turbidity development in native, defatted and heat-moisture treated starch pastes during storage (4°C for 24 h and then at 40°C for 34 days) followed the order : native > defatted > heat-moisture treated. In all three starches, the highest rate of turbidity development was observed during the first 24 h of storage (at 4°C). The above results in conjunction with SEM observations showed that turbidity development is influenced by the interaction between leached starch components (AM-AM, AM-AMP, AMP-AMP), and interaction between granule remnants and leached starch components (amylose and amylopectin). In alkali treated gelatinized native, defatted and heat-moisture treated starch pastes, turbidity development (native > defatted > heat-moisture treated) was influenced by aggregation of granule remnants. In native, defatted and heat-moisture treated starch pastes, both the rate and extent of turbidity

development (on storage) decreased after hydroxypropylation. This decrease was due to the interplay of : 1) steric effects imposed by hydroxypropyl groups on chain aggregation, 2) aggregation between small granule remnants, and 3) settling of large granule remnants beneath the path of the spectrophotometer beam.

The intensity (at 5.2Å) of the 'B' type X-ray pattern of gelatinized pastes of native, defatted and heat-moisture treated starches increased during storage (native > defatted > heat-moisture treated). The same 'B' pattern was also evident (at the end of the storage period) after alkali treatment and hydroxypropylation of the above starches. However, the peak at 5.2Å was reduced in intensity after alkaline treatment and hydroxypropylation. The extent of the decrease being greater in the latter.

Fresh pastes of gelatinized native, defatted and heat-moisture treated potato starches were hydrolyzed by porcine pancreatic α-amylase nearly to the same extent (75.3-77.2%). Storage (at 4°C for 24 h) of the above gelatinized pastes decreased (native > defatted > heat-moisture treated) their susceptibility towards α-amylase. However, storage times longer than 24 h did not seem to have any further influence on the enzyme susceptibility of the starch gels. Alkaline treatment increased the susceptibility of freshly gelatinized starch pastes (native > defatted > heat-moisture treated) towards hydrolysis by α-amylase. However, storage (24 h at 4°C) of alkali treated starch gels, decreased their susceptibility towards hydrolysis by α-amylase. Hydroxypropylation decreased

the accessibility of α-amylase towards the glycosidic linkages of freshly gelatinized pastes of native, defatted and heat-moisture treated starches (defatted > native > heat-moisture treated). However, the extent of this decrease was not altered during storage (at 4°C for 24 h).

The retrogradation endotherm (monitored by DSC) of starch gels (native, defatted and heat-moisture treated) occurred after 2 days of storage (4°C for 1 day and then at 40°C for 1 day). A similar trend was also observed after alkaline treatment. However, hydroxypropylated native, defatted and heat-moisture treated starch gels [at nearly the same MS level (0.10-0.11)], showed a measurable retrogradation endotherm only after 7 days (4°C for 1 day and then at 40°C for 6 days). This showed that hydroxypropyl groups are effective in hindering starch chain realignment during gel storage. Both alkaline treatment and hydroxypropylation decreased the retrogradation enthalpies of native, defatted and heat-moisture treated starch gels.

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LIST OF ABBREVIATIONS

ν	- Volume fraction
ΔH	- Enthalpy of gelatinization
ΔH_R	- Enthalpy of retrogradation
AM	- Amylose
AML	- Amylose leaching
AMP	- Amylopectin
BU	- Brabender units
C*	- Entanglement concentration
CL	- Chain length
CP MAS NMR	- Cross polarization magic angle spinning nuclear magnetic resonance
CTAB	- Cetyltrimethylammonium bromide
db	- Dry basis
DP	- Degree of polymerization
Dp_n	- Degree of polymerization - number (average)
Dp_w	- Degree of polymerization - weight (average)
DSC	- Differential scanning calorimetry
F-AM	- Lipid free amylose
FTIR	- Fourier transform infrared spectroscopy
GMP	- Glyceryl monopalmitate
GMS	- Glyceryl monostearate
HPLC	- High performance liquid chromatography
L-AM	- Lipid amylose
MG	- Monoacylglycerol
MS	- Molar substitution
NIR	- Near infrared reflectance spectroscopy
PPA	- Porcine pancreatic α-amylase
PW	- Propanol water
SF	- Swelling factor
SP	- Swelling power
SSL	- Sodium stearoyl-2- lactylate
To	- Onset of gelatinization temperature
Tp	- Peak gelatinization temperature
Tc	- Conclusion of gelatinization temperature
Tc-To	- Gelatinization transition temperature range
Tg	- Glass transition temperature
w/v	- Weight / volume

DEDICATED TO MY LOVING PARENTS

CHAPTER 1

INTRODUCTION

Modified starches are important in processed foods because of their improved functional properties over their unmodified counterparts. Reaction of starch with propylene oxide to form the hydroxypropyl starch derivatives is used primarily in the food industry. This modification process improves the freeze - thaw stability, clarity and textural properties of the starch paste. Hydroxypropyl groups are hydrophilic in nature and when introduced into the starch granule, weaken or strain the internal bond structure holding the granule together. Hydroxypropylated starches have been shown to alter physicochemical properties compared to their native starches (Butler *et al.*, 1986; Hoover *et al.*, 1988; Kim & Eliasson, 1993), primarily depending on the molar substitution (MS). Hood & Mercier (1978) have shown by enzyme hydrolysis and gel chromatography, that in manioc starch the hydroxypropyl groups in amylose were distributed at/or near the reducing end or along the entire amylose molecule. Kim *et al.* (1992) have shown by light microscopy studies, that in potato starch granule, hydroxypropyl groups are mainly distributed in the central region. Previous studies have shown that physical modification alters starch granule structure. This suggests that the distribution of hydroxypropyl groups within the granule interior could change on physical modification.

Presently hydroxypropyl starches for commercial purposes are produced by reaction of a highly concentrated slurry of starch granules with propylene

oxide under alkaline conditions (NaOH and Na_2SO_4). These alkaline conditions increase the reaction efficiency of hydroxypropylation. The changes in physicochemical properties on hydroxypropylation have been attributed solely to the level of MS. No attempt has been made to investigate the possibility that the alkaline conditions could also influence starch properties.

The reactivity and the mode of action of α-amylase have been primarily studied on unmodified starches from various botanical sources, and the results have shown that the action of α-amylase is influenced by granule structure. It has been postulated (Marsden & Gray, 1986; Franco *et al.*, 1988) that α-amylase preferentially hydrolyzes amorphous regions of the starch granule. However, studies have not been conducted to examine how the arrangement of starch chains within the amorphous regions of the starch granule influences the degree of accessibility of α-amylase into the granule interior. Previous studies (Hoover & Vasanthan, 1994a,b; Hoover & Manuel, 1996a) have shown that starch chain interactions occur within the amorphous region during thermal treatment. Furthermore, chemical modification has been shown (Mohd Azemi & Wootton, 1985; 1995) to reduce the accessibility of α-amylase into the amorphous regions. Thus, a comparative study of α-amylolysis of native starches with their physically and chemically modified counterparts may provide a deeper insight into the mechanism of α-amylolysis.

Starch gelatinization is a process that takes place when starch granules are heated in the presence of water, resulting in the disruption of molecular order within the starch granule. The process is manifested by irreversible changes in granular swelling, crystallite melting, loss of birefringence and starch solubilization. Starch retrogradation is a process that occurs when molecules composing gelatinized starch begin to reassociate. Retrogradation is accompanied by increases in turbidity, gel firmness, degree of crystallinity and the appearance of a 'B' type X-ray pattern.

The retrogradation of starches from cereal, tuber and legumes has been subjected to detailed studies. However, there is a dearth of information on the influence of physical modification such as defatting and heat-moisture treatment on the rate and extent of retrogradation of starches from different plant origins. Recently, Vasanthan & Hoover (1992a) and Hoover & Vasanthan (1994a) have shown that defatting and heat-moisture treatment cause structural changes to occur within the amorphous and crystalline domains of tuber and cereal starches. However, no attempt has been made to explain how these changes influence granule swelling, amylose leaching, granule rigidity and starch chain interaction during gelation and crystallization.

Hydroxypropylation has been shown to reduce starch retrogradation. However, there is a dearth of information on the influence of alkaline conditions used during hydroxypropylation on starch retrogradation. Furthermore, most of the present information on starch retrogradation has come from differential

scanning calorimetry and X-ray diffraction studies. It is important to measure the retrogradation mechanism of a particular starch using a wide variety of techniques (turbidity development, enzyme digestibility, differential scanning calorimetry, X-ray diffraction and scanning electron microscopy), since one technique alone cannot unravel the different molecular processes occurring during starch gelation and crystallization. Thus, in this study, different physical probes (turbidity development, enzyme digestibility, differential scanning calorimetry, X-ray diffraction and scanning electron microscopy), have been used in an attempt to study how the rate and extent of starch retrogradation is influenced by defatting, heat-moisture treatment, alkali treatment and hydroxypropylation (at different levels of MS).

The hypothesis and objectives underlying this research investigation are outlined below :

Hypothesis 1 : The thermal energy imparted to starch chains during defatting (1-propanol water, 3:1v/v, 7 h, ~82°C) and heat-moisture treatment (30% moisture, 100°C, 16 h), and the added moisture content (30% moisture) within starch granules during heat-moisture treatment would increase starch chain mobility, resulting in either increased interaction between amylose - amylose (AM-AM), amylose - amylopectin (AM-AMP), amylopectin - amylopectin (AMP-AMP) chains and/or disruption of starch crystallites. These structural changes would, in turn, radically alter the physicochemical properties of the starch granule.

Objective 1 : To determine (by physical and chemical methods) the influence of defatting and heat-moisture treatment on starch structure and properties.

Hypothesis 2 : Hydroxypropylation has been shown to occur within the amorphous regions of the starch granule. Therefore, any changes within the amorphous regions during defatting and heat-moisture treatment could either increase or decrease the accessibility of hydroxypropyl groups into the granule interior. Thus, hydroxypropylation at the same level of propylene oxide should result in different levels of molar substitution (MS) in native, defatted and heat-moisture treated starches.

Objective 2 : To determine the degree of accessibility of hydroxypropyl groups into the amorphous regions of potato starch before and after defatting and heat-moisture treatment.

Hypothesis 3 : Changes in starch properties on hydroxypropylation have been attributed solely to the hydroxypropyl group. However, it is also likely, that the alkaline reagents used during hydroxypropylation could also influence starch properties by altering starch chain arrangements within the amorphous and crystalline domains of the starch granule.

Objective 3 : To study the influence of the reagents (NaOH and Na_2SO_4) used during hydroxypropylation on the structure and properties of native, defatted and heat-moisture treated starches.

Hypothesis 4: The structural arrangement of amylose and amylopectin chains within the amorphous and crystalline regions of native, defatted and heat-

moisture treated starches could influence the extent to which hydroxypropyl groups are able to modify starch properties.

Objective 4 : To determine the influence of hydroxypropylation at various levels of MS on the thermal and rheological properties of native, defatted and heat-moisture treated starches.

Hypothesis 5 : Changes within the amorphous and crystalline regions of starch granules on defatting, heat-moisture treatment and alkaline treatment could influence the reactivity of the starch granule towards α-amylase. Furthermore, steric effects imposed by bulky hydroxypropyl groups could hinder the accessibility of α-amylase towards the glycosidic linkages.

Objective 5 : To study the reactivity of α-amylase towards native, defatted and heat-moisture treated potato starches before and after hydroxypropylation.

Hypothesis 6 : Starch chains within gelatinized granules and in the continuous medium interact during gel storage. Thus, any change in starch structure on defatting, heat-moisture treatment and alkaline treatment could indirectly influence the rate and extent of starch chain interactions during gel storage. Furthermore, bulky hydroxypropyl groups could hinder this interaction (amylose-amylose [AM-AM], amylose-amylopectin [AM-AP], amylopectin-amylopectin [AP-AP]) sterically and/or by decreasing the mobility of the interacting chains.

Objective 6 : To study how the rate and extent of potato starch retrogradation is influenced by defatting, heat-moisture treatment, alkaline treatment and hydroxypropylation (at different MS levels).

CHAPTER 2

LITERATURE REVIEW

2. 1 Starch - Introduction

Starch is a major reserve polysaccharide of green plants, which is deposited in granular form in seeds, roots, tubers, stems and leaves of plants. The granules are partially crystalline, insoluble in cold water and their size, the shape (Table 2.1), and the composition are essentially genetical. In general, cereal starch granules are small and polyhedric, whereas, tuber starch granules are large, spherical or ellipsoid. In contrast to most starches which have single size distribution of granules, rye, wheat, triticale and barley starches show a bimodal distribution : spherical 'B' granules (1-10μ) and lenticular 'A' granules (15-35μ). The granule size and morphology have received much attention recently, since small granules are important in determining the taste and mouthfeel of some starch based fat mimetics (Alexander, 1992). Pure starch is a mixture of two α-(1$\rightarrow$4) glycosidic bonded glucose polymers, namely essentially linear amylose and branched amylopectin (Table 2.2) in which branching occurs through α-(1$\rightarrow$6) linkages. The ratio of amylose and amylopectin differ depending on the botanical source of starch. The amount of amylose present in starches is in the range of 17 (rice) - 38 (lentil) %, while amylopectin ranges from 62 to 83%. Granule composition, morphology and supermolecular organization are to a certain extent under genetic control; i.e. they are influenced

Table 2.1 Size and shape of starch granules from different botanical sources.

Starch source	Shape	Size (μm)
Cereal		
Wheat	spherical, lenticular	1-45
Maize	spherical, polygonal	2-30
Waxy maize	polyhedric	3-26
Rice	polyhedric	3-8
Sorghum	polyhedric, spherical	4-24
Barley	lenticular	2-5
Root and tuber		
Potato	oval, spherical	5-100
Tapioca	truncated, spherical, oval	4-35
Legumes		
Smooth pea	reniform (simple)	5-10
Wrinkled pea	reniform (compound)	30-40
Chick pea	spherical, oval	8-54
Cow pea	spherical, oval	15-85
Lentil	spherical, oval	15-36
Black beans	oval, spherical	8-34

Adapted from Swinkels (1985); Blanshard (1987); Hoover & Sosulski, (1985).

Table 2.2 Characteristics of amylose and amylopectin

Characteristic	Amylose	Amylopectin
Molecular structure	essentially linear with α-(1-4) linkages, slightly branched	branched with α-(1-4) and α-(1-6) linkages
Molecular weight	700-5000	10^4-10^5
Average CL	100-550	18-25
Branch linkage (%)	0.2-0.6	4.0-5.5
Iodine binding -λ max	640-660 nm	530-570 nm
- color	deep blue	purple
Stability of aqueous solutions	retrogrades	stable
Film properties	strong	brittle
Solubility in water	variable	soluble
Digestibility		
α-amylase	~100	~90-100
β-amylase	70-96	50-60
β-amylase and debranching enzyme	~100	~75
Gel properties	stiff, thermally irreversible below 100°C	soft, thermally reversible below 100°C

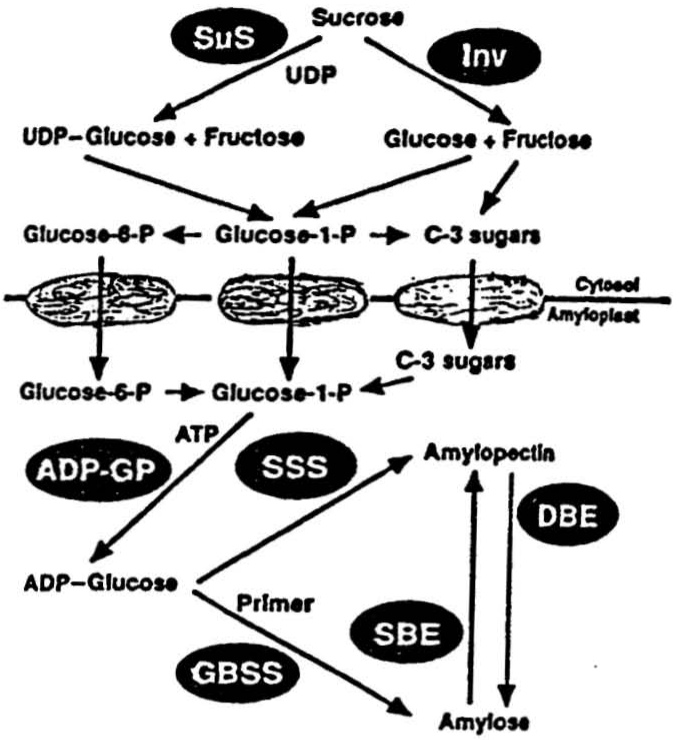
Adapted from Manners (1985); Biliaderis (1991); Hizukuri (1996).

by the biochemistry of the amyloplast (starch synthesizing organelle) as well as by the physiology of the plant (Banks & Greenwood, 1975; French, 1984; Biliaderis, 1991). However, the exact mechanism by which plant genetic information is translated into a specific granule composition, morphology and organization is unknown. Even the exact role and *in vivo* regulation of various enzymes involved in starch biosynthesis is still in dispute (Preiss & Lori, 1980; Robyt, 1984). Only when this information is available it might be possible to manipulate the amount, composition and properties of starch by genetic engineering (Biliaderis, 1991). The fine structure and the molecular order of amylose and amylopectin determine the physicochemical properties of starches.

2.2 Starch Biosynthesis

Biosynthesis of starch takes place in a specialized subcellular organelle, the amyloplast, which has a limiting lipoprotein membrane. Figure 2.1 shows the enzymes involved and the metabolic pathways of starch biosynthesis. The enzymes which catalyze the biosynthesis are present within the amyloplast. These enzymes include phosphorylases, starch synthetase and branching and debranching enzymes(Jansson *et al.*, 1995). Sucrose, the starting material of starch synthesis is transported from the photosynthetic tissue to the storage organ. The assembly of starch chains occurs on a lipoprotein matrix. At a certain moment, a minute amount of insoluble polysaccharide deposits and this acts as the nucleus (hilum) of the granule, around which the granule is developed. As

Fig. 2.1 Schematic representation of the metabolic pathway for starch synthesis (with permission, Jansson *et al.*, 1995).



SuS = sucrose synthetase
ADP-GP = ADP-glucose pyrophosphorylase
GBSS = granule-bound starch synthase
DBE = debranching enzyme

Inv = sucrose invertase
SSS = soluble starch synthase
SBE = starch branching enzyme

the dissolved glucose units are linked to the growing polymer, they simultaneously solidify (Swinkels, 1985). As the granule develops within the amyloplast, it occupies an increasing proportion of the volume, until the internal volume of the amyloplast is completely occupied by starch (Galliard & Bowler, 1987).

2.3 Starch production and uses

Although starch occurs throughout the plant world, there are only a limited number of plants utilized extensively for the production of commercial starches. Sources of commercial starches are maize, wheat, rice, potato, tapioca, arrowroot and sago (Swinkels, 1985). Maize is the major commercial starch produced and in the US, maize accounts for more than 95% of commercial starches. In addition to maize the US produces limited amount of potato and wheat starches as well. However, in Europe, use of potato in starch production is much greater than that in the US. Tapioca and sago starches are mainly produced in tropical countries such as East Indies and Brazil. Tapioca is imported into the US for use in industrial and food applications and also to produce modified starches (Wurzburg, 1987). In the US, 70% of the starch is utilized in industrial applications and the remaining 30% is used in food products. In contrast, European countries use 69% of starch in food applications and the remaining 31% is consigned to industrial purposes (Lillford & Morrison, 1997).

Starch, in granular form as well as in the paste form has its function in food and non food applications. In food applications, granular starch is utilized as a dusting agent for candy and carrying agent for baking powder. Small granular starches (< 2 μ in diameter) are proposed for fat mimetics. A mixture of small particles dispersed in a starch gel matrix resembles the texture of butter in which fat micelles are dispersed in a liquid fat matrix (Jane, 1997a). In non food uses, granular starch is utilized as a dusting agent in pharmaceuticals (tablets), antiperspirant, and in facial powder substitutes (oat, maize). Granular starch has been used in various products depending on their size and shape. The large granular wheat starch has been shown desirable as "silt material" for coating on carbonless copy paper. Spherical aggregates of small granular starch are used as flavour carriers.

In the paste form, starch is used in food products as a thickener in semi solid foods, pie fillings, sauces, oil mimetics and to provide texture (body) to beer and soft drinks (Jane, 1997a). In non food applications, starch paste has its functions as a coating agent in pharmaceuticals, encapsulating agent in agrochemicals (pesticides), textiles, adhesive, paper and board industry. Starch based products are being considered for use in surfactants (detergents), bleaching boosters (bleach under low temperature) and degradable plastics (Entwistle *et al.*, 1998).

In these applications, the ability of starch to produce a viscous paste when heated in water is its most important property. In addition, the appropriate paste

quality with respect to clarity, stability towards heat, shear, pH and resistance to syneresis may also be important depending on the application.

2.4 Structure of starch

2.4.1 Major components

2.4.1.1 Amylose

2.4.1.1.1 Structure

Amylose is found with a molecular weight ranging from $1x10^5$ to $2x10^6$ g/mole and the number of glucose residues per molecule, degree of polymerization (DP), ranging from 930-4920 (Table 2.3). Although considered to be essentially linear, amylose is not completely hydrolyzed by β-amylase (Hizukuri *et al.*, 1981). Greenwood & Thompson (1962) reported that β-amylolysis limits of amylose extracted from various starches range from 72-95% and some of the limits are presented in Table 2.3 The incomplete β-amylase hydrolysis indicates, that a certain degree of branching is present in amylose. According to Hizukuri *et al.* (1981), the branching occurs through α-(1→6) links and the amount of branching depends on the origin of amylose and is in the range of 25-55% on a molar basis. The average number of branch linkages per branch molecule is 4-18, whereas the percentage of branch linkages is 0.27-0.68%. The branch chains are usually moderately long, but a few may be as small as glucosyl to maltotetraosyl (G_1 -G_4). Amylose leached from granules immersed in water just above their gelatinization temperature has a lower

Table 2.3 Properties of amylose from different botanical sources

Starch source	Content (%)	Iodine affinity (%)	β-amylolysis limit (%)	Degree of polymerization	Branched molecule (%)	Number of branch linkages	
						whole	branched molecule
Wheat	26-31	19.9	81	2100	40	4.8	12.0
Maize	28	20.1	82	940	48	2.4	4.4
Amylomaize	52-80	19.4	75-76	1300	44	2.0	4.5
Oats	27	19.5	78	1300	-	-	-
Rice	14-32	20.5	77	1110	36	2.4	6.5
Barley	22-29	6.1	87	1850	-	5.4	-
Kuzu	20.3	19.5	75	1540	53	3.7	6.8
Sago	19.9	19.3	80	2490	62	10.4	18.3
Tapioca	16-17	20.0	75	2260	42	6.8	16.1
Yam	22	19.9	86	2000	29	2.8	-
Sweet potato	20.7	20.2	76	4100	70	7.8	12.6
potato	23	20.4	80-87	3200	-	6.3	-
Smooth pea	33	19.1	82	1300	-	-	-
Wrinkled pea	70	18.8	85	1100	-	-	-
Navy beans	36	18.5	86.2	1300	-	-	-
Faba beans	31.3-42.1	19.6	85.6	1400	-	-	-

Adapted from Colonna *et al.* (1992); Blanshard (1987); Hizukuri (1996).

molecular weight and higher β-amylolysis limit (90-100%), than more extensively branched amylose which is leached at higher temperatures (β-amylolysis limit 70-80%) [Banks & Greenwood, 1975; Takeda *et al.*, 1986]. Despite the slight branching, the branched amylose appears to behave like a linear polymer forming films and helical inclusion complexes with ligands. Amylose of some starches has been shown to contain phosphate groups [maize 0.02-0.03%, potato 0.04-0.13% of dry starch (Galliard & Bowler, 1987)] probably attached to C-6 of glucose residue (Banks & Greenwood, 1975).

2.4.1.1.2 Conformation

It has been postulated that the conformation of amylose is slightly helical due to the natural twist present in the chair conformation of glucose (Kowblansky, 1985). Amylose in solid state shows two polymorphs; 'A' (Fig. 2.2a) and 'B' (Fig. 2.2b) [Wu & Sarko, 1978a], which give similar X-ray patterns as the amylopectin crystallites in native starches. Imberty *et al.* (1987;1988) have shown that 'A' and 'B' polymorphs are right handed sixfold double helices. However, conflicting hypothesis still exists concerning the molecular conformation of amylose in aqueous solution (Banks & Greenwood, 1971; Senior & Hamori, 1973; Cheetham & Tao, 1997). Banks & Greenwood (1971) proposed three model conformations for amylose in aqueous solution (Fig 2.3 a-c). In both

Fig. 2.2 ^{13}C CP/MAS NMR spectra of highly crystalline (A) 'A' type amylose, (B) 'B' type amylose (adapted from Horii *et al.*, 1987).

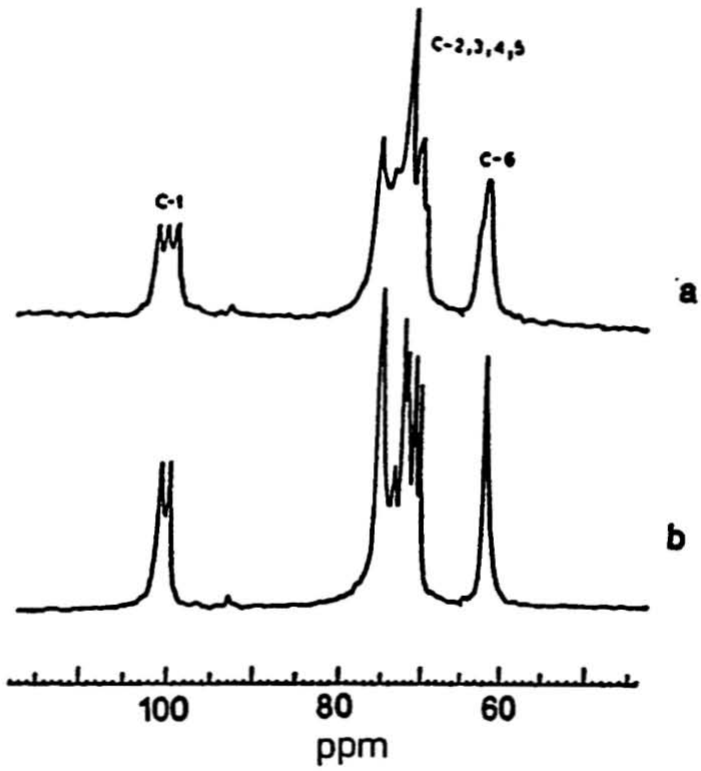
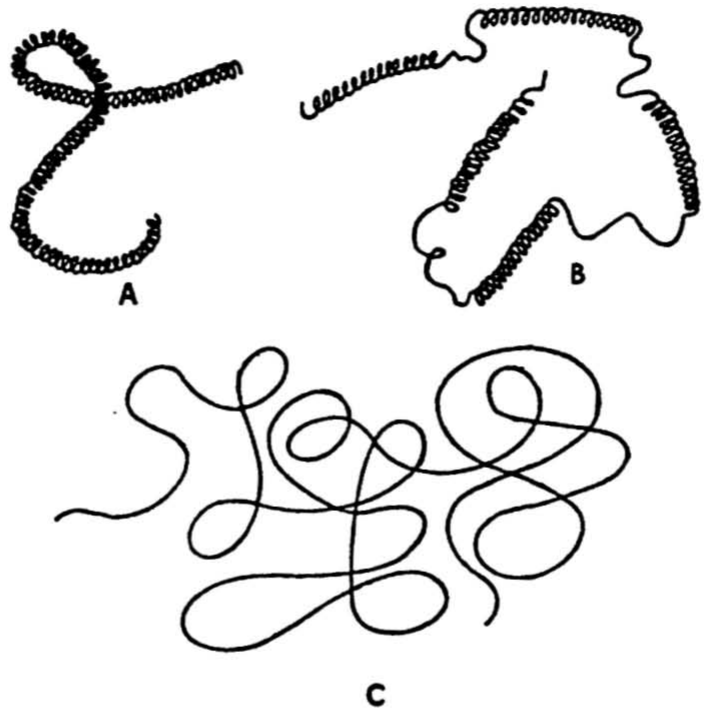

C-2,3,4,5
C-6
C-1
a
b
100
80
60
ppm

Fig. 2.3 Models proposed for the amylose in aqueous solution (with permission, Banks & Greenwood, 1971).

A) random helical coil (6 glucose units per turn).

B) interrupted helix.

C) random coil with no helical character.


A
B
C

random helical coil (Fig. 2.3a) and interrupted helix (Fig 2.3b), the helical portions are thought to be stabilized by intramolecular hydrogen bonds. The random coil conformation (Fig 2.3c) exists in neutral aqueous solutions as well as in solvents such as dimethyl sulphoxide (DMSO), whereas the helical conformation is attained in neutral or alkaline solutions in the presence of complexing agents (lipids, iodine) [Banks & Greenwood, 1971]. Senior & Hamori (1973) suggested that amylose conformation shows regions of loose and extended helices which alternate with shorter random coil segments. When amylose forms a complex, the loose helix is forced into a tighter helical conformation by the complexing agent. In contrast to Banks & Greenwood (1971), Cheetham & Tao (1997) have shown [using the changes in chemical shifts of ^{13}C NMR (nuclear magnetic resonance), optical rotation and limiting viscosity measurements] that amylose conformation in 100% DMSO is helical rather than a random coil. With the addition of water, the intramolecular hydrogen bonding in amylose (when amylose is in DMSO) is gradually replaced by amylose - water intermolecular hydrogen bonds (amylose / DMSO / water), leading to conformational changes. When the concentration of DMSO is decreased from 100% to 66.6%, amylose conformation changes from tight helix → loose helix. Further decrease in DMSO (66.6 to 33.3%) causes conformational change from loose helix → random coil [Cheetham & Tao, 1997].

Amylose in solution presents two features which are important in the functionality of starch based products; namely the ability to form inclusion complexes with desirable ligands and the ability to form interchain associations in polysaccharide chains leading to gelation or precipitation.

Amylose combines with a variety of compounds like iodine, aliphatic alcohols, aliphatic hydrocarbons, fatty acids and monoacylglycerol (MG) to form so-called inclusion complexes (Fig. 2.4) which are insoluble at room temperature (Teitelbaum *et al.*, 1978; Carlson *et al.*, 1979; Swinkles, 1985). Inclusion complexes are not formed as a result of a chemical reaction, but have been defined as addition compounds (complexes) in which 'guest' entity fits into and is surrounded by the lattice of the 'host' molecule. The bonds involved in inclusion complex are Van der Waals attractive forces which are quite weak but sufficient to provide the formation of stable complexes (Osman-Ismail, 1972). In the helical form, the interior of the helix is built up by C-H groups and glycosidic oxygen atoms forming a lipophilic core, while the polar hydroxyl groups are positioned on the outer surface of the helix (Banks & Greenwood, 1971). Carlson *et al.* (1979) have shown that the helical space in 'V' amylose conformation is too narrow to accommodate the bulky polar group of the lipid molecule. Hence, the polar group must exist outside the amylose helix.

Iodine, in the form of polyiodide ions (up to I^-_{13}, but mainly as I^-_3 or I^-_5), can bind with amylose (Teitelbaum *et al.*, 1978). The complex produces a deep blue colour which is used to identify amylose containing starches and to measure

Fig. 2.4 Schematic illustration of amylose - lipid complex (adapted from Carlson *et al.*, 1979).

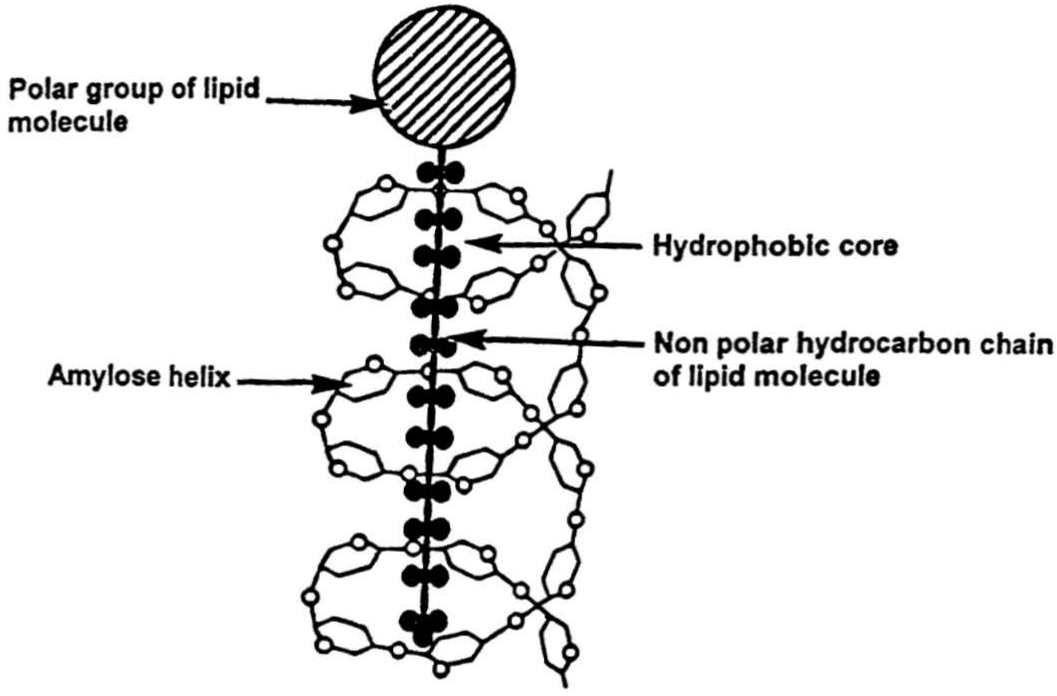

Polar group of lipid molecule
Hydrophobic core
Non polar hydrocarbon chain of lipid molecule
Amylose helix

Fig. 2.5 'V' X-ray diffraction pattern of amylose - lipid complex showing characteristic d- spacings (4.4-4.3, 6.5-6.8, 11.3-12).

(adapted from Zobel, 1988a).

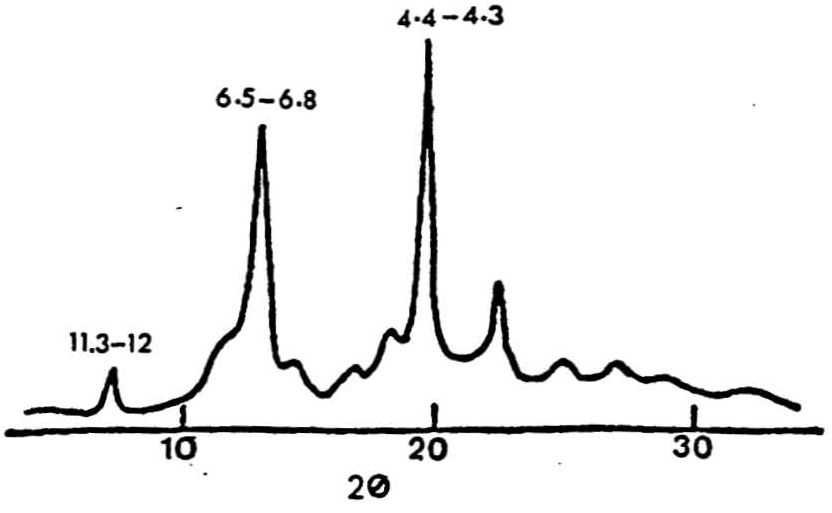

4·4–4·3
6·5–6·8
11.3–12
10
20
30
2Ø

Table 2.4 Properties of amylopectin from different botanical sources

Starch source	Iodine binding (mg/100mg)	β-amylose limit	Average CL	A- CL	B- CL	A : B chain ratio
Wheat	0.2	57	19-20	17	56	1.5 : 1
Maize	0.9	59	25-26	17	60	1.2 : 1
Waxy maize	-	58	20-22	18	51	1.5 : 1
Amylomaize	0.1	61	23	19	65	1.7 : 1
Rice	0.6	59	58-59	-	-	1.5 : 1
Waxy rice	-	-	23	15	62	1.1-1 . 5:1
Barley	0.7	60	26	-	-	-
Barley (skx)	0.82	59	20	-	-	-
Sago	0.43	59	22	-	-	-
Tapioca	-	57	21	17	52	1.2 : 1
Yam	0.07	-	24	19	55	
potato	0.6	51-56	24	20	62	1.3 : 1
Sweet potato	0.44	56	30	16	66	1.8 : 1
Lentil	-	-	23	16	50	-
Faba bean	-	-	21	-	-	-
Smooth pea	1.7	61	26	-	-	-
Wrinkled pea	0.9	57	27	-	-	-

Adapted from Swinkels (1985); Blanshard (1987); Colonna *et al.*(1992); Hizukuri (1996).

inner and outer chain lengths (Robin *et al.*, 1974; Hizukuri, 1986). Although the main structural features of amylopectin have been known for some 50 years, details of the fine structure are still lacking. There is still uncertainty about the detailed arrangements of the constituent linear chains of (1-4) linked α-glucose residues.

Investigation of amylopectin structure is a case in point of where progress is dependent on methodology development (both enzymatic and instrumental methods). Hydrolysis of amylopectin with a debranching enzyme and separation of the digest (containing linear chains) using exclusion gel filtration technique normally gives a bi- or trimodal elution profile, indicating the presence of chains with different chain length or molecular weight (Hizukuri, 1986).

A number of models have been proposed for the amylopectin molecule, including comb-like model and laminated structure (Staudinger & Husemann, 1937). However, currently accepted structural models are those derived from the cluster models of Nikuni (1969) and French (1972) [Fig. 2.6a]. These models exhibited the presence of chain segments which are designated as 'A', 'B' and 'C'. The 'A 'chains are joined to the remainder of the molecule with a single 1,6 bond, 'B' chains are joined through a 1,6 bond but may carry one or more 'A' and/or 'B' chains on primary hydroxyl groups; 'C' chain carries the sole reducing group (Zobel, 1988b).

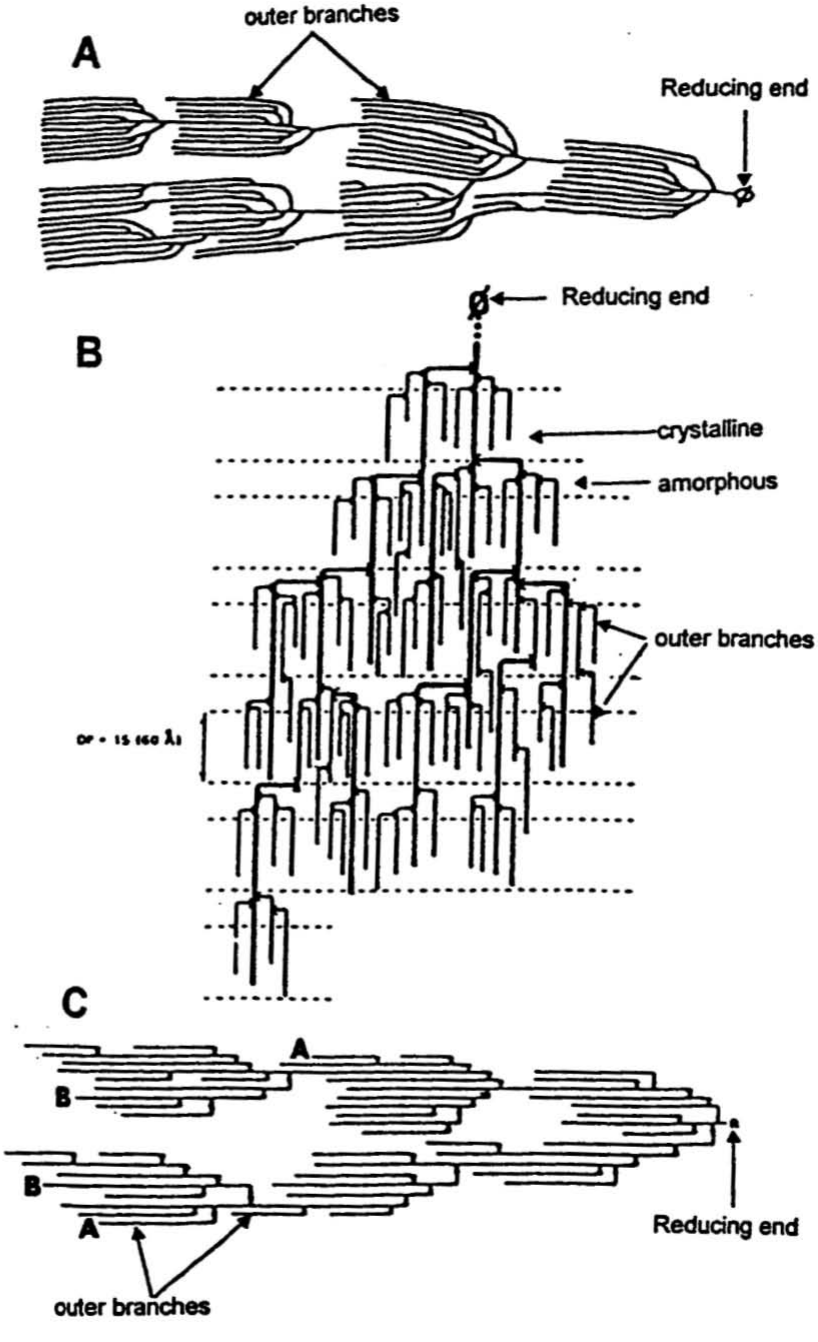
A similar model (Fig 2.6b) was proposed by Robin *et al.* (1974), from data derived from sequential treatment of potato amylopectin with debranching

Fig. 2.6 Models proposed for amylopectin (with permission).

A) Cluster model of French (1972).

B) Robin *et al.* (1974).

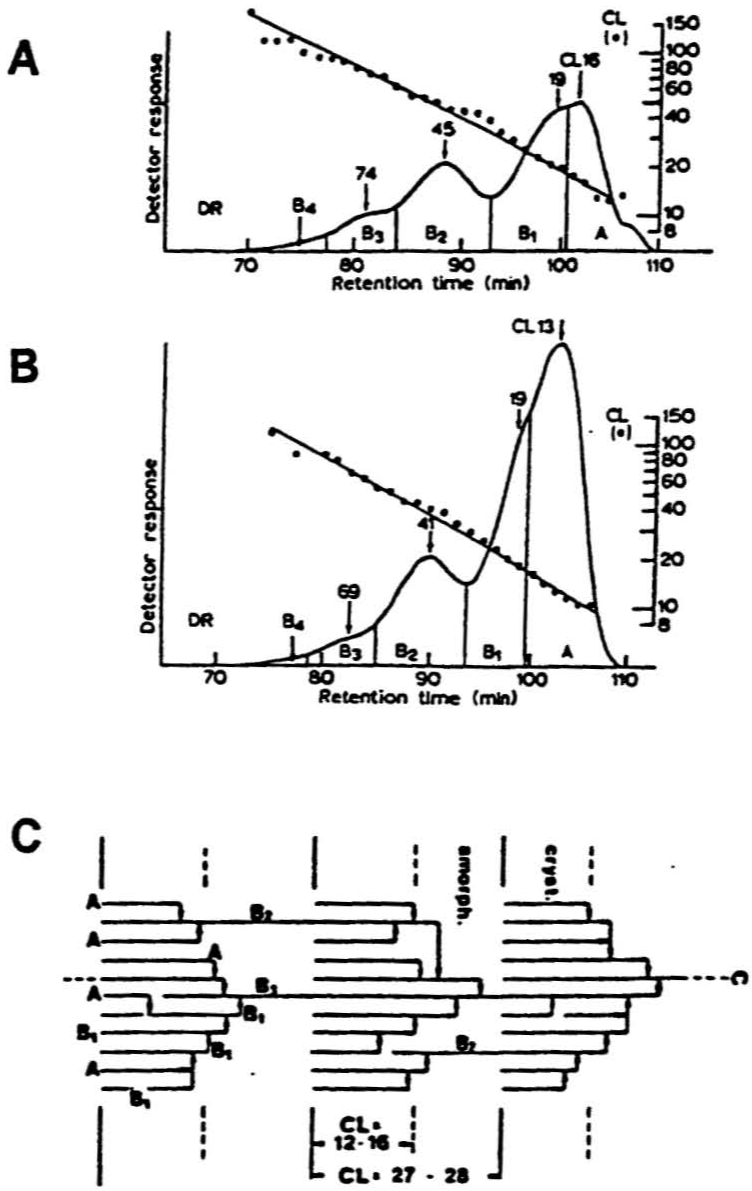
C) Manners & Matheson (1981).


A
outer branches
Reducing end
B
Reducing end
crystalline
amorphous
outer branches
C
A
B
B
A
Reducing end
outer branches

enzymes and β-amylase, its β-limit dextrin and a derived acid-resistant amylodextrin. The significance of this model was the presence of three chain populations with CL of 15-20, 45 and 60. The chains with CL of 15-20 are in highly ordered clusters, which are linked to each other by much longer chains, which would correspond to the longer 'B' chains (CL 45) found in elution profiles.

Based on the polymodal distribution of chain profile (Fig. 2.7a,b) elucidated from gel permeation HPLC (high performance liquid chromatography) of amylopectin, Hizukuri (1986) proposed a refined cluster model (Fig. 2.7c). 'A' chains are represented by fraction A which eluted last while various B chains are represented by fractions 'B_1 - B_4'. Moreover, 'A' and 'B_1' makes a single cluster. Chains in fraction 'B_2' extend into two clusters, those in fraction 'B_3' extend into three clusters, and the chains in fraction 'B_4' extend into more than four clusters (Fig. 2.7a,b). Hizukuri (1986) showed that amylopectins isolated from potato, tapioca, kudzu and waxy rice the average CL of the fraction 'B_1', 'B_2' and 'B_3' were 20-24, 42-48 and 69-75 respectively; the relative lengths being ~ 1:2:3. The CL of 'A' chains are in the range of 12-16 which was in agreement with the exterior CL of amylopectin as reported by Manners (1985). Similar CL values have been reported by French (1972), Robin *et al.* (1974) and Hood & Mercier (1978) and the values were 12, 15 and 15, respectively. The sum of 'A' and 'B_1' fractions represents 80-90% of total chains and constitute a single cluster and the 10-20% are in inter-cluster connections (Hizurkri, 1986).

Fig. 2.7 Chain distribution of (A) potato, (B) waxy rice and (C) the proposed model for amylopectin (adapted from Hizukuri, 1986).

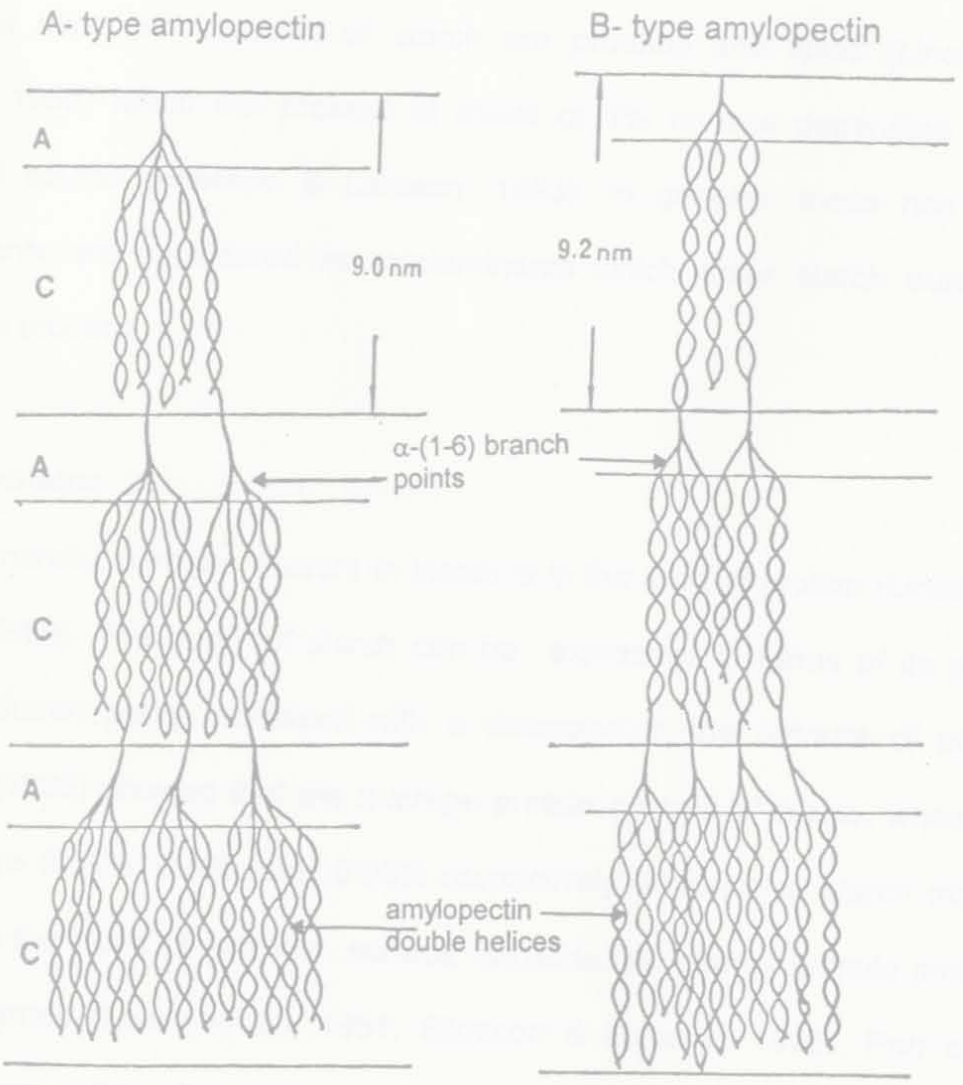

A
Detector response
CL (°)
150
100
80
60
40
20
10
8
CL16
19
45
74
DR
B4
B3
B2
B1
A
70
80
90
100
110
Retention time (min)
B
CL13
19
41
69
DR
B4
B3
B2
B1
A
Retention time (min)
C
A
B2
B1
CL = 12-16
CL = 27 - 28

The ratio of 'A' to 'B' chains (2:1) was first reported by Marshall & Whelan (1974). However, a critical examination by Manners & Matheson (1981) revealed that the 'A' to 'B' chain ratio was about 1:1. Hizukuri (1986) reported that the A : B ratios are 0.8-0.9:1 for tapioca, kuzu and potato, and 2.2:1 for waxy rice amylopectin.

The cluster model is in accordance with the relatively high viscosity of amylopectin, the crystallinity of the macromolecule as revealed by X-ray analysis and the relative resistance of parts of the molecule to attack by acid and amylolytic enzymes (Manners, 1985). All these structures have the concept that the branch points are arranged in clusters of short chains.

On the basis of the structures of Naegeli dextrins (obtained by high performance anion exchange chromatography) and data obtained from other studies (Jenkins *et al.*, 1993; Jenkins & Donalds, 1995; Jane *et al.*, 1997b), Jane (1997b) proposed two structurally different models of 'A' and 'B' type amylopectin (Fig. 2.8). The structures show that α- (1$\rightarrow$6) branch linkages of 'A' starches are more scattered. Substantial amounts of branch linkages are located within the crystalline regions (which are protected from acid hydrolysis), whereas the branch linkages in the amorphous region are hydrolysed during acid treatment. The branch linkages in the 'B' type amylopectin are clustered in the amorphous region and are susceptible to acid hydrolysis. These models show that the repeating distance of 'A' and 'B' type amylopectin are 9.0 and 9.2 nm (Fig. 2.8), respectively (Jane, 1997b).

Fig. 2.8 Proposed models for 'A' (from waxy maize) and 'B' (from potato) type amylopectin branching patterns. A and C represent amorphous and crystalline regions, respectively (adapted from Jane, 1997b).


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

2.4.2 Minor components of starch

The minor components of starch exist either as surface materials on the granules or as internal components within the granule matrix. The most important minor components of starch are proteins and lipids (Lineback & Rasper, 1988) which are present at levels of 1% or less depending on the botanical source (Eliasson & Larsson, 1993). In general, these non starch components are considered as contaminants which enter starch during the extraction process.

2.4.2.1 Proteins

Generally, nitrogen present in starch is in the form of protein (Lineback & Rasper, 1988). The purity of starch can be expressed in terms of its protein content. Starch purity increases with a decrease in the amount of protein. Swinkels (1985) showed that the average protein content of maize, wheat and potato were 0.35%, 0.4% and 0.06% respectively. Proteins in starch may be present in the form of granule surface proteins, as internal granule proteins, or as enzymes (Lowry *et al.*, 1981; Eliasson & Larsson, 1993). Part of the nitrogen may also be present in association with starch lipids (e.g. lysophosphatidylcholine in wheat starch). Lowry *et al.* (1981) have shown that the protein content of well washed pure 'A' wheat starch was 0.1%, while ~ 10% of the protein was associated with the granule surface. Proteins which are associated with the surface of the granule can be readily extracted with dilute

salt under mild conditions that cause no disruption of granules. The internal proteins are not released by dissociating agents (e.g. sodium dodecyl sulphate [SDS]) until the granules have been gelatinized by heating. The requirement for disruptive conditions to release these proteins indicates, that they are buried within the matrix of the granules. The sub-units of internal proteins obtained on sodium dodecyl sulphate - polyacrylamide gel electrophoresis are of higher molecular weight than the surface proteins (Lowry *et al.*, 1981). It is likely that the internal proteins may represent residual material from lipoprotein membranes of the original amyloplasts or of membrane bound starch synthesizing systems employed during development (Galliard, 1983).

2.4.2.2 Lipids

Lipids associated with isolated cereal starch granules have been found to occur on the surface as well as inside the granule (Morrison, 1981). The surface lipids are mainly triacylglycerol, followed by free fatty acids, glycolipids and phospholipids and they include those that may have been present on the granule surface *in situ* in the plant tissue as well as the non starch lipids, which are absorbed into the surface layer of starch granule during isolation (Morrison, 1981; Galliard & Bowler, 1987; Vasanthan & Hoover, 1992b). Non-starch lipids occur as spherosomes, concentrated in the sub-aleurone region, and also as components of membranes and organelles associated with storage protein (Morrison, 1981). Since it is not possible to distinguish between these two types

of surface lipids on the basis of solvent extraction techniques, it has been suggested that all lipids found on the surface have to be considered as starch lipids (Galliard & Bowler, 1987). The internal lipids of cereal starches are predominantly monoacyl lipids, with the major component being lysophospholipids (of which lysophosphatidylcholine is the major component) and free fatty acids (Hargin & Morrison, 1980; Morrison, 1981). 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 or linked via ionic or hydrogen bonding to hydroxyl groups of the starch components. Free lipids are easily extractable by solvent systems at ambient temperatures (Morrison, 1981), whereas prolonged extraction with hot aqueous alcoholic solvent systems (Morrison, 1981) or disruption of the granular structure by acid hydrolysis (Goshima *et al.*, 1985) is required for the efficient removal of bound lipids. The amount of total starch lipids (surface and bound) has been found to be in the range of 0.7-1.2% in cereals (Morrison & Milligan, 1982; Vasanthan & Hoover, 1992b; Takahashi & Seib, (1988), 0.01-0.87% in legumes (Hoover & Sosulski, 1985), and 0.08-0.19% in tubers and roots (Vasanthan & Hoover, 1992b; Emiola & Delarosa, 1981; Goshima *et al.*, 1985).

Zobel (1988a) suggested that lipids in starches may be responsible for effecting an amylose separation within the granule. This would imply that the starch polymers of low lipid containing starches (potato, lentil, cassava) may be more associated with each other in the native granule than those of high lipid

containing starches (wheat, corn). Morrison & Laignelet (1983) showed that the presence of lipid decreases the iodine binding capacity of cereal starches by 20-30% which may be interpreted as the proportion of amylose complexed with lipid and hence unavailable to complex with iodine.

2.4.2.2.1 Amylose-lipid inclusion complex

Amylose - lipid inclusion complexes (Fig 2.4) have been shown to influence the texture and the structural stability of cereals and starch based products (Lund, 1984). These complexes are effective in decreasing the rate of bread staling (Krog & Jensen, 1970), improving the texture in extruded starch containing products (Mercier *et al.*, 1980), improving structural integrity in cereal kernels (parboiled rice) during cooking (Biliaderis *et al.*, 1993) and preventing stickiness in mashed potato granules (Hoover & Hadziyev, 1981).

2.4.2.2.2 Occurrence of amylose-lipid complex

The existence of naturally occurring amylose-lipid complexes in starch granules has been the subject of much controversy. For many years, it has been observed that native starches with < 30% amylose do not exhibit the naturally occurring 'V' X-ray pattern (Galliard & Bowler, 1987) except for wrinkled pea, amylomaize and some other maize genotypes [sugary (*su*), and dull (*du*) (Gernat *et al.*, 1993)]. The absence of a 'V' X- ray pattern does not necessarily indicate the lack of amylose-lipid complexes in native starches; it suggests that the

helices are not organized in a three dimensional array. Recently ^{13}C CP/MAS NMR (cross polarization magic angle spinning nuclear magnetic resonance) studies provided the evidence for the presence of amylose-lipid complex in granules of native oat, maize rice, barley and wheat starches (Morrison *et al.*, 1993a, 1993b; Morgan *et al.*, 1995). The spectral features (Fig. 2.9) of cereal starches indicative of the single 'V' amylose helix were : 1) the signal of C-1 at 103-104 ppm representing 'V' form, and 2) the broad peak at 31 ppm (represent the mid-chain methylene carbons of monoacyl lipids) which reflects a near-solid state structure of lipids due to steric constrains in the helical cavity. Morrison *et al.* (1993a, 1993b) showed that amylose exist partially as lipid-complexed amylose (L-AM) with a lysophospholipid to L-AM ratio of 1: 7 and partially as lipid-free amylose (F-AM). Table 2.5 shows different barley starches with their L-AM and F-AM contents. Waxy barley starches used in the study contained 0.8-4.0% L-AM and 0.9-6.45% F-AM, whereas in normal barley starches corresponding values were 6.1-7.2% and 23.1-25.0%, respectively. Gernat *et al.* (1993) have also shown the existence of amylose - lipid complexes in native starch granules by X-ray diffraction studies of enzymatically degraded wheat starch.

The ability of lipids to form complexes depends on the type of lipid. Using the iodine binding capacity of starch, it has been shown that saturated MG are more effective in complex formation (Lagendiijk & Pennings, 1970; Krog, 1971) than unsaturated MG (Eliasson & Larsson, 1993). The amount of fatty acid

Fig. 2.9 ^{13}C CP/MAS NMR spectra of 'V' amylose in waxy barley starch (Chalky Glen), with inset of the 10-50 ppm region at x 20 scale expansion. The marked resonance is for midchain methylene carbons of fatty acids in lysophospholipid with a chemical shift of 31.2 ppm (A). Resonance from polysaccharide carbons (B-E) are B = C-6 (61.3 ppm), C = unresolved C-2, C-3, C-5 (71.4 ppm), D = C-4 (80.9 ppm), E = C-1 (100.5 ppm) with some helical amylose (103 ppm) [With permission, Morrison *et al.*, 1993b].

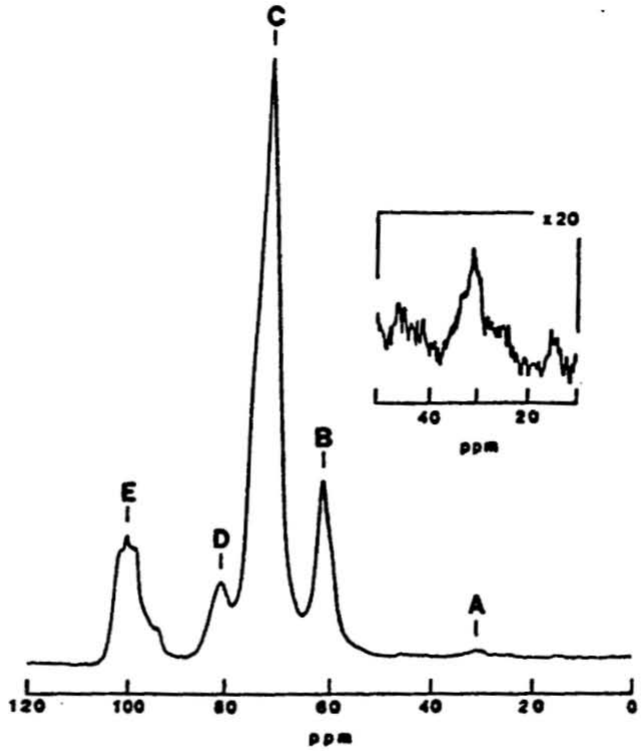

C
x20
40
20
ppm
B
E
D
A
120
100
80
60
40
20
0
ppm

Table 2.5 Lipid complexed amylose (L-AM) and lipid-free amylose (F-AM) in waxy and normal barley starches

Variety	L.AM (%)	F.AM(%)
waxy		
Summier Mochi	0.8	0.9
Dango Mugi	1.2	0.9
Masan Naked	1.5	0.9
Tokushima Mochimugi (a)	1.7	1.4
Tokushima Mochimugi (b)	2.1	1.5
Chalbori	1.6	1.8
Iyatomi Mochi	2.1	1.8
Waxy Oderbrucker	2.8	2.4
Bozu Mochi	3.2	2.2
Wapana	3.4	3.1
Wanupana	3.5	3.0
Washonupana	4.0	3.4
Normal (non waxy)		
Ckalky Glen	6.1	23.1
Midas	6.2	24.0
Hector	5.4	25.0
Shopana	7.0	23.5
Compana	7.2	23.3
Glen	6.8	25.9

Adapted from Morrison *et al.* (1993b).

bound by amylose increases with an increase in the chain length of the fatty acid. Furthermore, the increase in unsaturation decreases the ability of complex formation (Hahn & Hood, 1987), possibly due to the fact that saturated fatty acids with straight hydrocarbon chains may fit into the amylose helical cavity more easily than unsaturated fatty acid hydrocarbon chains which may be bent at various angles. Moreover, unsaturated fatty acids have greater solubility in water and may therefore, exist in the free state in higher amounts than saturated fatty acids which are less soluble (Hahn & Hood, 1987).

The acyl chain of amylose-lipid complex is considered as a straight "rod-like" structure. In linoleic acid, approximately 50% of fatty acid chains are of *cis*-9, *cis*-12, 18 : 2. Therefore, it might be expected that the kinks introduced by two cis-double bonds would interfere with complex formation. Galliard (1983) showed that there is evidence to show the complex formation between amylose and linoleic acid is slightly less stable than that between amylose and the corresponding fully saturated fatty acid, the free energies determining the conformation of the complexes with saturated or unsaturated fatty acids are presumably relatively similar. Furthermore, Riisom *et al.* (1984) have shown that *cis* unsaturated compounds were more effective than *trans* unsaturated monoelaidate and the saturated monopalmitate in complex formation. However, surprisingly, naturally occurring amylose-lipid complexes in starch consist of lysolecithin which is rich in *cis-cis* linoleic acid.

Formation of 'V' amylose has been observed during swelling and gelatinization of starch granules (Morrison & Milligan, 1982; Eliasson & Larsson, 1993) on heat-moisture treatment (Zobel, 1988a), on extrusion cooking of lipid containing cereal starches (Mercier *et al.*, 1980), and after addition of monoacyl lipids to starch under appropriate conditions (Hoover & Hadziyev, 1981; Biliaderis *et al.*, 1986a). The hydrothermal conditions induce the mobility of amylose chains to complex with naturally occurring or added monoacyl lipids, leading to the formation of larger assemblies detectable by X-ray diffraction. Extrusion of cassava starches (Mercier *et al.*, 1980) with 2-4% monoacyl lipids at 22% moisture, showed formation of two types of structures depending on the extrusion temperature. When starches were extruded below 170°C, they showed X-ray pattern termed 'hydrated - V' pattern [d spacings (the distance between the planes in the crystal) at 4.4, 6.8 and 12.0 Å], whereas when the extrusion temperature was above 185°C and the moisture content was < 13%, the 'extruded' type X-ray pattern was observed. Extruded type was characterized by the slight displacement of diffraction peaks to a lower angle (e.g. the peak at 9°54' in hydrated 'V' type appears at 9°03' in 'extruded' type).

X-ray, DSC (differential scanning calorimetry) and structural analysis data (Biliaderis & seneviratne, 1990; Biliaderis & Galloway, 1989) have shown that the amylose-lipid complex in the solid state shows the existence of two structurally different forms; form I and form II (Fig. 2.10), based on the crystallization conditions (temperature, type of ligand, etc.). Form I is obtained under conditions

Fig. 2.10 A typical DSC thermal curve (50% solid) of a cereal starch showing the different melting transitions and the corresponding structural domains undergoing a phase change.

M_1 & M_2 : melting of amylopectin crystallites at intermediate moisture content.

M_3 : melting of Form I amylose - lipid complex

M_4 : melting of Form II amylose - lipid complex (with permission, Biliaderis & Galloway, 1989; Biliaderis & Seneviratne, 1990).

favouring rapid nucleation. This gives an amorphous X-ray pattern showing the lack of properly packed ordered systems, whereas form II shows the typical 'V' pattern, reflecting the well developed long range order (Biliaderis & Galloway, 1989).

2.4.2.2.3 Stability of amylose-lipid complex

Starch - lipid complex formation takes place when both amylose and lipid are in solution. Therefore, in order to form complexes, lipids must be in a suitable dispersed state. The optimal conditions for amylose - lipid complex formation are directly related to the lipid monomer concentration (Larsson, 1983). The most effective state of lipid in complex formation is the micellar solution (Fig. 2.11a), since its monomer concentration in equilibrium with a micellar solution is high. Furthermore, lipids in the lamellar phase (Fig. 2.11a,b) are excellent in complex formation (Riisom *et al.*, 1984) because of their ability to form fine dispersions (Larsson, 1983).

2.4.2.2.4 Thermal and rheological properties of amylose-lipid complex

Amylose-lipid complex has been shown to alter gelatinization parameters, granule swelling, solubility, amylose leaching and viscosity of starches. In general, formation of amylose-lipid complex decreases gelatinization enthalpy, granule swelling, solubility and amylose leaching of starch.

Fig. 2.11 Structures in binary lipid-water systems.

A) Binary lipid-water system characteristic of polar lipids which form micellar solutions. The horizontal axis defines the composition and the vertical axis the temperature. Lipid molecules are illustrated by the polar head (a circle) and one attached chain tail (adapted from Larsson, 1983).

B) Structure of a fragment of a particle of the lamellar phase dispersed in water (adapted from Larsson & Dejmek, 1990).

DSC studies show that, the melting transition of amylose-lipid complex occurs in the temperature range of 85-130°C. In Figure 2.10, M_3 and M_4 transitions show the melting of form I (with low melting temperature) and form II (with high melting temperature) complexes respectively. The melting temperature of the complex is influenced by hydrocarbon chain length (Eliasson & Krog, 1985; Biliaderis & Galloway, 1989), complex concentration (Biliaderis *et al.*, 1985), crystallization temperature (Biliaderis & Galloway, 1989) and moisture content (Biliaderis *et al.*, 1985). Eliasson & Krog (1985) have reported that melting temperature of potato amylose-lipid complex increased when the chain length of monoacyl lipid increased from C_{12} to C_{18}. Furthermore, Biliaderis *et al.* (1985) have shown that peak melting temperature of amylose-monopalmitin, amylose-lysolecithin and amylose-lauric acid complexes increased with increase in complex concentration.

Influence of moisture content on amylose - lipid complex formation showed that at moisture contents > 80%, a single endotherm was observed for melting of amylose-lipid complex, whereas for the moisture contents < 50%, melting was shown by two endotherms which were separated by an exothermic peak. Such nonequilibrium melting is due to partial melting followed by recrystallization and final melting of the complex (Biliaderis *et al.*, 1985).

Lonkhuysen & Blaknestijn (1976) reported that the stability of swollen granules was greatly enhanced when MG was added to the starch after gelatinization, but the swelling power was not affected. Hoover & Hadziyev

(1981) reported that, swelling power and solubility of potato starch decreased when the starch was complexed with saturated 1-monoacylglycerols. Solubility decreased by 8% with C_8 and 90% by C_{14}. Swelling power dropped steadily when the MG chain length increased from C_8 to C_{14} (Hoover & Hadziyev, 1981).

Evans (1986) showed that viscosity of heated waxy maize starch increased in the presence of SDS or cetyltrimethylammonium bromide (CTAB). Eliasson *et al.* (1988) reported that the addition of lipids (CTAB, saturated MG) into normal maize, cross-linked waxy maize and acetylated high amylose maize increased the dynamic viscosity of starches. The above authors suggested that the additives with two (lecithin) or three (soybean oil) hydrocarbon chains affect the viscoelastic properties of maize starch independent of amylose content.

2.4.3 Super molecular order of the starch granule

The super molecular order of starch granule (the organization of amylose and amylopectin within the granule) which governs most of the physicochemical properties of starch, is an important aspect of starch structure determination. The structure of starch has been subjected to many investigations and much speculation, nevertheless, the detailed arrangement of amylose and amylopectin within the starch granule is still under investigation. Different techniques have been employed to study the structural organization of starch granules. Quantitative structural analysis has been previously relied on a combination of enzymatic and chemical methods. Transmission and scanning electron

microscopy as well as small and wide angle X-ray diffraction have highly contributed to the structure determination. Today, high resolution nuclear magnetic resonance in the form of ^{1}H NMR, ^{13}C NMR and ^{13}C CP/MAS NMR have proven to be more effective, non invasive quantitative analytical tool.

The growth of starch granule by concentric deposition of layers has been known for many long years. Nikuni (1978) proposed a model which incorporated the amylose and amylopectin components including the appearance of concentric rings. Lineback (1984) proposed a modified version (Fig. 2.12) of Nikuni's (1978) model, which incorporated the cluster model of amylopectin. The current models (Fig. 2.13a) of granule are based on the fact that the crystalline structure of granule consists of radially arranged amylopectin clusters (Jenkins *et al.*, 1993). However, the exact arrangement of starch chains within the granule remains unclear.

It is now accepted that the starch granule is composed of both crystalline and amorphous regions. The fact that the crystalline component primarily consists of amylopectin was affirmed by the crystallinity, shown by granules after amylose was leached out, and waxy maize starch with no amylose being semi crystalline (Zobel, 1988b). The crystalline nature of native starch granules display a "Maltese cross" when viewed under polarized light. The positive birefringence indicates that there is a high degree of molecular orientation in the granule (Imberty & Perez, 1996). Electron or optical microscopy has

Fig. 2.12 Schematic model of starch granule proposed by Lineback (with permission, Lineback, 1984)

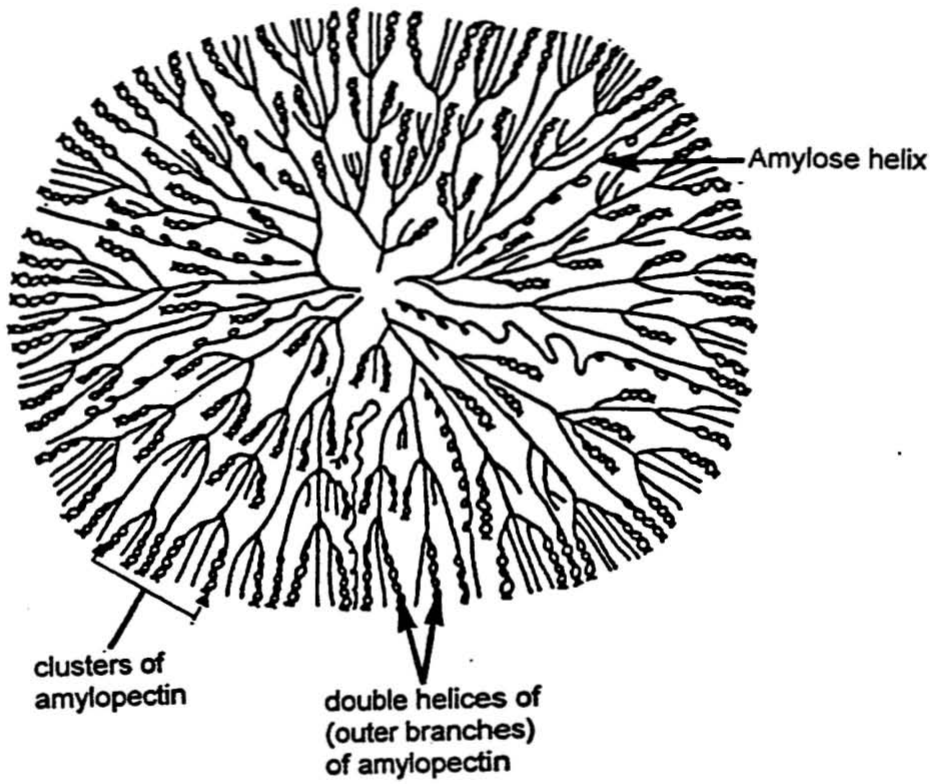

Amylose helix
clusters of amylopectin
double helices of (outer branches) of amylopectin

Fig. 2.13 Inner structure of starch granule showing crystalline and amorphous regions.

(A) stacks of semi crystalline lamella are separated by amorphous growth rings.

(B) magnified view of a stack made up of alternating crystalline and amorphous regions.

(C) the crystalline lamellae consist of double helices made up of amylopectin branches (adapted from Donald *et al.*, 1997).

confirmed the presence of well defined lamella which are also known as growth rings (Fig. 2.13a), especially in the granules treated with acid or amylolytic enzymes (Jenkins *et al.*, 1993; Eliasson & Larsson, 1993). These rings are alternately semi crystalline and amorphous and are generally 120-400 nm in size. These semi crystalline growth rings are composed of stacks (Fig. 2.13b) of alternating crystalline [double helices of short DP chains of amylopectin (Fig. 2.13c)] and amorphous [amylopectin branch points (Fig. 2.13c)] lamella [Kassenbeck, 1978]. This represents an average cluster of amylopectin (Fig. 2.13c) which is described and measured as repeat distance or periodicity.

Electron microscopy has shown that there is a periodicity of 6-7 nm along a radially oriented molecular axis (Kassenbeck, 1978; Yamaguchi *et al.*, 1979). This value is smaller than the value deduced (9-10 nm) from small angle neutron scattering (Blanshard *et al.*, 1984) and small-angle X-ray scattering (Oostergetel & Van Bruggen, 1989) studies of various starches. However, X-ray diffraction profile analysis showed that this periodicity is independent of the botanical source and the size is 9 nm for all the studied starches (Jenkins *et al.*, 1993; Jenkins & Donald, 1995). Oostergetel & Van Bruggen (1993) proposed a super-helical lamellar structure (Fig. 2.14) for potato amylopectin, using electron optical tomography and cryoelectron diffraction data from non disrupted granule fragments. The linear segments of double helices (Fig. 2.14a) were crystallized into lamella of 5 nm alternating with amorphous layers (Fig. 2.14b). The neighboring helices interpenetrate each other forming a continuous

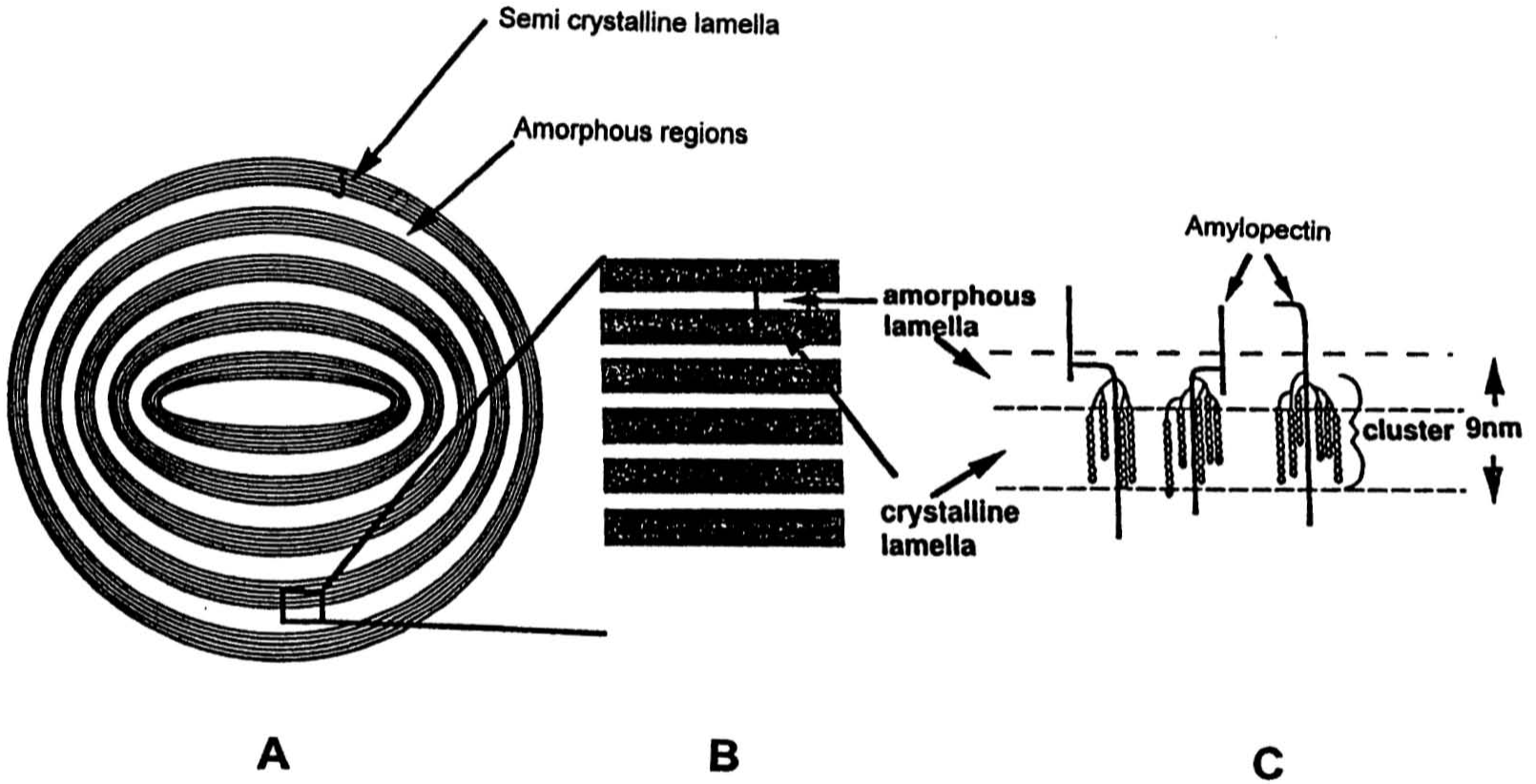
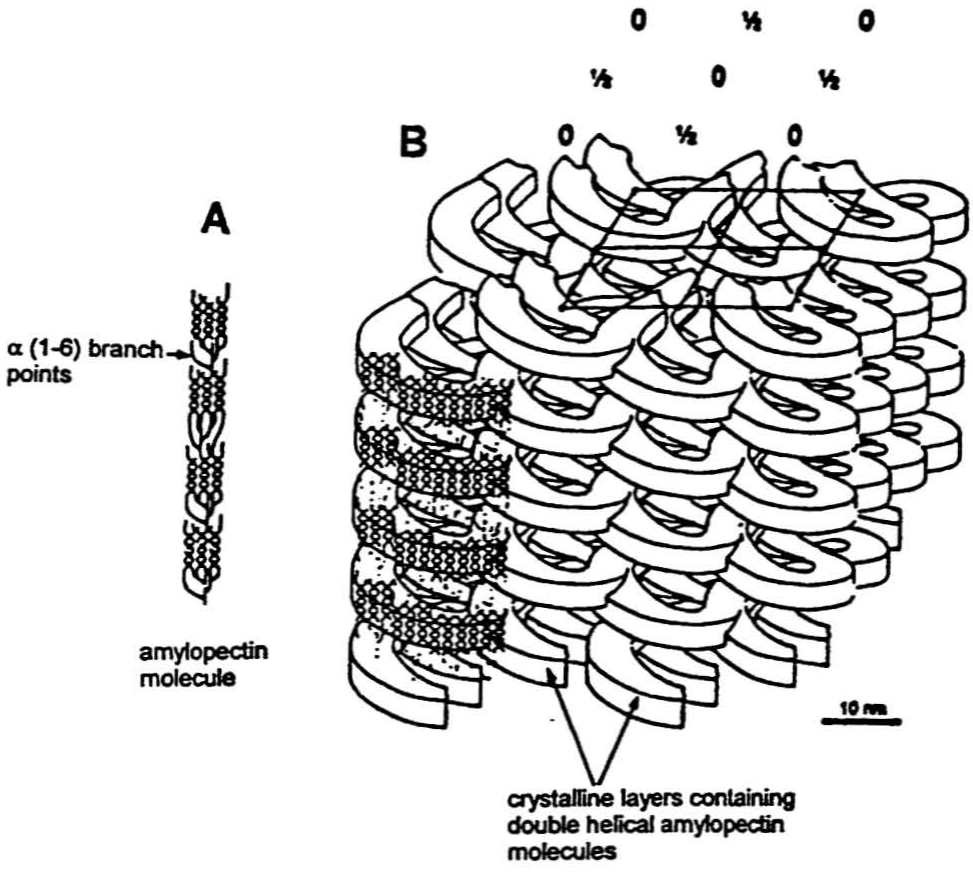

Semi crystalline lamella
Amorphous regions
amorphous lamella
crystalline lamella
Amylopectin
cluster
9nm
A
B
C

Fig. 2.14 Schematic model for the arrangement of amylopectin (super helical structure) in potato starch (with permission, Oostergetel & van Bruggen, 1993).
A) amylopectin molecule showing clustering of the α-(1-4), α-(1-6) branch points and the double helical linear glucan chains.
B) crystalline layers containing linear double helical segments in the amylopectin molecules form a continuous network consisting of left-handed helices packed in a tetragonal array.


A
α (1-6) branch points
amylopectin molecule
B
0
½
0
½
0
½
0
½
0
10 nm
crystalline layers containing double helical amylopectin molecules

super-helical network of crystalline lamella with left handed helices packed in a tetragonal array. The helical arrangement of crystalline lamellae leaves a void of 8 nm in diameter.

2.4.4 Molecular organization of crystalline regions

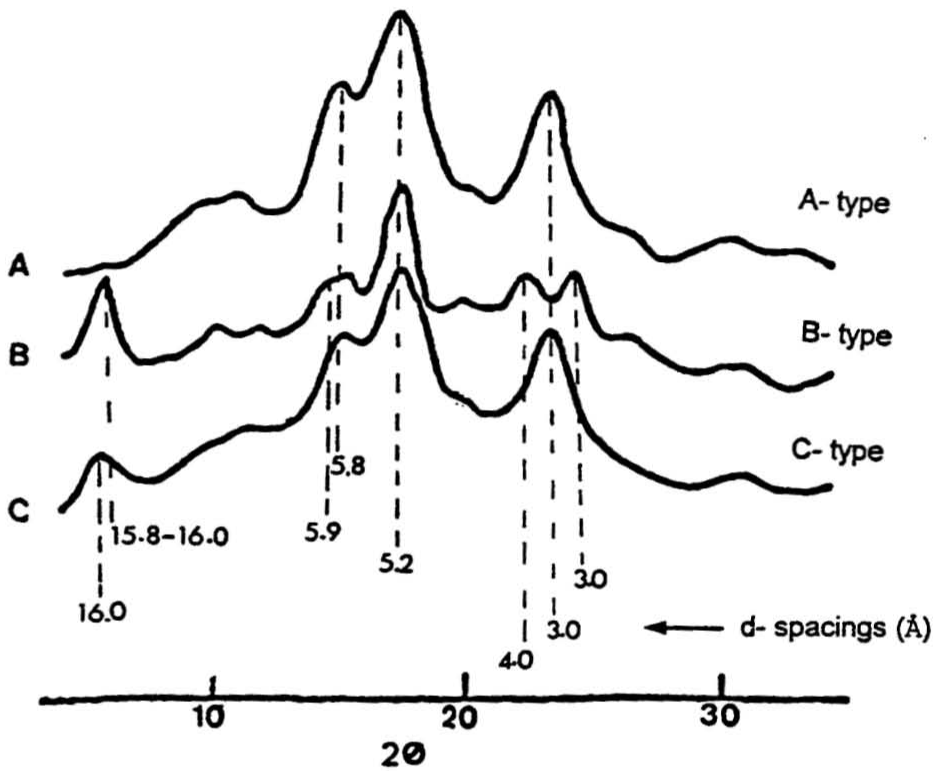
Based on the characteristic d- spacings (the distance between planes in the crystal) of wide angle X-ray diffraction patterns, common native starches can be classified into 3 main categories, namely 'A', 'B' and 'C' type [Fig. 2.15]. Cereal starches (rice, normal maize, wheat) show 'A' type X-ray pattern (Fig. 2.15a), whereas tuber starches (potato, tulip, canna, lily) and high amylose maize show 'B' type X-ray pattern (Fig 2.15b). However, some root starches such as tapioca, taro and sweet potato (Takeda *et al*, 1986) have been found to exhibit 'A' pattern. 'C' pattern (Fig. 2.15c), which is a mixture of 'A' and 'B' type unit cells, has been found in legume (Gernat *et al.*, 1990) and rhizome starches (Zobel, 1988a). In legume starches, 'A' and 'B' type unit cells in starch crystallites have been found in varying proportions. It has been shown that pea starch contains 38.6% 'B' type and 61.4% 'A' type, whereas broad bean starch is composed of 17% and 83% of 'A' and 'B' type crystallites respectively (Gernat *et al.*, 1990). Furthermore, Colonna *et al.* (1981) have reported that pea starch showed X-ray pattern more towards 'B' whereas in broad bean starch, the X-ray pattern was more towards 'A' type. 'C' type has been subdivided into 'Ca', 'Cb' and 'Cc' based on their resemblance to 'A' and 'B' types or between the two

Fig. 2.15 X-ray diffraction patterns of 'A', B' and 'C' type starches with their characteristic d-spacings (adapted from Zobel, 1988b).

A) 'A' type

B) 'B' type

C) 'C' type


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
3.0
d- spacings (Å)
10
20
30
2Ø

types, respectively (Hizukuri *et al*, 1960). Hoover & Sosulski (1985) reported that even legume starches belonging to the same biotype show widely varied X-ray intensities for major peaks, possibly due to the differences in crystallite orientation and/or the amount of 'A' and 'B' crystallites. The distinguishing features for 'A', 'B' and 'C' X- ray patterns are : 'A' [three peaks at 5.8, 5.2 and 3.8 Å (Fig. 2.15a)]; 'B' [peak at 15.8-16.0 Å, a broad medium intensity line at about 5.9Å, a strong line at 5.2 Å and a medium intensity doublet at 4.0 and 3.7 Å (Fig. 2.15b)]; 'C' is the same as 'A', except for the addition of a medium to strong peak at ~16.0 Å (Fig. 2.15c); Appearance of this 16.0 Å peak depends on the presence of moisture, and may be missing in dry or partially dry specimens (Zobel, 1988a). The difference in X-ray pattern among starches derives from the way in which the double helices are packed into the unit cells. Therefore, crystallinity in starch is based on the packing arrangement of double helices.

The level of granular crystallinity as measured by X-ray diffraction is in the range of 15-45% (Table 2.6). Maize and waxy maize starches have the same crystallinity regardless of their amylopectin content [maize 73% and waxy maize 100%]. Thus, amylose content appears to have little effect on amylopectin crystallinity in the starches that give the 'A' pattern (Zobel, 1988b). 'B' starches show lower crystallinity at increased amylose contents (Table 2.6). Instead of the 'A' pattern (typical for cereal starches), a 'B' pattern is observed for amylomaize (55-75% amylose) [Table 2.6]. Therefore, it is apparent that in 'B' type starches low crystallinity is associated with a high amylose content. Zobel

Table 2.6 Crystallinity of 'A', 'B'- and 'C' type granular starches

Starch	Crystallinity (%)	Amylose (%)
	'A' starches	
Oat	33	23
Rye	34	26
Wheat	36	23
Waxy rice	37	-
Sorghum	37	25
Rice	38	17
Corn	40	27
Waxy maize	40	0
Dasheen	45	16
Nageli amylodextrin	48	-
	'B' starches	
Amylomaize	15-22	55-75
Edible canna	26	28
Potato	28	22
	'C' starches	
Sweet potato	38	20
Horse chestnut	37	25
Tapioca	38	18

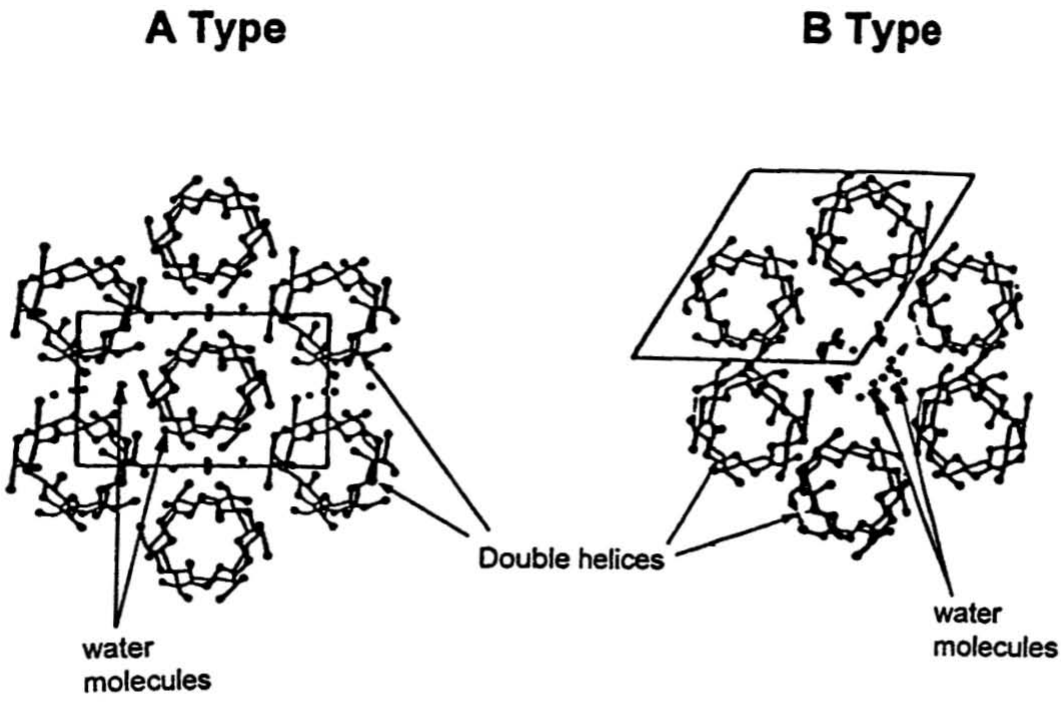
Adapted from Zobel (1988b).

(1988a) reported that 'B' type is more likely to result from the presence of the amylose extender (*ae*) gene that causes amylopectins to have longer side chains. A waxy genotype (*aewx*), with no amylose has been shown to give a 'B' pattern rather than the 'A' pattern of normal waxy starch. However, 'C' starches do not show a relationship between crystallinity and amylose content (Table 2.6) [Zobel, 1988b].

2.4.5 Crystal structure of 'A' and 'B' starches

The detailed structure of 'A' and 'B' unit cells was derived through the collective use of electron diffraction of single crystals, X-ray powder patterns, X-ray fibre diffraction data (from crystalline amylose) and extensive molecular modeling (French, 1984; Imberty *et al.*, 1988; Imberty & Perez, 1996). Kainuma & French (1972) were the first to suggest that the crystalline orientation of starch was due to both parallel and anti parallel arrangement of double helices. This suggestion was further studied by Wu & Sarko (1978a,b) who postulated that both 'A' and 'B' polymorphs are right-handed, parallel-stranded double helices packed in anti parallel manner. The unit cells of 'A' and 'B' starches (Fig. 2.16) were orthogonal and hexagonal, respectively. However, 'A' and 'B' types differ depending on the water content, which is 8 and 36 molecules per unit cell, respectively. Unit cell of 'A' starch has the dimensions of a = 1.19 nm, b = 1.77 nm and c = 1.05 nm. The main objection to this structure was that such anti parallel packing is incompatible with the cluster model of amylopectin.

Fig. 2.16 Double helix packing arrangement in 'A' and 'B' type unit cells (adapted from Wu & Sarko, 1978a; 1987b).


A Type
B Type
Double helices
water molecules
water molecules

A new model (Fig. 2.17a) of crystalline 'A' starch was proposed by Imberty and co-workers (1987; 1988). The new 'A' unit cell consists of chains which are crystallized in a monoclinic symmetry (a = 2.124 nm, b = 1.172 nm, c = 1.069 nm and γ=123.5°) [Fig. 2.17a]. This unit cell has a maltotriose residue as the asymmetric unit, and within the maltotriose residue, all the glucosyl residues are nearly identical. The density calculated for the crystalline region (d = 1.48) was reasonably close to the observed density of the fibre (d = 1.51), and indicates that there are 12 glucose residues and 4 water molecules per unit cell. The chain structure is left-handed parallel stranded sixfold double helices packed parallel in the crystalline lattice (Imberty & Perez, 1996). Each strand repeats in 2.138 nm, but is related to the other strand by a two-fold rotation axis, yielding the apparent fibre repeat distance of 1.069 nm.

There are no intrachain hydrogen bonds, but there is an O-2....O-6 hydrogen bond between the two strands that contributes about 40% of the stability of the double helix. The remaining energy of stabilization comes from van der Waals' forces. The double helix is very compact and there is no room for water or any other molecule in its centre (Imberty & Perez, 1996).

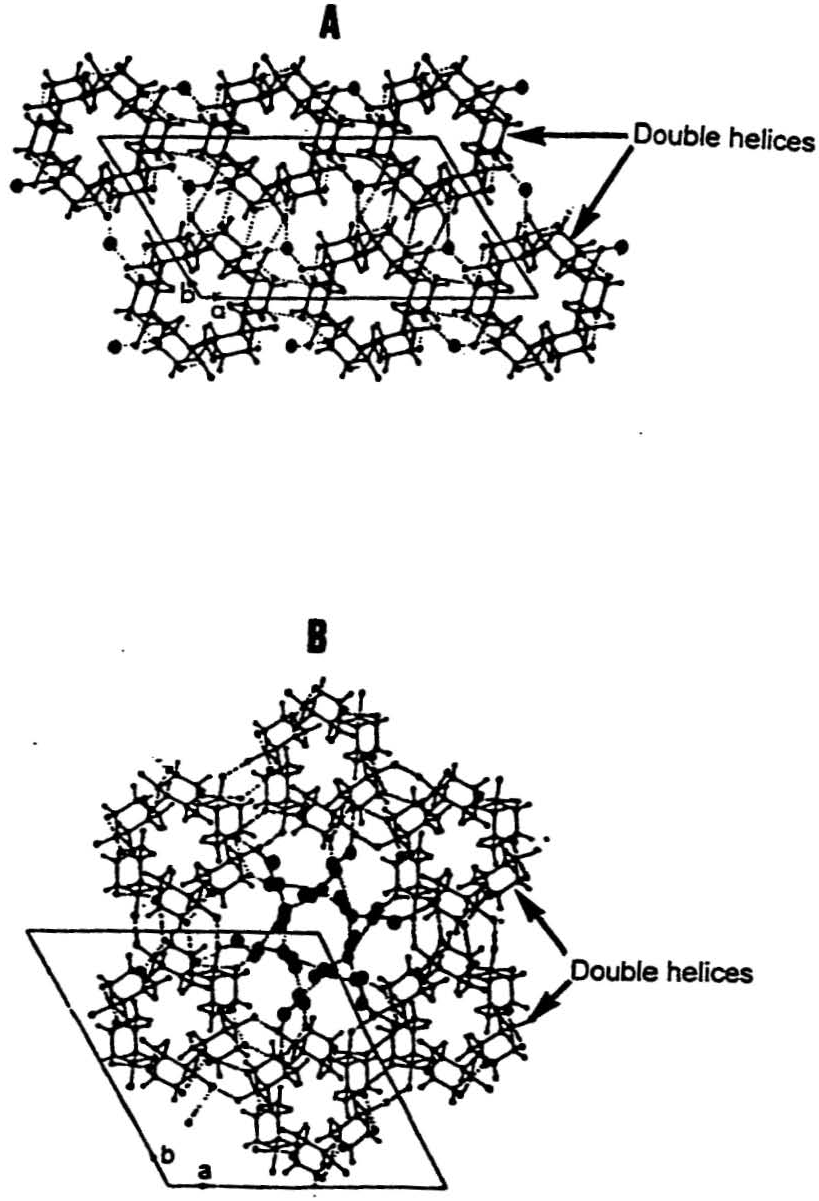
The double helical packing and lattice parameters (Imberty & Perez, 1988) of the currently accepted 'B' type unit cell (Fig. 2.17b) are in agreement with Wu & Sarko (1978a) [Fig. 2.16b]. The double helices are left handed, parallel stranded and connected through a network of hydrogen bonds that leaves a channel in the centre of the hexagonal arrangement of six double

Fig. 2.17 Structure of 'A' and 'B' unit cells.

A) structure of 'A' unit cell. For each unit cell 4 water molecules (•) are located between the helices (with permission, Imberty *et al.*, 1988).

B) structure of 'B' unit cell. Thirty six water molecules (•) represent 27% of hydration (with permission, Imberty & Perez, 1988).

Hydrogen bonds are shown as broken lines.

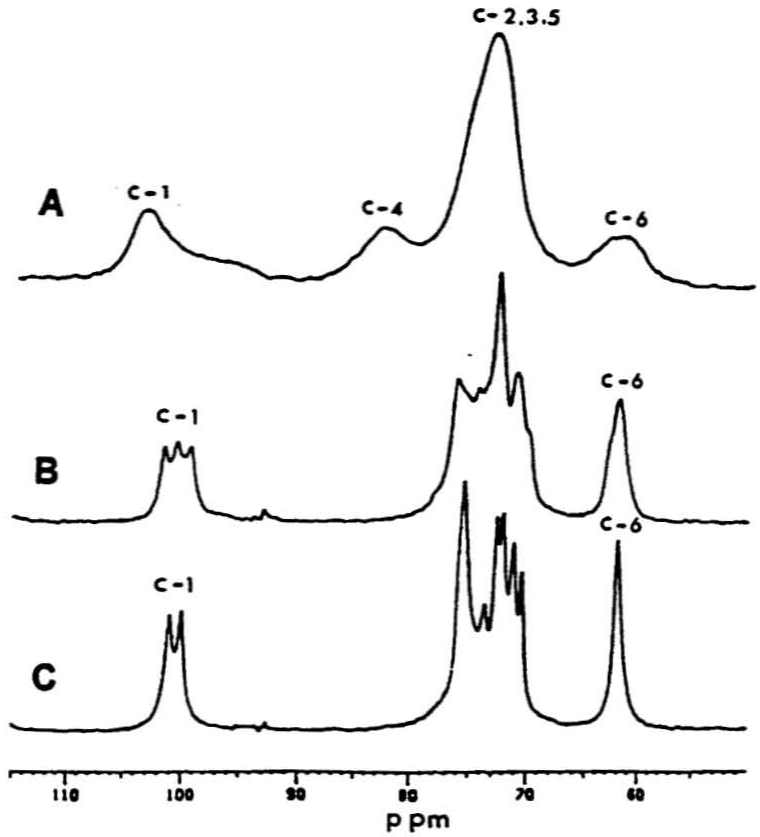

A
Double helices
b
a
B
Double helices
b
a

helices. This unit cell (a = b = 1.85 nm and c = 1.04 nm) [Fig. 2.17b] has more open packing of double helices, an asymmetric unit with a maltose residue and 36 water molecules (6 per maltose unit) at 27% (w/w) hydration. The water molecules are located in fixed positions within this channel. Half of the water molecules are hydrogen bonded to the amylose chains and the other half to other water molecules (Imberty & Perez, 1988; Imberty & Perez, 1996). There is no sign of disorder of these water molecules, agreeing with an NMR study which indicates that "freezable" water can be observed only when the hydration is above 33% (Imberty & Perez, 1996). The calculated and experimentally determined densities of the unit cell were 1.41 and 1.45, respectively.

In both 'A' and 'B' polymorphs, there is a pairing of double helices that corresponds to a 1.1 nm distance between axes of two double helices. The dense association of this type, which is strengthened by O2...O6 and O4...O3 hydrogen bonding, corresponds to the most energetically favoured interactions between two double helices (Perez *et al.* 1990).

Evidence is also provided by solid state ^{13}C NMR to confirm the asymmetry assignments for 'A' (Fig. 2.18a) and 'B' types (Fig. 2.18b) [Gidley & Bociek, 1988]. The C-1 signal in 'A' starch spectra (Fig. 2.18a) gives a triplet (~99.3, 100.4 and 101.5 ppm) representing 3 residues in maltotriosyl unit, whereas in 'B' starch spectra (Fig. 2.18b), C-1 produces a doublet having shifts at ~ 100.9 and 100.0 ppm representing the 2 residues in maltosyl unit.

Fig. 2.18 ^{13}C CP/MAS NMR spectra of (A) amorphous starch, (B) crystalline 'A' type and (C) crystalline 'B' type starches (adapted from Gidley & Bociek, 1988).


C-2,3,5
A
C-1
C-4
C-6
B
C-1
C-6
C
C-1
C-6
110
100
90
80
70
60
P pm

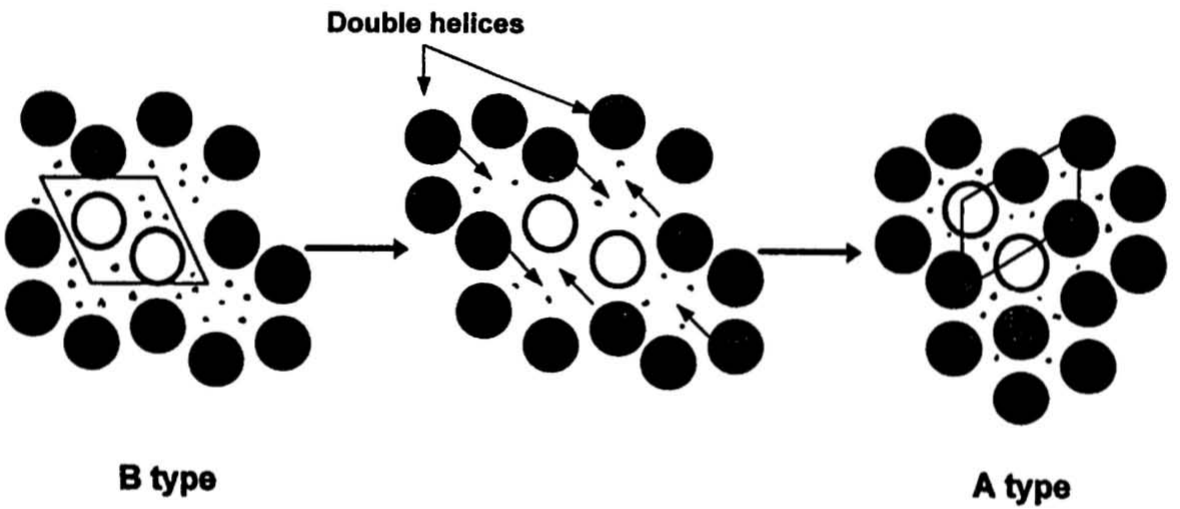
As described before, in the 'A' unit cell (Fig. 2.17a), the centre is filled with a double helix, and the adjacent double helices in crystallites are mainly bonded through hydrogen bonding. In the B structure (Fig. 2.17b) the central open space is filled with water molecules and the double helices are linked by hydrate water bridges. Thus, the 'B' to 'A' transition (Fig. 2.19) can take place by shifting of helices following removal of water (Zobel, 1988a; Imberty *et al.*, 1991). Under high temperature and low humidity, 'B' starch may irreversibly be converted to 'A' starch while remaining in the solid state as fibres or granules. The 'A' to 'B' transition is energetically less favourable, therefore, only after 'A' is melted to an amorphous state, crystallization to 'B' type is possible (Zobel, 1988a).

The factors affecting the crystalline type of native starches have been examined. Sair (1967) reported that crystallization of starches into 'A', 'B' and 'C' patterns depends mainly on the temperature and water content. Hizukuri (1969) showed that the 'A' type appears in relatively warm (30°C) conditions and the 'B' type in cold (13°C) conditions. Using linear maltooligosaccharides as model compounds. Gidley (1987, 1992) showed that the crystallization of an 'A' type polymorph over 'B' type was favoured, under conditions of shorter chain length, higher temperature, higher concentrations, and presence of salts and water-soluble alcohols.

The effect of chain length on the polymorphic form may be rationalized from entropy considerations since with longer chains the entropy changes on

Fig. 2.19 The transition from 'B' starch to 'A' starch.

Model of the polymorphic transition from 'B' to 'A' starch. The water molecules are shown as dots (.) (adapted from Imberty *et al.*, 1991).


Double helices
B type
A type

crystallization will become larger and favour the polymorph of highest entropy; i.e. the 'B' type. Hizukuri *et al.* (1983) and Hizukuri (1986) have also shown that the average chain length of amylopectin is the major determinant of crystalline polymorphism. Based on a study with a series of short chain amyloses of uniform length, Pfannemuller (1987) showed that the degree of crystallinity and the formation of 'A' and 'B' type amylose is largely dependent on chain length. The most remarkable observation of this study was an abrupt change from 'B' to 'A' pattern in going from DP 13 to DP 12.

2.4.6 Amorphous region of the granule

The amorphous region has received scant attention, though it accounts for ~ 70% of the granule (Oostergetel & Van Bruggen, 1993). It has been shown that the amorphous regions are less dense, thus more susceptible to chemical and enzyme attack (Biliaderis, 1982). Absorption of cold water by amorphous regions allows the limited reversible swelling of starch granules (French, 1984). Diffusion of small water soluble molecules (< 1000 dalton) into the granule occurs through the amorphous region. Gidley & Bociek (1988) reported that ^{13}C CP/NMR spectra of amorphous starch (Fig. 2.18c) showed substantial differences compared to crystalline starch (Fig. 2.18a,b). Conformational differences between amorphous and crystalline regions were shown by chemical displacements, especially at the C-1 and C-4 sites. In C-1 region, peak intensity in amorphous region (Fig. 2.18c), was shifted to a low field compared to

crystalline region (Fig. 2.18a,b), and the amorphous spectrum (Fig. 2.18c) showed a peak at 81-83 ppm, which was absent in crystalline material. The signals at 81-83 ppm (C-1), 94-98 ppm and 102-105 ppm (C-4) were assigned to amorphous sites (Fig. 2.18c).

The arrangement of amylose and amylopectin within the amorphous regions has been the subject of much controversy. Blanshard (1987) and Zobel (1988a) reported that in corn and wheat starches amylose is separated from amylopectin, whereas in potato starch, part of the amylose seems to be co-crystallized with amylopectin. Light cross linking and characterization of products by molecular sieve chromatography (Jane *et al.*, 1992; Kasemsuwan & Jane, 1994) showed that in potato and corn starches, amylose was cross linked with amylopectin, but no cross linking occurred between amylose molecules. This observation suggested that amylose molecules in the amorphous regions are interspersed among amylopectin, but do not exist in the form of bundles. Thus, it is likely that some amylose may form double helices with amylopectin and become less prone to complex formation with iodine or leaching in the presence of warm water. Vasanthan & Hoover (1992a) suggested that the extent to which the starch components are associated with each other within the native granule may also depend on their respective average CL. Long amylose chains may facilitate easier association with the short chain (DP 20-25) amylopectin molecule. The above authors postulated that in potato starch, the degree of

association between starch components may be higher than in other starches due to its long amylose CL (Table 2.4).

2.5 Starch properties

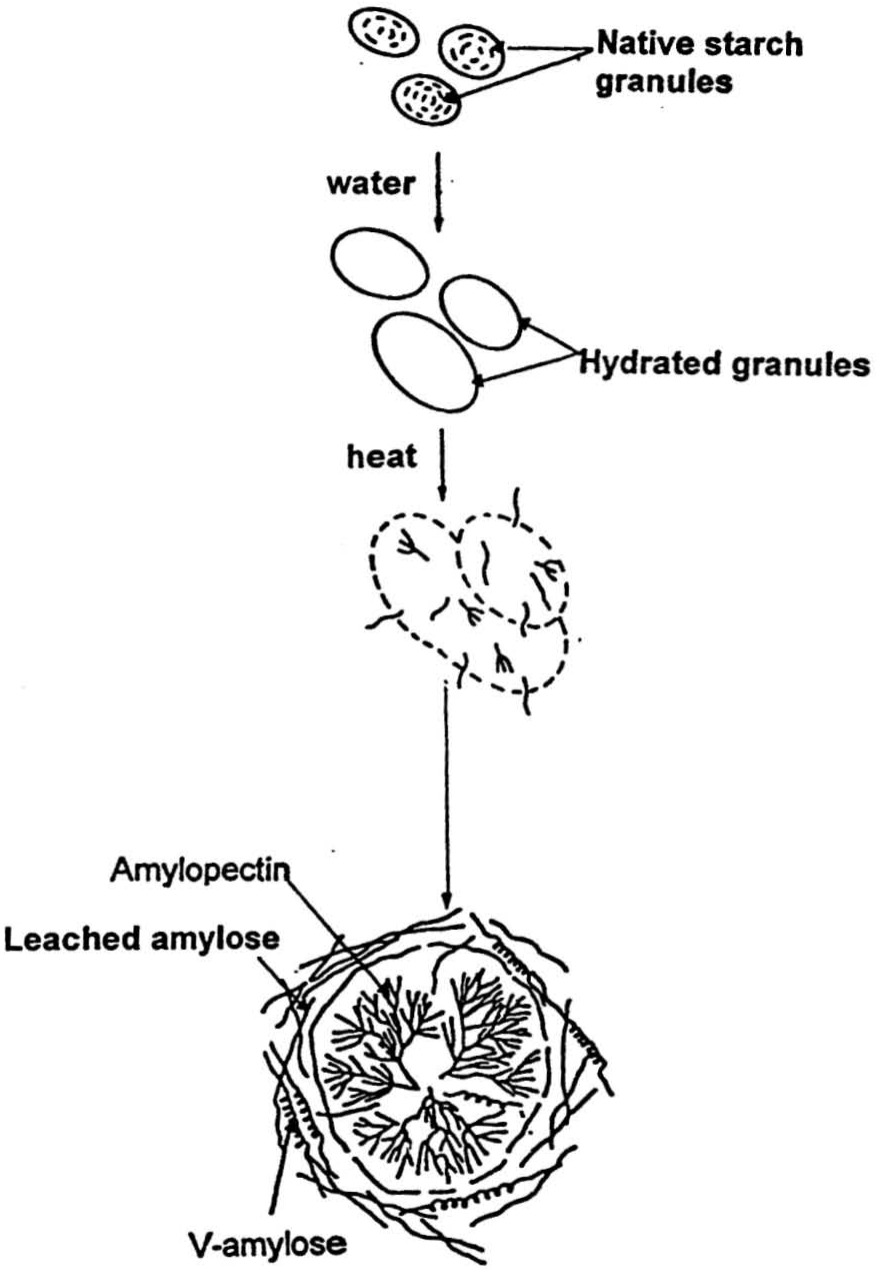
2.5.1 Gelatinization

Native starches are generally insoluble in cold water, but granules undergo slight reversible swelling (10-20%) due to absorption and diffusion of water into the amorphous regions (Biliaderis, 1991). On heating, starch granules in an aqueous starch suspension undergo an order-disorder phase transformation termed gelatinization (Fig. 2.20) [Donovan, 1979]. This phase transition is associated with a significant uptake of water which results in irreversible granular swelling, loss of birefringence, loss of crystalline order, leaching of amylose into the solution and increase in viscosity (Donovan, 1979; Hoover & Hadziyev, 1981). An individual granule gelatinizes over a narrow temperature range of 0.5-1.5°C, whereas a population of starch granules gelatinizes over an approximate range of 10°C (Gough & Pybus, 1971).

In studying the gelatinization phenomenon, many researchers (Biliaderis *et al.*, 1986b; Russell, 1987) have applied the Flory-Huggins (Flory, 1953) equation to relate melting of starch crystallites to the amount of water, assuming that starch-water system is homogenous and gelatinization occurs under equilibrium conditions. This theory describes the depression of the true melting point of a polymer (T_m^o) to the melting point of the polymer diluent mixture (T_m) as a function of the volume fraction of the diluent.

$$1/T_m - 1/T_m^o = (R/\Delta H_u)\,(V_u / V_1)\,(v_1 - \chi_1 v_1^2) \quad \text{--------} \quad \text{Flory-Huggins equation}$$

Fig. 2.20 Schematic presentation of the changes which occur in the starch granule during gelatinization (adapted from Aguilera & Stanley, 1990; Biliaderis, 1991).


Native starch granules
water
Hydrated granules
heat
Amylopectin
Leached amylose
V-amylose
Gelatinized granules

ΔH_u = change in enthalpy of fusion per repeating unit (glucose)

V_u / V_1 = ratio of the molar volume of the repeating unit (glucose) in the chain to that of the diluent (water)

R = gas constant

T_m = melting point of the diluent-polymer mixture

T_m^o = true melting point of the undiluted polymer

v_1 = volume fraction of the diluent [volume of water/(volume of water + volume of starch)]

χ_1 = polymer solvent interaction parameter

Since the Flory-Huggins theory is applicable only to equilibrium crystals, its application to phase transition of starch water system has only a limited success. However, Lelievre (1976) reported that equilibrium conditions in heated aqueous starch systems can be approached using very slow heating rates (~1°C /3h). Evans & Haisman (1982) reported that the starch-water system is not homogenous, due to the fact that it consists of individual granules suspended in a variable amount of liquid phase. Once these starch granules which are in osmotic equilibrium with external phase are fully swollen, further addition of the liquid phase will not affect granule composition. Therefore, volume fractions for the Flory-Huggins equation should be based on granule composition rather than the composition of the entire system. Whittam *et al.* (1991) suggested that even with highly crystalline preparations of A- or B- type starch crystals (DP ~15), the

estimates of T_m° obtained for starch crystallites using Flory-Huggins approach may be somewhat low.

The amorphous regions and the crystallites are not independent of each other, but are interconnected in the starch granule. The amorphous regions are in a glassy state and their transition temperature (the glass transition temperature: T_g) is higher than the melting temperature of the crystallites in native starch granule. However, the amorphous regions must first undergo a transition from a glassy state to a rubber-like state before the crystallites can melt, i.e. the crystallites are kinetically stabilized in the native starch granule by the amorphous region. The amorphous regions are always hydrated first (van den Berg, 1986) and the water acts as a plasticizer and depresses the T_g below the melting temperature of crystallites. These are now less kinetically constrained, and melt at temperature slightly higher than the T_g. The glass transition always precedes gelatinization and determines the start of the gelatinization process (Slade & Levin, 1987; Slade & Levin, 1988). Since water has to penetrate the starch granule from the outside, the amorphous regions are only partially hydrated at the beginning of gelatinization and the 'effective T_g' (which determines the start of gelatinization) of each type of starch is therefore, more or less the same (Biliaderis, 1990).

Several analytical techniques including viscometry, X-ray diffraction, DSC, light and electron microscopy and NMR have been employed to understand the mechanism of starch gelatinization. Because gelatinization is an endothermic

process, DSC has been widely used to study phase transitions of aqueous starch suspensions. DSC can provide the characteristic temperatures and enthalpies of the various transitions as well as allowing measurements over a wide range of starch concentrations.

At relatively high moisture levels (volume fraction of water > 0.7), DSC thermograms of starches show a single endotherm at about 60°C (Fig. 2.21a). The position of the peak depends on the starch variety being investigated (Biliaderis *et al.*, 1986b). As the water content is reduced (at intermediate moisture levels, volume fraction ~ 0.6), two endothermic transitions become evident (Fig. 2.21b) [Donovan, 1979; Hoseney *et al.*, 1986; Blanshard, 1987]. Donovan (1979) designated the initial peak and the second peak as G and M, respectively. The G endotherm occurs at the same temperature as before. The temperature of the second endotherm (M) increases as the water content decreases. If the volume fraction (ν) of water is decreased still further ($\nu < 0.45$), the lower temperature endotherm (G) disappears (Fig. 2.21c), while the temperature of the second peak continues to rise (Fig. 2.20d) [Donovan, 1979]. In addition to the above endotherms, transitions due to lipid- amylose complexes are found at about 120°C (Biliaderis *et al.*, 1986ac; Biliaders & Seneviratne, 1990). The exact temperature depends on the moisture content.

Donovan (1979) suggested that the single peak (G endotherm) at excess moisture content (Fig. 2.21a) results from the 'stripping' or unfolding of polymer chains from the surfaces of crystallites due to stress developed by hydration and

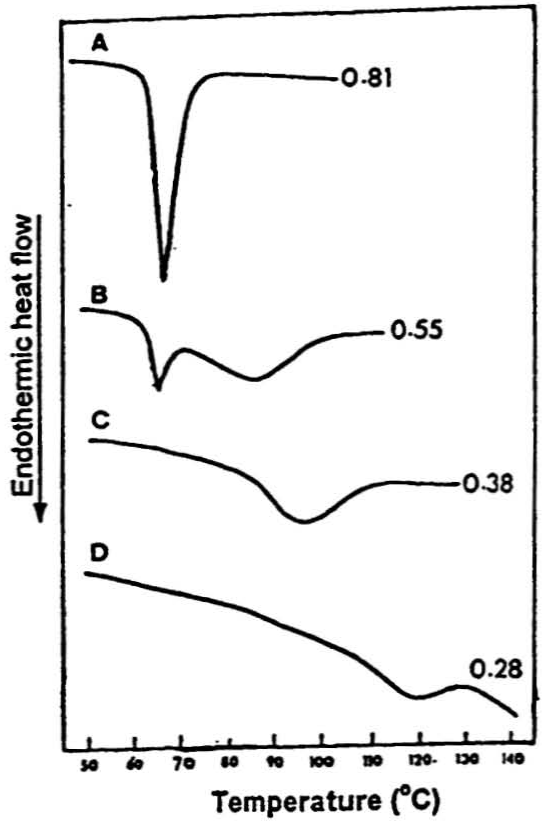
Fig. 2.21 DSC melting profiles of potato starch at various volume fractions of water (adapted from Donovan, 1979).

A) 0.81

B) 0.55

C) 0.38

D) 0.28


A
0·81
B
0·55
C
0·38
D
0·28
Endothermic heat flow
50
60
70
80
90
100
110
120
130
140
Temperature (°C)

swelling of amorphous regions, while the second peak represents melting at low diluent volume fractions. Since Donovan (1979) considered different mechanisms to be responsible for each section of the biphasic endotherm, the peaks were designated as G and M, respectively. However, Zobel *et al.* (1988) have postulated that the X- ray data do not indicate that different molecular processes are responsible for each peak.

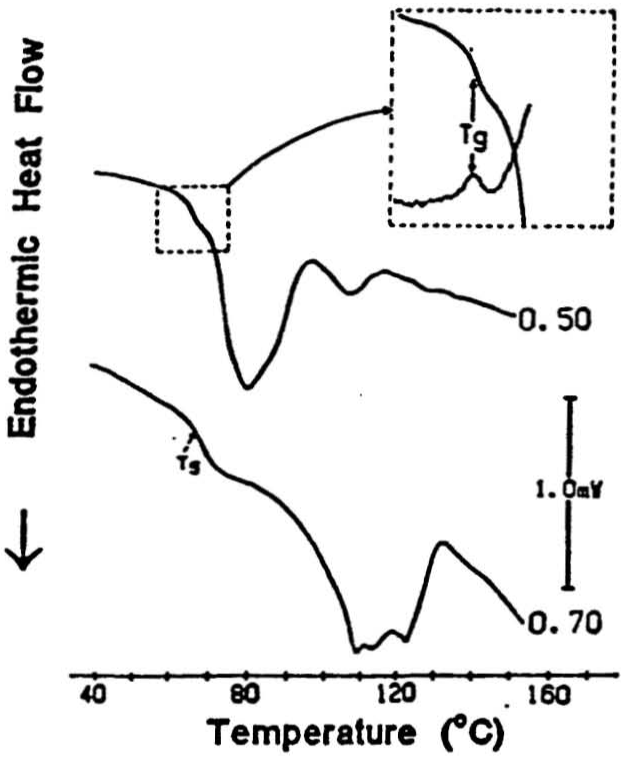
Evans & Haisman (1982) explained the appearance of the biphasic endotherm based on water migration. Water migrates within the sample from one location to another, and the peaks represent the order-disorder transitions occurring at different diluent levels. Starch granules can take up only a limited amount of water and the additional water added to the system forms a separate phase. The crystallites in granules are of a range of stabilities and the least stable crystallites melt first when heat is applied. When crystallites melt, the deformed polysaccharide chains absorb more water from the separate phase because it is slightly more flexible. Then, the additional water will lower the stability of the remaining crystallites, thereby the crystallites will melt at a higher temperature. This theory was supported by Liu *et al.* (1991) who reported that water gradient within the sample is responsible for the biphasic transition and the crystallinity is lost when gelatinization proceeds. The theory based on the migration of water is also in agreement with the study conducted by Zobel *et al.* (1988), which shows the occurrence of X-ray intensity changes corresponding to the development of first and the second peaks, based on the

melting of crystallites with different stabilities. Russell (1987) ascribed the biphasic endotherm to disruption of double helices associated with short-range order involving amylose and amylopectin followed by melting of crystallites. Biliaderis (1990) suggested that a process of partial melting followed by recrystallization and final melting is the reason for biphasic thermal profiles. Recently, Svensson & Eliasson (1995) attributed the biphasic endotherm to a slow plasticization of the amorphous granular regions under restricted water conditions which forces the melting of crystallites to higher temperatures.

Several workers have postulated that the enthalpy of gelatinization isrelated to the disruption or melting of organized structures (Donovan, 1979; Zobel *et al.*, 1988; Whittam *et al.*, 1990; Gidley, 1992). However, Cooke & Gidley (1992) using ^{13}C MAS NMR, showed that double helix melting rather than loss of crystallinity, could be primarily responsible for gelatinization enthalpy.

Some researchers have attempted to explain the development of biphasic endotherm based on the glass transition which defines the temperature region in which the chain motion of a polymer commences. This theory suggests that the initial (G) peak is a result of chain mobilization in the amorphous regions of the starch granule. With the increase in mobility, the polymer chains are transferred from a glassy to a rubbery aqueous gel (Biliaderis *et al.*, 1986b; Yost & Hoseney, 1986). Several investigators (Biliaderis, 1991; Zeleznak & Hoseney, 1987) suggested that glass transition is located at the leading edge of the first peak (Fig. 2.22) and it is associated with a change in heat capacity. The completion of

Fig. 2.22 Thermal profiles of rice starch (at volume fractions of water 0.50 and 0.70) showing both glass transition (Tg = glass transition temperature) and melting transition (with permission, Biliaderis, 1986b).


Endothermic Heat Flow
Tg
0.50
Tg
1.0mW
0.70
40
80
120
160
Temperature (°C)

glass transition allows the crystalline domains to undergo a non equilibrium melting process giving the second endothermic peak (Slade & Levine, 1988). However, some studies have provided data contrary to this theory, showing that heat capacity changes occur throughout the temperature range of both endotherms (Lelievre, 1992). Liu *et al.* (1991) demonstrated, using X-ray diffraction studies, that the heat capacity change is not related to glass transition due to two reasons; firstly, the X-ray data do not show that a significant endothermic transition occurs without a corresponding change in crystallinity, secondly, the X-ray data suggest that the volume expansion measured by other investigators (Biliaderis *et al.*, 1986b) using thermomechanical analysis are attributable to the increase in the quantity of amorphous polymer with the temperature rather than to a glass transition followed by melting.

A study (Jang & Pyun, 1996) of wheat starch in the presence of limited water (40, 50% moisture) showed the appearance of four endotherms (Fig. 2.23a) : G (water mediated melting of starch crystallites), M_1 (melting of the remaining crystallites or amylopectin crystallites), M_2 (melting of amylose-lipid complex) and M_3 (melting of amylose). These results were in agreement with the study conducted by Donovan & Mapes (1980). For moisture contents less than 30%, (Fig. 2.23b) the G and M_1 endotherms shifted to a higher temperature and at moisture contents below 20%, G and M_1 coalesced into a single band ($G+M_1$) and M_2 and M_3 coalesced into a single band [M_2+M_3] (Jang & Pyun, 1996). Biliaderis *et al.* (1986a) and Biliaderis & Galloway (1989) attributed the M_2 and

Fig. 2.23 DSC thermograms of wheat starch at various water contents (adapted from Jang & Pyun, 1996).

A) DSC profiles of wheat starch at water contents varying from 30-90%.

B) DSC profiles of wheat starch at water contents varying from 2.8-25%.

G & M_1: melting of amylopectin crystallites at intermediate moisture content.

M_2 : melting of amylose - lipid complex

M_3 : melting amylose

M_3 to melting of amylose - lipid complexes (differ in the degree of helical chain organization), since waxy or defatted starches do not exhibit the M_2 or M_3 transitions (Donovan *et al.*, 1983).

2.5.1 Factors influencing Gelatinization

It has been shown that melting parameters (transition temperatures and enthalpy) are influenced by factors such as botanical source (granule structure), moisture content, heating rate, starch modification, lipids, sugars, mechanical damage, etc.

2.5.1.1 Botanical source

Crystal size, perfection of ordered chains and the amorphous domains contribute to the thermal stability of granular starch. Granular 'A' starches usually exhibit lower melting temperatures than 'B' starches (Table 2.7) [Whittam *et al.*, 1990]. However, highly crystalline 'A' and 'B' spherulites obtained from potato starch lintners (DP ~ 15) showed similar melting enthalpy (35 J/g) and a difference of 15-20°C in a fixed water content (Whittam *et al.*, 1990). In contrast to native starch crystals, 'A' crystals of lintners showed a higher melting temperature than 'B' type crystals (Whittam *et al.*, 1990). Variations in melting parameters (Table 2.7) have been observed among different genotypes of rice, barley, corn and legume starches (Hoover & Sosulski, 1985; Morrison *et al.*, 1993a; Yuan *et al.*,1993,). Yuan *et al.* (1993) studied the gelatinization

M_3 to melting of amylose - lipid complexes (differ in the degree of helical chain organization), since waxy or defatted starches do not exhibit the M_2 or M_3 transitions (Donovan *et al.*, 1983).

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Table 2.7 Gelatinization parameters (at excess water) of some cereal tuber and legume starches

Starch	X-ray diffraction pattern	Gelatinization peak temperature	Enthalpy of gelatinization (J/g)	Source
Oat (Svea)	A	58	10.6	Gudmundsson & Eliasson, 1989
Oat (Chichauaua)	A	62	10.1	Gudmundsson & Eliasson, 1989
Rye	A	58	11.9	Gudmundsson & Eliasson, 1991
Wheat	A	58	9.7	Gidley, 1992
Maize	A	58	14.3	Cooke & Gidley, 1992
Waxy maize	A	72	16.0	Cooke & Gidley, 1992
Waxy rice (RD6)	A	64	13.4	Tester & Morrison, 1990b
Waxy rice (IR29)	A	66	14.2	Tester & Morrison, 1990b
Amylomaize	B	82	10.0	Gudmundsson & Eliasson, 1989
Edible canna	B	70	-	Zobel 1988b
Potato	B	58	16.2	Cooke & Gidley, 1992
Kidney beans	C	70	15.1	Hoover & Sosulski, 1985
Navy beans	C	68	13.4	Hoover & Sosulski, 1985
Black beans	C	66	12.6	Hoover & Sosulski, 1985
Smooth pea	C	64	14.7	Biliaderis *et al.*, 1980
Smooth pea	C	61	13.4	Colonna *et al.*, 1987
Lentil	C	57	14.2	Biliaderis *et al.*, 1980
Sweet potato	C	70	-	Zobel 1988b
Tapioca	C	66	-	Zobel 1988b

parameters of three genotypes (wx, aewx, duwx) of waxy corn starch and reported that the aewx (T_p = 79°C) had the highest melting temperature and ΔH (4.1 Cal/g) among the three genotypes (for *wx*, T_p = 66.9°C, ΔH =3.2 Cal/g; for duwx, T_p = 70°C, ΔH =3.2 Cal/g). The higher melting temperature of aewx was attributed to aewx having amylopectin with longer chains, which could account for the 'B' type crystallites.

2.5.1.2 Heating rate

Calorimetric studies of Shiotsubo & Takahashi (1984) has shown that the peak temperature of gelatinization endotherm increases with increasing heating rate. The peak temperature remained constant for heating rates below 0.5°C / min. Biliaderis *et al.* (1986b) reported that the biphasic endotherm observed at 3-20°C/min merged into a single endotherm at increased heating rates. Since reorganization process is time-limited, the melting probably occurs as a single stage process at high heating rates (Biliaderis *et al.*, 1986b).

2.5.1.3 Lipids

The effect of lipids on gelatinization has been discussed in section 2.4.2.2.4.

2.5.1.4 Sugars

In general, sugars hinder granule swelling and increase gelatinization temperatures (Evans & Haisman, 1982; Eliasson, 1992; Bello-Perez & Paredes-Lopez, 1995). Sucrose hindered gelatinization and increased melting temperature from 57 to 92°C at concentrations of 55-60% (Kim & Walker ,1992). Scanning electron micrographs showed that the granules isolated from baked products with high sucrose contents were less deformed compared to those obtained from baked products with low sucrose contents (Hoseney *et al.*, 1978). However, unaffected and increased gelatinization enthalpies in the presence of sugars have also been reported by Evans & Haisman (1982) and Eliasson (1992), respectively. Several theories have been presented for the influence of sugars on retarding gelatinization, which include the competition between starch and sugar for water (D'Appolonia, 1972) and sugar-starch interactions (Lelievre, 1976).

2.5.1.5 Starch modification

Physical or chemical modification of starch by changing the molecular order while maintaining the granular form is applied in production (atomization and heating in aqueous monohydric alcohol solutions) of granular cold-water soluble starch which gelatinize in warm water (Jane *et al.*, 1986). Heat-moisture treatment, annealing, defatting, acetylation, hydroxypropylation, cross linking etc. have been shown to alter gelatinization parameters of starches (Wootton &

Bamunuarachchi, 1979; Hoover & Sosulski, 1985,1986; Hoover & Vasanthan, 1994a; Hoover & Manuel, 1996a,b). Effect of physical and chemical modification on gelatinization parameters will be discussed in section 2.6 in detail.

2.5.2 Retrogradation

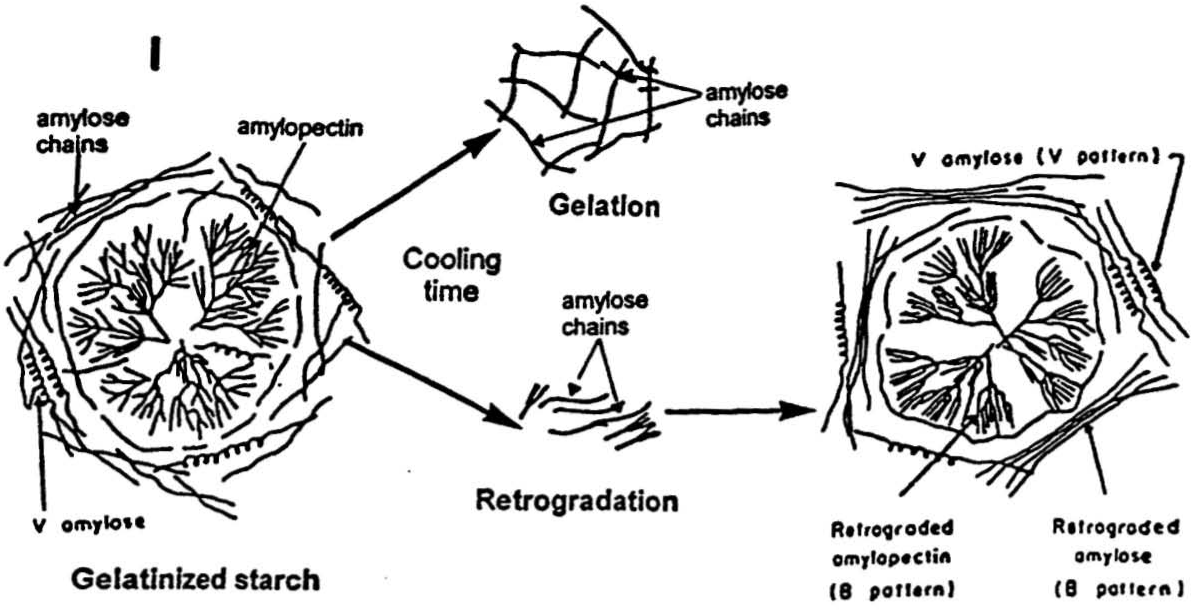
Starch granules heated in excess water undergo an order-disorder phase transition called gelatinization over a temperature range characteristic of the starch source. This phase transition is a non equilibrium process associated with the diffusion of water into the granule, hydration and swelling of starch granules, uptake of heat, loss of crystallinity and amylose leaching (Donovan, 1979; Hoover & Hadizeyv, 1981; Biliaderis, 1990). On cooling, amylose and amylopectin chains in the gelatinized paste associate, forming a more ordered structure (Fig. 2.24-I). These molecular interactions are termed collectively "retrogradation" and have important textural and dietary implications. In order to understand the process of aging, the starch/water system has been extensively investigated using physical methods such as turbidity (Miles *et al.*, 1985a; Ring *et al.*, 1987; Jacobson *et al.*, 1997), DSC (McIver *et al.*, 1968; Longton & LeGrys, 1981; Russell, 1987), rheology (Miles *et al.*, 1985a,b; l'Anson, *et al.,* 1988), X-ray diffraction (l'Anson, *et al.,* 1988), microscopy (Jacobson *et al.*, 1997), FTIR (Wilson *et al.*, 1991; Van Soest *et al.*, 1995) and NMR spectroscopy (Wu & Eads, 1993) to measure different properties occuring during retrogradation. For example turbidity measures distribution of refractive index (hence density), DSC

Fig. 2.24 Mechanisms of starch retrogradation.

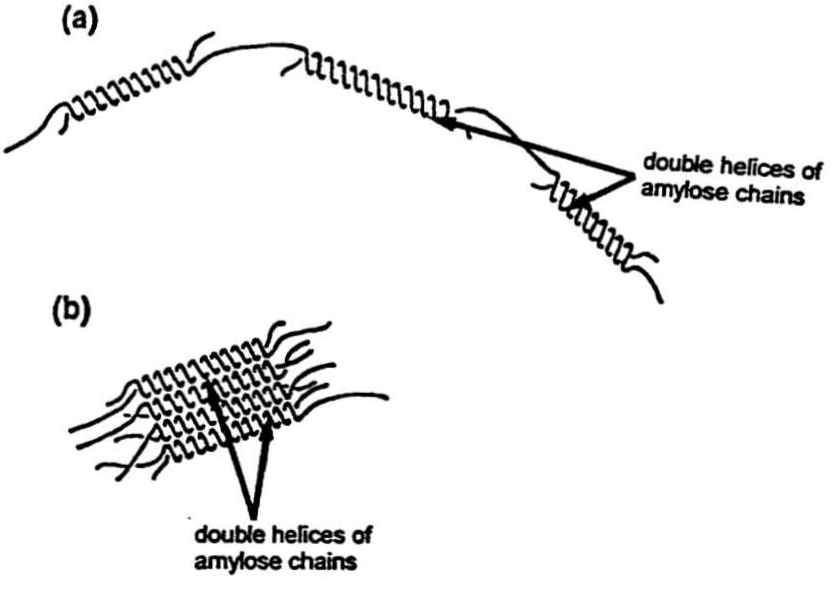
(I) Schematic illustration of changes during storage of gelatinized starch paste (adapted from Aguilera & Stanley ,1990; Biliaderis, 1991).

(II) double helix formation and association : (a) helix formation and chain elongation; (b) lateral association of helical regions (with permission, Morris, 1990),

I


amylose chains
amylopectin
V amylose
Gelatinized starch
Gelation
amylose chains
Cooling time
amylose chains
Retrogradation
V amylose (V pattern)
Retrograded amylopectin (B pattern)
Retrograded amylose (B pattern)

II


(a)
double helices of amylose chains
(b)
double helices of amylose chains

measures melting transitions, X-ray diffraction monitors long-range order in crystalline starch domains, microscopy is used to understand the spatial distribution of refractive mass and NMR monitors chain segmental motions, conformation dependent chemical shifts (resonance frequencies) and degree of crystallinity.

The molecular structures and transformations that occur during retrogradation of starch and its components have been subjected to several investigations. Despite the numerous investigations that have been carried out to study retrogradation, the exact mechanism of retrogradation, particularly at the molecular level still remains unclear. The rate and extent of retrogradation is influenced by many factors such as botanical source (Orford *et al.*, 1987; Jacobson *et al.*, 1997), chain length of amylose and amylopectin (Gidley & Bulpin, 1989; Clark *et al.*, 1989), water content in gel (Longton & LeGrys, 1981; Zeleznak & Hoseney, 1986), cooking and cooling conditions (Kim *et al.*, 1993), storage temperature (Jankowski & Rha, 1986; Jang & Pyun, 1997) and the presence of solutes such as sugars, lipids and salts (Russell & Oliver, 1989; Katsuta *et al.*, 1992a,b; Huang & White, 1993; Conde-Petit & Escher, 1994). This review summarizes the contribution of amylose and amylopectin to starch retrogradation.

2.5.2.1. Amylose Gelation

Amylose gelation is characterized by the formation of a permanent elastic network and the development of opacity (Miles *et al.,* 1985a). Amylose gelation occurs above its critical overlap or entanglement concentration [C* ~ 1.5%]; which defines the minimum concentration for gelation (Miles *et al.,* 1985a). Below C*, amylose precipitates forming insoluble crystals which melt at 150-160°C (Miles *et al.,* 1985a; Stute & Konieczny-Janda, 1983). However, amylose gelation was found to proceed even at a concentration below C*, (1%) regardless of the molecular weight (Dublier & Choplin, 1989). Amylose gelation is favoured by long CL (DP >1100), high concentrations and fast cooling rates, whereas precipitation of amylose is favoured by shorter CL (DP < 110), low concentration and slow cooling rates (Gidley & Bulpin, 1989). Both gelation and precipitation occur for CL of 250-660 residues (Gidley & Bulpin, 1989). The above authors postulated that gelation involves extensive cross linking (*via* hydrogen bonding and/or hydrophobic interactions) between long amylose chains, which leads to the formation of a macromolecular network. Thus, if the total chain length is greater than the length of the chain segments which participate in the above interactions, then cross linking would probably involve more than two regions within a single amylose chain. For intermediate chains (DP 250-660), both chain alignment and cross linking can occur. However, if the length of interacting chains are approximately of the same length as the total chain length, then chain alignment would occur in preference to cross linking.

Cross linking is favoured by higher concentrations (greater interchain contact) and rapid cooling (increases nucleation). Whereas slow cooling may lead to annealing of the structure favouring chain alignment (Gidley & Bulpin, 1989). Chain alignment followed by lateral association predominates in shorter chains (DP < 110). This leads to precipitation of amylose (Gidley & Bulpin, 1989).

Amylose gels are also characterized by the development of turbidity, which is caused by phase separation which forms polymer rich and polymer deficient regions (Miles *et al.*, 1985a). This process depends on polymer concentration, molecular size and cooling temperature. Gidley (1989) suggested that amylose gelation is due to interchain associations in the form of double helices, followed by aggregation of helices which act as junction zones. Morris (1990) postulated that in amylose gelation, double helix formation can occur between the ends of molecules favoring chain elongation (Fig. 2.24-IIa). Once the helices are formed, lateral association may occur through crystallization (Fig.2.24-IIb). Based on the results of stored amylose (21°C for 5 weeks), Muller *et al.* (1995) postulated that aged amylose gels may contain double helices, small aggregates of double helices (junction zones), crystallites and their aggregates.

Amylose gels are poorly crystalline structures, largely composed of amorphous regions (Miles *et al.*, 1985a). The development of crystallinity in polymer rich phase was shown to be a slow process (Miles *et al.*, 1985a) in which the rate was concentration independent. The overall level of crystallinity

was shown to be concentration dependent (Miles *et al.*, 1985a). Miles *et al.* (1985a) reported that the development of crystallinity is accompanied by stiffening of amylose gels. X-ray diffraction (Leloup *et al.*, 1991; Cairns *et al.*, 1995) and ^{13}C NMR (Colquhoun *et al.*, 1995) have shown that retrograded amylose is composed of 'B' type crystals. In the crystalline domains, amylose double helical structures aggregate into compact arrays (Imberty & Perez, 1988). Helical packing becomes more perfect for shorter chain lengths (Gidley ,1989). The crystalline regions of amylose gel are resistant, whereas the amorphous regions are easily degraded by acidic and amylolytic hydrolyse (Leloup *et al.*, 1991). The amount of the crystalline region in retrograded amylose can be as high as 65-83% of the gel (Leloup *et al.*, 1991; Cairns *et al.*, 1995).

Clark *et al.* (1989) reported that although all gelling amylose exhibit turbidity development, variations exist with respect to the time scale of modulus (which measures gel stiffness) and absorbance development as a function of chain length. For short chains (DP 250 & 300) the increase in turbidity precedes modulus development, suggesting that some non cross linking aggregation (precipitation) occurs. However, for longer chains (DP >1100) modulus increases before significant turbidity is apparent. This suggests that the processes which lead to gelation and turbidity in aqueous amylose systems are not directly related, although turbidity development was ascribed to helix - helix aggregation (Gidley, 1989).

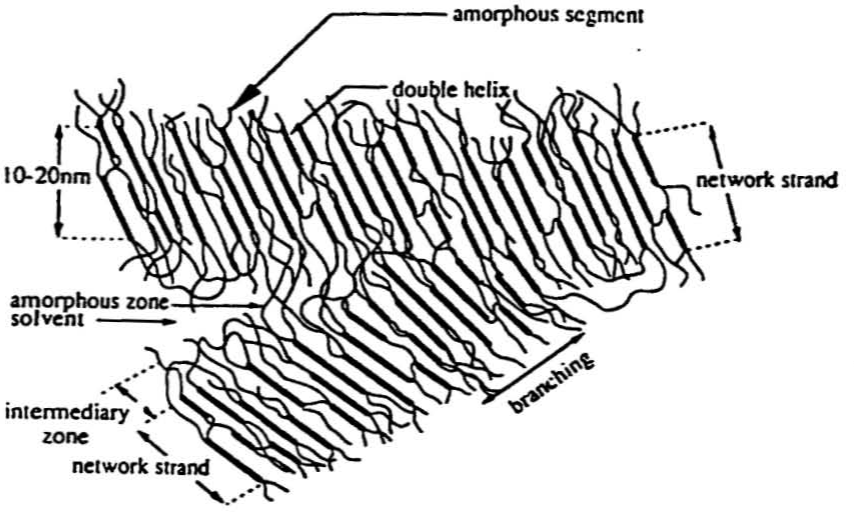
Leloup *et al.* (1992) investigated the structural characteristics of amylose gels (2-8%w/v) of smooth pea starch by electron microscopy, mild acid hydrolysis, DSC and size exclusion chromatography. The results showed a macroporous structure in gel (mesh size 100-1000 nm) with filaments 20 ± 10 nm wide (Fig. 2.25). The filaments were composed of association of amylose chains with DP_n 26-31 and DP_w 56-73. The double helices in the filaments linked to each other by loops of amorphous amylose segments, which are dangling in the gel pores. The aggregation of these filaments generates a three dimensional network consists of network strands, amorphous zone and intermediary zone (Fig. 2.25).

2.5.2.2. Amylopectin Gelation

Compared to amylose, amylopectin retrogradation is a very slow process. Amylopectin gels are turbid and elastic, and the gels form on cooling of substantially higher polysaccharide concentrations (> 10% w/w) to 2 °C (Ring *et al.*, 1987). The development of gel stiffness was attributed to associations involving crystallization process, which gives an X-ray pattern characteristic of the 'B' type (Ring *et al.*, 1987; Eerlingen *et al.*, 1994). Crystallization and the increase in stiffness in amylopectin gels can be reversed by heating to 100 °C (Miles *et al.*, 1985b; Durani & Donald, 1995).

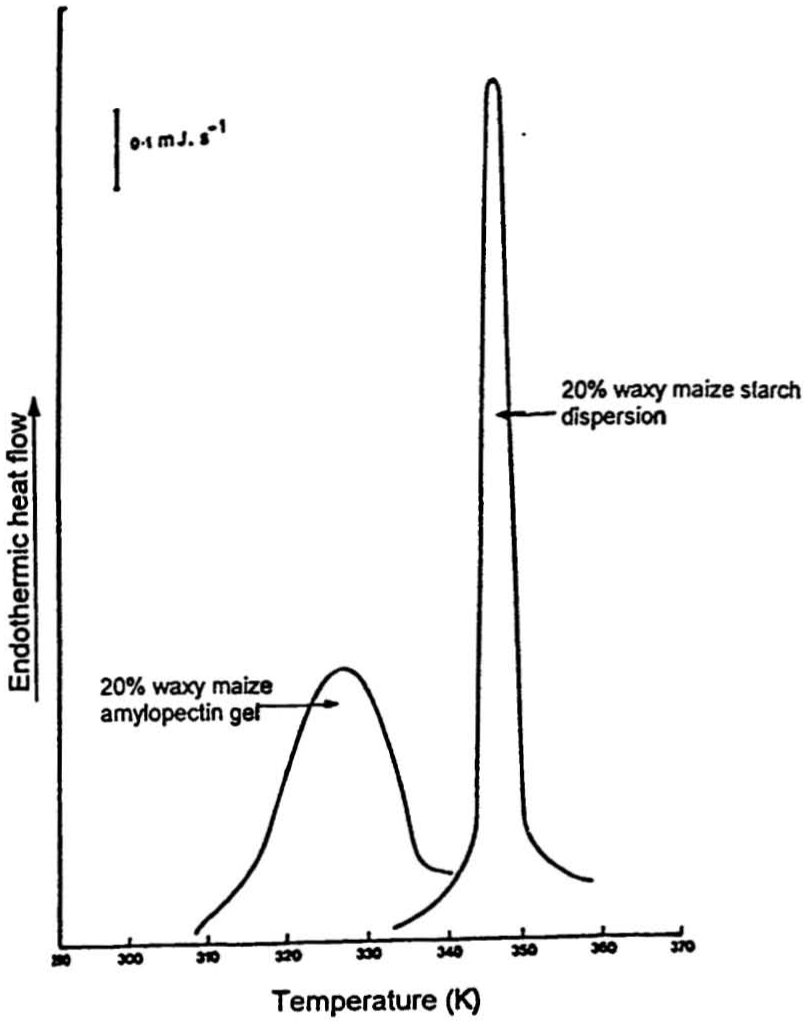
Gelling behaviour of amylopectin is influenced by the fine structure (botanical source) of amylopectin (Kalichevsky *et al.*, 1990). Amylopectins from

Fig. 2.25 Continuous model for amylose gel (with permission, Leloup *et al.*, 1992)


amorphous segment
double helix
10-20nm
network strand
amorphous zone
solvent
branching
intermediary
zone
network strand

potato, pea and canna were found to exhibit higher rates of retrogradation than those from wheat, barely and maize, presumably due to the shorter CL in cereal starches (Table 2.4) [Kalichevshy *et al.*, 1990]. Ward *et al.* (1994) demonstrated that corn amylopectin retrogrades faster than wheat amylopectin. This was attributed to the greater proportions of chains with DP 15-20 in corn amylopectin. The above authors postulated that the extent of retrogradation is increased by high molar proportion of unit chains with DP 14-24, and decreased by a low molar proportion of short chains with DP 6-9. This was in agreement with the finding of Ring (1987) and Wursh & Gumy (1994) who reported that retrogradation is hindered in the presence of short amylopectin chains with DP 11 or less. A study with starches from different rice cultivars showed that amylopectin gels from Japonica and low amylose Indica had higher retrogradation rates compared to amylopectin gels of waxy rice (Lu *et al.*, 1997). This was attributed to greater proportion (63-66%) of short chains (DP 10-15) in japonica and indica. Furthermore, waxy rice amylopectin also contained a greater proportion of very short chains (DP 6-9), which were shown to hinder retrogradation (Lu *et al.*, 1997). In a study with waxy maize amylopectin, Ring *et al.* (1987) observed a broad retrogradation endotherm for 20% amylopectin gel stored for 4 weeks at 1°C, and a sharp peak for gelatinization of waxy maize starch dispersion (Fig. 2.26). The mid point transition temperatures for gel and starch were 54 and 75°C, respectively. Both retrogradation (1.5 mJ/mg) and gelatinization (1.54 mJ/mg) transitions gave similar enthalpy values. Durani &

Fig. 2.26 DSC thermograms showing gelatinization of waxy starch and melting of 20% waxy-maize amylopectin gel stored for 4 weeks at 1°C (adapted from Ring *et al.*, 1987)


0·1 mJ. s⁻¹
20% waxy maize starch dispersion
20% waxy maize amylopectin gel
Endothermic heat flow
290
300
310
320
330
340
350
360
370
Temperature (K)

Donald (1995) reported that amylopectin gels (formed with amylopectins of different molecular weights) stored at 4°C melt over a broad range of temperatures. The melting enthalpies of gels made from higher molecular weight amylopectin were higher than those of their lower molecular weight counterparts.

FTIR spectroscopic studies revealed chain ordering at the very early stages of amylopectin gelation (complete within 0.2h for a 20% waxy maize gel) which was not detectable by other methods (Goodfellow & Wilson, 1990). The above authors attributed this chain ordering to coil to double helix transitions in short DP chains of amylopectin. This transition was followed by a slow aggregation of the helices to produce crystallites. Biliaderis & Zawistowski (1990) studied the storage modulus and melting enthalpy of aging waxy maize (amylopectin) gels (40%w/w). The results showed that the storage modulus reached a limiting value after 36 h at 6°C, but ΔH continued to increase over a long time scale. Based on the results, the above authors postulated that once the gel network is developed, subsequent ordering and crystallization of amylopectin chains do not contribute significantly to gel rigidity. Similar time dependence was reported for a 20% amylopectin solution stored at 1°C by Ring *et al.* (1987) who observed that the increase in turbidity values stopped after 4-5 days, whereas DSC and X-ray diffraction data continued changing over a long period of time (30-40 days). The above authors suggested that increase in turbidity reflected the aggregation of amylopectin chains prior to gelation and the long term gel network development (as measured by DSC and X-ray diffraction)

showed the ordering and crystallization of short (DP 10-20) amylopectin chains. Studies on aging waxy maize (20,40%w/w) starch gels using ^{1}H NMR (Wu & Eads, 1993) showed that the polymer chains in starch gels exhibit three types of mobile fractions : 1) a highly mobile chain fraction, 2) highly rigid (the motion of the segments is highly restricted) regions corresponding to crystalline domains, and 3) a fraction with intermediate mobility (motions are intermediate between those in crystalline and dissolved states). The component with intermediate mobility has been shown to correspond to unassociated or partially associated chains in polymer rich regions. During the aging process the immobile fractions of starch increase and the mobile fractions decrease (Wu & Eads, 1993). Cameron *et al.* (1994) demonstrated the establishment of network structures, involving only short range intermolecular associations possibly *via* double helix formation.

2.5.2.3. Retrogradation of starch

Starch gels are formed when gelatinized starch dispersions (> 6.0%w/w) are cooled to room temperature (Ring, 1985). On cooling the paste, the exuded amylose forms an interpenetrating network in which the gelatinized granules rich in amylopectin are embedded. Such a matrix is regarded as a composite material and its mechanical properties depend on characteristics of amylose matrix, interactions between the dispersed and continuous phase and the rigidity of gelatinized granules (Eliasson, 1985).

During storage of concentrated gels, stiffness increases due to rearrangement of starch chains. The short term development of gel structure was found to be dominated by the gelation of amylose within the continuous phase (Miles *et al* .,1985b). In starch gels, increase in crystallinity and gel stiffness over longer time periods was attributed to the reordering of amylopectin molecules which occurs at a much slower rate (Ring *et al.*, 1987).

Starch gels develop 'B' type crystallinity on storage (Miles *et al.*, 1985b; Russell, 1987; Van Soest *et al.*, 1994) regardless of the initial crystalline pattern of the native starch. The intensity of the 'B' pattern has been shown to increase within the time of gel storage (Roulet *et al.*, 1988; Eerlingen *et al.*, 1994). Even though starch gels regain some of the structural order during retrogradation process (Miles *et al.*, 1985b; Eerlingen *et al.*, 1994; Van Soest *et al.*, 1994), Keetels *et al.* (1996) showed that this order is different from the super-helical structure (Fig. 2.14) [Oostergetel & van Bruggen, 1993] in the crystalline domains of native starch granules [since the size of semi crystalline clusters observed in retrograded starch gels was smaller (5 nm) than that in native starch (9 nm)]. Based on these results, the above authors (Keetels *et al.*, 1996) stated that the long range order in starch granules is not regained during starch retrogradation.

Non invasive methods such as FTIR and near infrared reflectance (NIR) spectroscopy have been used to monitor staling of bread (Wilson *et al.*, 1991) and starch retrogradation (Van Soest *et al.*, 1994, 1995). In bread crumbs, the increase in scattering of NIR radiation as the crumb structure changes during

storage indicates the development of crystallinity in the amylopectin fraction (Wilson *et al.*, 1991). FTIR measures short-range ordering in the gel system (Wilson *et al.*, 1991). In the spectra, C-C and C-O regions (1300-800 cm^{-1}) are sensitive to the retrogradation process (Van Soest *et al.*, 1994, 1995). The spectra of potato starch gels (10%w/w) showed that a broad band at 1022 cm^{-1} resolved into three bands (~ 1053, 1022 and 1000 cm^{-1}) when the gels were stored for several weeks. The most pronounced changes of the spectrum occurred at 1000 (peak), 1035 (valley) and 1053 (peak) cm^{-1} (Van Soest *et al.*, 1994). FTIR absorbance band at 1047cm^{-1} is sensitive to the amount of ordered or crystalline starch, whereas the band at 1022cm^{-1} is characteristic of amorphous starch (Van Soest *et al.*, 1995). Based on these observations, the above authors proposed a multi-stage process for retrogradation of starch gels. These stages are listed below :

Stage 1 - conformational ordering : a) formation of double helices between amylose chains and / or between the outer branches of amylopectin chains, b) amylose chain aggregation and crystallization.

Stage 2 - onset of amylopectin helix aggregation and crystal growth.

Stage 3 - amylose aggregation and crystallization.

Stage 4 - phase separation of water (syneresis) due to excessive retrogradation.

2.5.2.4. Factors influencing retrogradation

2.5.2.4.1 Botanical source

Russell (1987) studied the retrogradation of 4 starches with different amylose and amylopectin contents. DSC thermograms showed that after a given time, retrogradation endotherm for waxy maize starch was significantly greater than that for amylomaize starch while potato and wheat starches produced intermediate values. This suggests that the amylopectin fraction is responsible for the development of retrogradation endotherm. The initial development of modulus (Orford *et al.,* 1987) observed for 30% starch gels from various botanical sources followed the order : pea > maize > wheat > potato. The dependence of initial rate of increase of the modulus on the botanical source can be accounted for by the different amounts of amylose that are solubilized during gelatinization. However, long term increase in modulus showed the order : pea > potato > maize > wheat. The above authors suggested that higher long term modulus increase in pea and potato was due to the their low lipid contents, which prevent amylopectin crystallization. Jacobson *et al.* (1997) reported that retrogradation rates of 2% starch gels stored at 4°C for 56 days followed the order : wheat ~ corn > rice ~ tapioca, potato >> waxy maize. Upon storage, networked amylose transformed into a dense aggregated state, whereas amylopectin showed very little changes. Yuan & Thompson (1998) studied the retrogradation of 3 waxy maize genotypes and reported that ΔH_R of *duwx* was greater than that of *wx* or *wxshl*. Storage modulus of *duwx* increased rapidly

during the first 4 days of storage, whereas in *wx* and *wxshl* gels, the increase was gradual over 25 days of storage. The greater retrogradation tendency in *duwx* was attributed to the large proportion of DP 20-30 chains in amylopectin.

2.5.2.4.2 Storage temperature

Crystallization follows the three step mechanism of nucleation, propagation and crystal perfection (Wunderlich, 1976). Both nucleation and propagation (Fig. 2.27) depend exponentially on temperature, within the temperature range of glass transition (T_g) and melting temperature (T_m). Thus, nucleation rate increases with decrease in temperature down to T_g while the propagation rate increases with increasing temperature up to the T_m (Wunderlich, 1976). Several studies (Colwell *et al.,* 1969; Fearn, & Russell, 1982; Jankowski, & Rha, 1986) have shown that the rate limiting step for starch retrogradation is nucleation and the rate of retrogradation as well as the properties of retrograded starch gel depend on the storage temperature (Jankowski, & Rha, 1986).

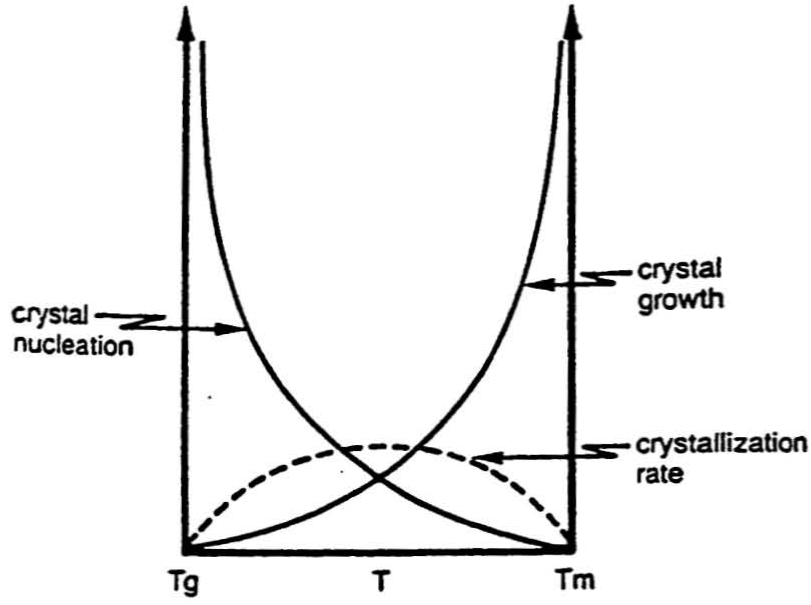
Differential thermal analysis of wheat starch gels stored at temperatures varying from -1 to 43°C, (Colwell *et al.,* 1969) demonstrated that instantaneous nucleation followed by rod-like crystal growth occurs throughout the temperature range. However, at elevated temperatures (>10°C) more perfect crystallites are formed and the rate of aging is inversely related to storage temperature. Similar results were reported for potato starch gels stored at refrigeration temperature

Fig. 2.27 Effect of storage temperature on crystallization of partially crystalline polymers (adapted from Wunderlich, 1976).

Tg - Glass transition temperature

Tm - Melting temperature

T - Temperature


crystal
nucleation
crystal
growth
crystallization
rate
Tg
T
Tm

which formed less perfect crystallites than those stored at room temperature (Nakazawa *et al.*,1985).

Jankowski & Rha (1986) observed that retrogradation endotherm of cooked wheat grains shifted to higher temperatures, when the storage temperature was increased from 4 to 20°C. Similar findings were reported by Jang & Pyun (1997) for wheat starch gels (40-60%) stored at 4 and 32°C. A study with rice starch gels (50%w/w) stored at different temperatures showed that, the extent of retrogradation followed the order : refrigerated > room temperature > frozen. Jacobson & BeMiller (1998) showed that waxy maize starch gels (2.5%) subjected to freeze thaw treatment showed a retrogradation rate which was inversely correlated to the rate of freezing. The peak temperature of retrogradation endotherm increased with increase in thawing temperature showing the formation of more perfect crystals.

2.5.2.4.3 Moisture content of gels

Gel moisture content was shown to influence crystal formation in starch gels. DSC showed that crystallization is greatest in gels with 50-60% starch (Longton & LeGrys, 1981; Zeleznak & Hoseney, 1986), whereas it does not occur in very dilute (10% starch) or very concentrated (80% starch) gels (Longton & LeGrys, 1981). Orford *et al.* (1987) reported that, in maize starch gels stored for 7 days at 20°C, development of shear modulus was rapid for gels having 30% and 40% starch, whereas the increase was very low for 20% gel.

2.5.2.4.4 Lipids

Lipids and emulsifiers have been known to affect retrogradation, hence the texture of starch based products (Germani *et al.*, 1983; Eliasson, 1985; Conde-Petit & Escher, 1994). It appears that texture modification of starch is brought about by the formation of inclusion complexes particularly with monoacyl lipids (Eliasson, 1985; Conde-Petit & Escher, 1994). However, it has also been suggested that reduced retrogradation may be due to hindered crystallization caused by the surface adhesion of the lipids on amylopectin chains (Van Lonkhuysen & Blankestijn, 1974) or on the starch granule surface (Germani *et al.*, 1983). In emulsifiers, the anti-firming effect is mainly caused by amylose complexation, which in turn weakens the cohesion between the amylopectin rich starch granules (Conde-Petit & Escher, 1994). Decrease in granule swelling (Hoover & Hadziyev, 1981), amylose leaching (Eliasson & Krog, 1985), and changes in gel volume (Eliasson, 1985) have been shown to occur in the presence of MG and emulsifiers. The extent of the decrease depends on the nature of lipid (Hoover & Hadziyev, 1981; Eliasson & Ljunger, 1988). Germani *et al.* (1983) showed that the efficiency of lipid in decreasing retrogradation increased with decrease in chain length and increased degree of unsaturation. This is in agreement with the findings of Huang & White (1993), who reported a greater inhibition of retrogradation in waxy corn gels with shorter MG chain length. Eliasson & Ljunger (1988), demonstrated that MG hinder retrogradation more effectively than di- or triacylglycerols. The inhibition of retrogradation of

rice starch gels in the presence of emulsifiers was shown to follow the order : glyceryl monopalmitate [GMP] > glyceryl monostearate [GMS], sucrose esters of palmitic acid, diacetyl tartaric acid, esters of MG > sucrose esters of stearic acid, sodium stearoyl-2- lactylate [SSL] (Miura *et al.,* 1992). In 40%(w/w) starch gels of potato, maize and wheat, the effect of added emulsifiers on inhibition of retrogradation (as measured by modulus of elasticity) followed the order : calcium stearoyl-lactyl-2-lactylate > GMS > lecithin (Conde-Petit & Escher, 1994). Ward *et al.* (1994) showed (as monitored by DSC) that SSL did not significantly decrease the retrogradation of wheat and corn amylopectin gels. The above authors postulated that SSL affects the nature of crystallites formed but not the extent of crystallization.

2.5.2.4.5 Sugars

Effect of sugars on starch retrogradation is still in dispute. Several workers (Kohyama & Nishinari, 1991; Katsuta *et al.,* 1992a,b) support the idea that starch retards retrogradation whilst others (Chang & Liu, 1991; Wang & Jane, 1994) have provided evidence to the contrary. Germani *et al.* (1983) suggested that the mechanism of starch crystallization is instantaneous nucleation followed by a rod-like growth of crystals, regardless of the type of starch or the type of sugar used. However, Prokopowich & Biliaderis (1995) demonstrated that the effect of sugars on retarding aging was solute specific (fructose accelerated retrogradation whereas maltotriose and ribose retarded the process), and more

pronounced in starches with low amylose content compared to starches with high amylose. Slade & Levin (1987) reported that the retrogradation of starch : sugar : water (1:1:1) mixture stored at 25°C followed the order : fructose > mannose > water alone > glucose > galactose > maltose > sucrose > xylose > lactose. The above authors suggested that sugars inhibit aging by decreasing the chain mobility and diffusion in the water / solute plasticized amylopectin matrix which raise the network T_g of the starch gels. Thus, the rate of recrystallization at ambient temperatures (T) diminishes because of the lower temperature difference ($T-T_g$). Using X-ray diffraction, Cairns *et al.* (1991) showed that xylose and ribose decreased crystallization of wheat starch gels with increasing sugar concentration. However,. in gels containing fructose two effects were noted : addition of fructose led to both thermally reversible and irreversible crystallization upon storage. Similar findings were reported by Biliaderis & Prokopowich (1994). Kohyama & Nishinari (1991) observed that sucrose was more effective (sucrose > glucose > fructose) in inhibiting retrogradation of sweet potato starch gels. Among malto-oligosaccharides the effectiveness in reducing retrogradation followed the order : maltotriose > maltotetraose > branched oligosaccharides (Katsuta *et al.*, 1992b Miura *et al.*, 1992). It has been shown that hexoses (except galactose) are more effective than pentoses (Katsuta *et al.*, 1992a) in retarding retrogradation while disaccharides are better than monosaccharides in stabilizing gel structures (Katsuta *et al.*, 1992a; Miura *et al.*, 1992). The above authors suggested that the

ability of saccharides to stabilize starch-water systems might be influenced by the conformation of saccharides, whereas sugars with large number of equitorial hydroxyl groups are more effective in retarding crystallization. Moreover, based on the studies with a large number of polyhydroxy compounds, Biliaderis & Prokopowich (1994) and Prokopowich & Biliaderis (1995) postulated that the compatibility of sugar with the water structure (as governed by the stereochemistry of the main sugar conformers in solution) is important in controlling retrogradation kinetics. Seow *et al.* (1996) reported that recrystallization of rice starch gels increased to a maximum with increase in sucrose, xylose and arabinose concentration before decreasing with further addition of sucrose. However, fructose and maltose increased retrogradation over the concentration range of 0-100%.

2.5.2.4.6 Salts

Ciacco & Fernandes (1979) showed that retrogradation rate of wheat starch gels increased by anions in the order : $I^- < Br^- < Cl^- < F^-$; and cations increased retrogradation in the order :$K^+ < Li^+ < Na^+$. However, the crystallization mechanism (instantaneous nucleation followed by crystal growth) remained unchanged in the presence of anions or cations. Russell & Oliver (1989) studied the effect of NaCl concentration (0-4.43%) on aging of wheat starch gels by rheological and thermal measurements and observed that the increased salt concentration progressively increased the biphasic appearance of the

retrogradation endotherm. This implies that increased salt concentration reduced re-ordering of the amylopectin fraction. Bello-Perez & Paredes-Lopez (1995) showed that amaranth starch gels with NaCl produced an endotherm after storage of 4 weeks. The enthalpy of retrogradation decreased with increase in NaCl concentration from 0.05 to 0.2%. However, waxy corn starch gels with NaCl did not produce a retrogradation endotherm within the 4 week storage period.

2.5.2.4.7 Hydrocolloids

Hydrocolloids have been known to affect the gelling behaviour of starches which is important in food product development and specifically modification of the texture and stability of formulated food systems (Christianson *et al.*, 1981; Sajjan & Rao, 1987). In general, hydrocolloids have been shown to accelerate gelling (Christianson *et al.*, 1981; Alloncle & Doublier, 1991). However, the mechanism by which hydrocolloids such as guar gum, xanthan gum, carageenan influence the gelation and crystallization mechanism is still in dispute (Eidam *et al.*, 1995; Biliaderis *et al.*, 1997).

2.5.2.4.8 Physical modification

Orford *et al.* (1993) investigated the retrogradation of extrusion-cooked waxy maize starch gels (15-50%) stored at 1°C using DSC and gel firmness measurements. The results showed that extrusion cooked products formed thermoreversible gels, but caused less apparent changes in gelation kinetics.

Retrogradation of defatted and heat-moisture treated pigeon pea starch gels stored at -16°C was studied using freeze thaw stability measurements (Hoover *et al.*, 1993). The results showed that both treatments decreased the syneresis of starch gels compared to the unmodified gels. Hoover *et al.* (1994) showed that defatting and heat-moisture treatment did not alter the onset time of the retrogradation endotherm (endotherm appeared after 3 days in native and treated starches) of wheat, potato and lentil starches. However, in oat starch the above treatments accelerated the appearance of endotherm (endotherm developed after 3 and 6 days respectively, for defatted and heat-moisture treated starches). Defatting increased the enthalpy of retrogradation (ΔH_R) in all starches, whereas heat-moisture treatment increased ΔH_R in wheat, lentil and oat starches but decreased ΔH_R in potato starch. The gel strength of wheat and oat starches increased to a greater extent on defatting than on heat-moisture treatment, whereas in potato and lentil starches the increase was more pronounced on heat-moisture treatment. The above authors (Hoover *et al.*, 1993, 1994) explained the changes based on the structural changes that occur within the granule during defatting and heat-moisture treatment. Durani & Donald (1995) observed that amylopectin gels which were annealed (heating the sample at a temperature below its melting point) at a temperature below their melting point (after storage at 4°C) melted at a higher temperature and over a narrower range than those which were not annealed. Furthermore, the melting

temperature range of these gels were affected by the length of time of annealing as well as the annealed temperature.

2.5.2.4.9 Chemical modification

Introduction of phosphate (Bohlin & Eliasson, 1986), acetylated (Hoover & Sosulski, 1985) and hydroxypropylated (Hoover *et al.*, 1988; Yook *et al.*, 1993) groups into starch chains has been shown to influence starch retrogradation by retarding amylose and amylopectin chain aggregation during storage. Hoover *et al.* (1988) reported that hydroxypropylated field pea starch showed less retrogradation during frozen storage compared to the unmodified starch. Takahashi *et al.* (1989) studied the gel properties of wheat and corn starches cross linked after acetylation and hydroxypropylation. Both cross linked acetylated and cross linked hydroxypropylated wheat and corn starches showed a low gel firmness compared to their unmodified gels. Both modification methods reduced the gel firmness of wheat starch to a greater extent compared to modified corn starch. Furthermore, gel firmness decreased with increasing cross linking in both acetylated and hydroxypropylated wheat and corn starches. Freeze-thaw stability of hydroxypropylated field pea starches increased with increased substitution (MS 0.0 to 0.12). DSC showed that hydroxypropylation (Yook *et al.*, 1993) of rice decreased retrogradation of cooked rice.

2.5.3 Starch digestibility : Action of porcine pancreatic α-amylase

The digestibility of starch by porcine pancreatic α-amylase has been the subject of numerous investigations (Holm *et al.*, 1983; Seneviratne & Biliaderis, 1991; Cone & Wolters, 1990). The action of porcine pancreatic α-amylase on starch chains is known to occur by a multiple attack mechanism (Robyt & French, 1970) in which once the enzyme forms an enzyme-polymer complex (Fig. 2.28a), the enzyme may catalyze the hydrolysis of several bonds before it dissociates. The direction of multiple attack is from reducing to non reducing end of the molecule (Robyt & French, 1970). Being an endo-enzyme, porcine pancreatic α-amylase randomly cleaves α-(1-4) glycosidic linkages of linear amylose producing mainly G_2 and G_3 and finally G_1 and G_2 after prolonged incubation with a large amount of enzyme (Robyt & French, 1970). Porcine pancreatic α-amylase consists of an active site with 5 D- glucose sub sites (Robyt & French, 1970) and a catalytic site consisting of carboxylate anion (nucleophile) and imidazolium cation [electrophile] (Hoover & Sosulski, 1985). Porcine pancreatic α-amylase shows a wide variation in *in vitro* digestibility of native, gelatinized and modified starches. This wide variation has been attributed to several factors : starch crystallinity (Hoover & Sosulski, 1985; Ring *et al.*, 1988), amylose / amylopectin content (Atkins & Kennedy, 1985), granule size (Cone, & Wolters, 1990; Franco *et al.*, 1992), starch-lipid interactions (Seneviratne & Biliaderis, 1991; Holm *et al.*, 1983), starch - protein

Fig. 2.28 Starch hydrolysis by α-amylase : mechanism

A) Enzyme - substrate complex formation and hydrolysis of α (1-4) glycosidic bonds *via* multiple attack mechanism (adapted from Banks & Greenwood, 1975).

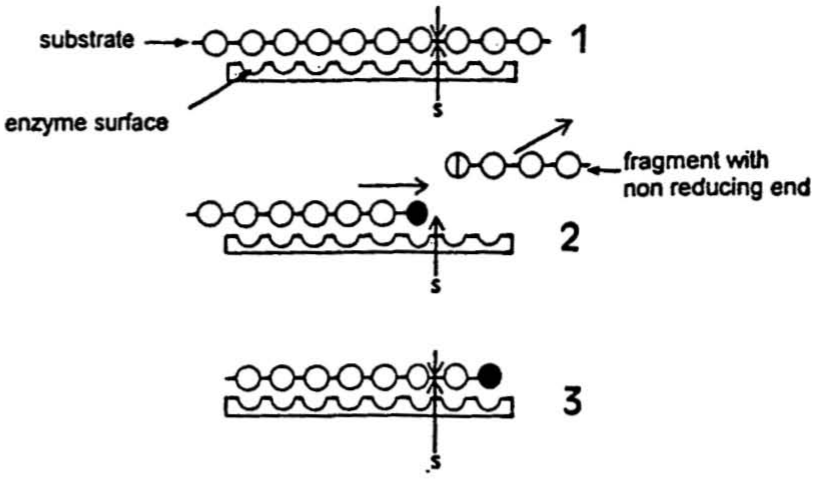
1) an internal segment of amylose is bound to the active site of the enzyme; hydrolytic scission occurs at catalytic site.

2) after hydrolytic scission, the fragment with non reducing end diffuses away; the remaining fragment is again bound to the enzyme.

3) the substrate rearranges itself and the second hydrolytic scission occurs.

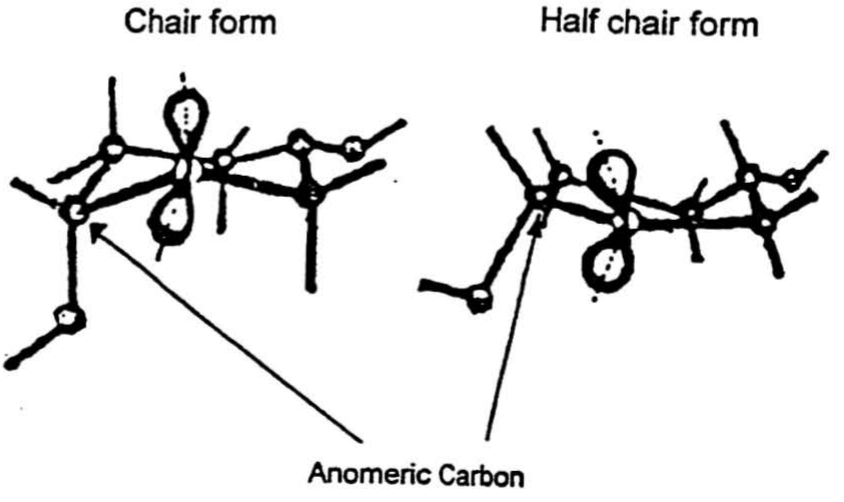
B) Schematic representation of 'chair' to 'half chair' conformations of the D-glucosyl residues of starch molecules during hydrolysis by α-amylase (Thoma, 1968).

A



S = catalytic site ● reducing end

B



interactions (Wursch *et al.*, 1986), starch modification (Wootton & Chaudhry, 1981; Hoover *et al.*, 1993; & Hoover & Manuel, 1996b), and retrograded starch (Jane & Robyt, 1984; Kim *et al.*, 1997).

The enzyme catalyzed hydrolysis of α-D glucose linkages has been shown to involve in an enzyme induced ring distortion of one of the D-glucosyl residues from the $^4C_1$ 'chair' conformation to 'half chair' conformation [Fig. 2.28b] (Thoma, 1968). This ring distortion decreases the enthalpy of activation and the susceptibility of the glucosyl residues to nucleophilic attack by functional groups on α-amylase and water. Laszlo *et al.* (1978) have shown that ring distortion or a 'half chair' conformation is involved in transition state of α-amylase.

Potato amylose complexed with lysolecithin and oleic acid showed reduced hydrolysis with porcine pancreatic α-amylase (Holm *et al.*, 1983). Eliasson & Krog (1985) reported that amylose complexed with saturated MG was more resistant to enzymatic degradation than that of unsaturated MG. Seneviratne & Biliaderis (1991) demonstrated that the rate and the extent of hydrolysis of helical inclusion complexes of amylose by porcine pancreatic α-amylase and α-amylase from *Bacillus subtilis* was higher in form I than form II amylose - lipid complexes. This was due to the fact that form I with less ordered structure (more open) in the solid state exhibits the highest susceptibility to α-amylase. This suggests that the super molecular structure

of 'V' amylose complex influences the accessibility of α-amylases to the solid substrate and thereby controls the digestion kinetics. Hoover & Manuel (1996b) suggested that a conformational change during α- amylase hydrolysis may be difficult for those amylose chains that are complexed by native lipids.

2.6 Starch modification

The physical properties of native starches and their colloidal sols produced on heating the suspension, limit the usefulness of starch in many commercial applications. Depending on the application, these drawbacks may include the lack of free flowing properties or water repellence of the starch granules, insolubility in cold water, excess or uncontrolled viscosity after cooking, cohesive or rubbery texture of the cooked starch, sensitivity to shear and pH, the lack of clarity and tendency of becoming opaque gels when cooled (Wurzburg, 1987). Therefore, native starches are modified by physical, chemical or enzymatic processes to overcome one or more of these drawbacks, thus expanding the usefulness of starch in many applications.

2.6.1 Physical modification of starch

Physical modification of starch generally involves the simultaneous action of factors such as temperature, moisture, pressure, and shear. The effect of

these factors on starches causes two types of transformations : 1) modification of the physical structure with either the conversion or the complete disorganization of the granule and 2) modifications at the molecular level involving either degradation of macromolecules or of the monomers (Colonna *et al.*, 1987). Physical modification methods which bring about these transformations include heat-moisture treatment, defatting (with various solvents), annealing, extrusion cooking and preglatinization.

2.6.1.1 Heat-moisture treatment

Physicochemical properties (swelling, amylose leaching, gelatinization, susceptibility to enzyme) of starches have been shown to change dramatically on heat-moisture treatment (18-27% moisture, 100°C) [Sair, 1967; Kulp & Lorenz, 1981; Hoover & Vasanthan, 1994a,b]. The extent of these changes are highly influenced by starch source, moisture content, time and temperature of heat-moisture treatment (Lorenz & Kulp, 1983; Stute, 1992; Hoover & Vasanthan, 1994a,b; Franco *et al.*, 1995). In general, physicochemical properties of tuber starches have been shown to approach those of untreated cereal starches after heat-moisture treatment (indicating structural changes within the starch granule). Physicochemical properties of tuber starches (potato, cassava) have been shown to improve on heat-moisture treatment, whereas in cereal starches (wheat, barley, triticale) they deteriorate after heat-moisture treatment (Lorenz & Kulp, 1981). Kulp & Lorenz (1981) reported that the disruption of granule

structure on heat-moisture treatment is more evident in wheat than in potato starch. Donovan *et al.* (1983) postulated that the effect of heat-moisture treatment may be either due to new crystal formation or recrystallization and perfection of the crystalline regions of the starch granules. Hoover & Vasanthan (1994a), Franco *et al.* (1995) and Hoover & Manuel (1996a) have shown that the extent of starch chain associations within amorphous regions and the degree of crystalline order are changed on heat-moisture treatment of cereal, tuber and legume starches. The magnitude of these changes was found to be dependent upon the moisture content (during heat treatment) and the starch source. Several researchers have shown that the X-ray pattern of potato and yam starches (Hoover, & Vasanthan, 1994a) change from a 'B' pattern to 'A+B' pattern on heat-moisture treatment . However, in cereal starches, the 'A' pattern remains unaltered on heat-moisture treatment (Lorenz & Kulp, 1983; Stute 1992; Hoover & Manuel, 1996a)

Imberty *et al.* (1991) have shown that in crystallites of both 'A' and 'B' starches double helices are found in pairs and all chains are packed in parallel arrays. The pairing of double helices is the same in both polymorphs and corresponds to the interaction between double helices that have the lowest energy. Starches exhibiting 'A' and 'B' X-ray patterns differ in their water content and the manner in which the pairs of double helices are packed within their respective crystals. In 'B' starches there are 36 water molecules present in a channel in the centre of a hexagonal arrangement of six double helices, while in

'A' starches there are only four water molecules between double helices (Imberty *et al.*, 1991). Furthermore, the centre of 'A' starches is occupied by an amylosic helix rather than a column of water. It has been suggested that adjacent double helices within crystallites of 'A' starches are mainly linked by direct hydrogen bonding (Leach *et al.*, 1959; Imberty *et al.*, 1991). However, in crystallites of 'B' starches, adjacent double helices are mainly linked by hydrated water bridges and to a limited extent by direct hydrogen bonding (Imberty *et al.*, 1991). The transformation of the X-ray pattern from 'B' to 'A+B' is probably initiated by rupture of the hydrated water bridges which enables helices to rearrange themselves into a crystalline array that contains an amylosic helix in the central channel of the unit cell (Hoover & Vasanthan, 1994a).

Hoover & Vasanthan (1994a) observed that the crystallinity of tuber starches decreased, whereas it increased in wheat, lentil and oat starches after heat-moisture treatment. Hoover & Manuel (1996a) observed increased crystallinity in normal, waxy, dull waxy and amylomaize V starches after heat-moisture treatment (at 30% moisture).

Heat-moisture treatment of cereal, tuber and legume starches at varying moisture contents for different lengths of time have been shown to alter gelatinization transition temperatures (onset, mid point and conclusion) and the enthalpy of gelatinization (Lorenz & Kulp, 1983; Stute, 1992; Hoover & Vasanthan, 1994a; Hoover & Manuel, 1996). The extent of these changes was found to be dependent upon the starch source and conditions of heat-moisture

treatment. Reported results of studies on HMT (95-110°C, 100% relative humidity for 16 h) of potato and corn starches have shown that heat-moisture treatment increases transition temperatures (Sair, 1967). These results were confirmed by Kulp & Lorenz (1981), Lorenz & Kulp (1983) [100°C, 18-27% moisture, 16 h], Donovan *et al.* (1983) [110°C, 20% moisture, 140-240 min], and Hoover & Vasanthan (1994a) [100°C, 30% moisture, 16 h] for wheat, barley, triticale, red millet, potato, arrowroot, and cassava starches. It was further shown that the gelatinization enthalpy decreases after heat-moisture treatment of potato starch (Donovan *et al.*, 1983; Stute, 1992; Hoover & Vasanthan, 1994a). Only a slight (Donovan *et al.*, 1983) or no decrease (Stute, 1992; Hoover & Vasanthan, 1994a; Hoover & Manuel, 1996a) was observed after heat-moisture treatment of cereal starches. Possible explanations for the effect of heat-moisture treatment on gelatinization parameters include : 1) increase in interactions between amylose and amylopectin or lipids (Hoover & Vasanthan, 1994a), resulting in a decrease in the destabilizing effect exerted by the amorphous regions on the melting of starch crystallites during gelatinization; 2) alteration of the interactions between crystallites and amorphous matrix (Stute, 1992); 3) changes in the stability of starch crystallites (Hoover & Vasanthan, 1994a; Eerlingen *et al.*, 1996) and 4) reorientation of double helices (Hoover & Vasanthan, 1994a).

2.6.1.2 Defatting

Defatting was shown to alter crystallinity, solubility, swelling, enzyme susceptibility, pasting and gelatinization parameters of cereal, tuber and legume starches. The extent of the above changes was influenced by the botanical source, nature and composition of the solvent, temperature and time of defatting, degree of associations between amylose and amylopectin chains in the native granule and on the lipid content (Takahashi & Seib, 1988; Vasanthan & Hoover, 1992a; Gibinski *et al.*, 1993).

Takahashi & Seib (1988) reported that defatting with methanol (30°C) and 75% n-propanol (at 100°C) removed 93% and 88% lipid from corn starch respectively. However, for wheat starch the corresponding values were 78% and 97%, respectively. Iodine binding capacity [IBC] has been used to indicate the efficiency of lipid removal. The above authors noted that wheat and corn starches showed the highest IBC after extraction with 75% n-propanol (100°C). Vasanthan & Hoover (1992b) reported that defatting with 1-propanol water 3:1 (v/v) [PW] removed almost all the lipids in wheat, corn, potato, lentil and cassava starches.

Defatting with 80% methanol was shown to produce unaltered X-ray patterns and decreased crystallinity in wheat and potato starches (Lorenz, & Kulp, 1983). Vasanthan & Hoover (1992a) observed an increase in crystallinity and a change in X-ray pattern from 'B' to 'A+B' on defatting potato starch with PW. However, in wheat, corn and cassava starche, the X-ray pattern and

crystallinity remained unchanged on deffatting. The change in X-ray pattern in potato starch was attributed (Vasanthan & Hoover, 1992a) to clustering of the outer branched of amylopectin chains [resulting in the formation of a closed pack arrangement of double helices].

Swelling of cereal starches is primarily a property of amylopectin. Amylose and lipids act as diluents (Tester & Morrison, 1990a). Partial removal of lipid in wheat starch (Tester & Morrison, 1990a) with anhydrous methanol at 100°C increased swelling factor [SF]. The above authors suggested that the natural lipids in starch cause a substantial suppression of swelling in cereal starches. Defatting with 80% methanol increased the solubility and swelling power [SP] of wheat starch, whereas in potato starch, solubility decreased, while SP remained unaltered (Lorenz & Kulp, 1983). Goshima and coworkers (1985) noted that in potato starch solubility and SP increased on defatting with 99% methanol. Vasanthan & Hoover (1992a) and Hoover *et al* (1994) reported that defatting of potato, wheat, oat, corn, lentil and cassava starches with PW decreased SF of all starches. Gibinski *et al.* (1993) observed that defatting of oat starch varieties (Halny, Komes and Santor) with cold chloroform-methanol-water (3:2:1, v/v/v) or hot 1-propanol, increased the water binding capacity (cold extraction > hot extraction) and solubility (hot extraction >> cold extraction) of all three varieties. The above authors postulated that the increase in water binding capacity was probably due to the opening of the capillaries (which contained lipid micelles) on the surface of granules for water uptake after defatting. The increase in solubility

was attributed to the damaged amylopectin shell of the granule by the joint action of alcohol and temperature (Gibinski *et al.*, 1993).

Goshima *et al.* (1985) observed no significant changes in gelatinization temperatures when potato starch was defatted with 99% methanol. Similar observations have been reported for defatted wheat starch with 80% methanol (Lorenz & Kulp, 1983) and defatted wheat and corn starches with 75% ethanol (Takahashi & Seib, 1988). In contrast, Vasanthan & Hoover (1992a) noted that defatting with PW increased the gelatinization temperatures of potato and lentil starches, but caused no significant changes in corn, wheat and cassava starches.

Lipid removal from potato starch with 99% methanol (15h) reduced the pasting temperature, increased paste consistency, but did not alter thermal stability during the holding period at 95°C (Goshima *et al.*, 1985). Reduced pasting temperature, increased paste consistency (PC) and pasting peak were also observed in wheat and corn starches when defatted with water saturated butanol (5h) at 70°C or with 85% methanol (72h) (Melvin, 1979). However, Takahashi & Seib (1988) noted that lipid removal from wheat and corn starches with 75% ethanol eliminated the pasting peak, decreased pasting temperature, paste consistency and set back. Vasanthan & Hoover (1992a) reported that defatting of cassava, potato, corn, wheat and lentil starches with PW eliminated pasting peaks of cereal starches and increased thermal stability and reduced paste consistency in all starches.

2.6.2 Chemical modification

Chemically modified starches have become important functional ingredients in a wide range of food and non food industrial applications, because of their improved functional properties over unmodified starches. Table 2.8 shows some of the properties and applications of chemically modified starches. The concept of chemical derivatization of starch to improve its functional properties for specific purposes began in late 1930s. An important outcome of this aspect was the discovery of a practical way to chemically modify starch in an aqueous slurry maintaining the integrity of the granule and making it possible to remove by-products by filtration and washing techniques. Alkaline catalyzed reactions of starch with alkylene oxide were carried out in solution or paste form. Kesler & Hjermstad (1950) showed that the reaction could be carried out to obtain hydroxyalkyl starches with undamaged granules. This discovery was especially important in the food industry for development of new starch products with improved properties (Kesler & Hjermstad, 1950).

Today, chemical derivatization of starch is commonly carried out using methods such as acid conversion, cross linking, esterification and etherification which include cationization, carboxymethylation, hydroxyalkylation [Fleche, 1985]. Acid converted starches are produced by hydrolyzing (with acids such as hydrochloric or sulfuric) a concentrated starch slurry (~40% starch) after heating at a temperature below its gelatinization temperature. During the process, acid hydrolyzes glycosidic linkages, thus shortening the chain length. The resulting

Table 2.8 Properties and applications of chemically modified starches

Modified starch	Treatment	Advantages over unmodified starch	Food applications	Non food applications
Oxidized	hypochlorite	reduced set-back increased paste clarity	thickeners, jellies	paper, textile, adhesives
Hydroxypropyl	propylene oxide	increased stability and clarity	pie fillings, salad dressings	textile and paper industry
Esterified	acetic anhydride	reduced set-back increased clarity	instant foods, frozen foods	textile, paper, packaging, film
Cross linked	phosphorus oxychloride	increased stability to pH, shear, heat and freeze thaw treatment	canned and frozen foods	paper, metal sequestrants
Acid converted	acid	high gel viscosity low hot paste viscosity	jellies, gums	laundry starch, textile
Cationic	reagents containing amino, imino, ammonium, sulfonium groups (e.g. 2-diethylaminoethyl chloride	improved clarity and stability of dispersions cold water swelling	not used	paper, textile, adhesives, detergents
Carboxy-methylation	sodium monochloroacetate	high viscosity	thickening agents	paints, adhesives

Adapted from Solarek (1986); Galliard & Bowler (1987).

product shows reduced hot paste viscosity, higher fluidity and solubility in hot water (Wurzburg, 1987).

Cross linking leads to a more rigid macromolecular network inside the granule by formation of inter- or intramolecular chemical bridges through hydroxy-substituted phosphate groups between starch chains (Fleche, 1985). Cross linking reaction is carried out in an aqueous starch suspension at temperatures varying from room temperature to ~ 50°C in the presence of a cross linking agent (e.g. phosphorous oxychloride, epichlohydrin, adipic acid). Cross linked starches show a great resistance to thermomechanical shearing. They can maintain their granule integrity in the presence of water under conditions that would rupture or destroy granules of unmodified starch (Fleche, 1985; Wurzburg, 1987).

Starch esters are formed by reacting starch suspension with acid anhydrides (acetic anhydride, malic anhydride) or carboxylic acids (acetic acid, formic acid) under appropriate pH and temperature conditions (Fleche, 1985). For example, acid anhydrides give optimum starch ester yield at pH 8.0-8.5 and 15-25°C (Wurzburg, 1987). Currently, starch acetates with low degree of substitution (> 0.1) are commonly used in many food and industrial applications (Table 2.8) since the low substitution level maintains the granule structure throughout the derivatization process. This gives commercial products with high purity which is necessary in food and pharmaceutical applications (Jarowenko, 1987). Starch esters with malic acid (starch malates) show low gelatinization

temperature and better water retention over unmodified starches (Jarowenko, 1987).

Cationic starches are produced by chemical reaction with reagents containing amino, imino, ammonium, and sulphonium groups which carry a positive charge (Solarek, 1987). Commercially significant derivatives of cationic starches include tertiary amino and quaternary ammonium starch ethers. Their affinity for negatively charged substrates make them useful in paper industry to improve sheet strength by fibre bonding through ionic or ionic and hydrogen bonding (Solarek, 1987). Cationic starches also show improved paste clarity, stability (resistant to retrogradation) and also high dispersibility and solubility which give cold water solubility (Paschall, 1967).

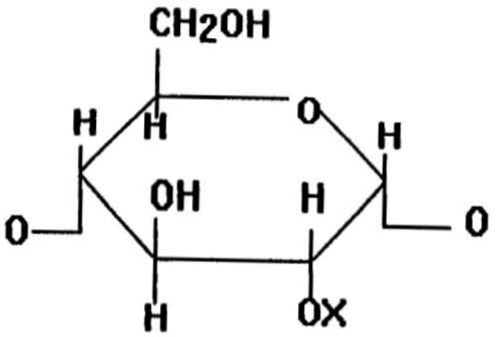
Carboxymethylation is carried out by reacting sodium monochloroacetate with starch (in an alkaline medium) in a solid phase or in a homogeneous paste. The reaction temperature of the medium is in the range of 40-50°C. Anionic nature of the group $-O-CH_2-COO^+Na^-$ gives a polyelectrolyte behaviour and high viscosity to the starch (Fleche, 1985). Thus, carboxymethyl starches are used as a thickener in many applications (Table 2.8). At a higher degree of substitution (~0.1), carboxymethyl starch becomes highly water soluble, resulting in cold water soluble starches (Hofreiter, 1987).

2.6.2.1 Hydroxypropylation

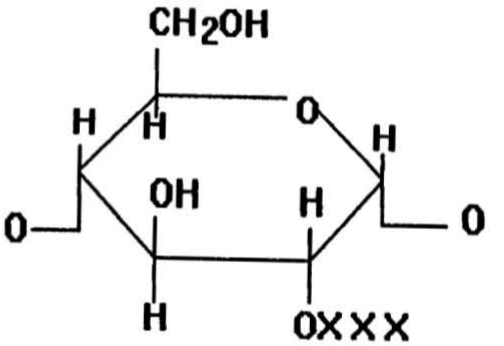
Among hydroxyalkyl starches (hydroxyethyl, hydroxypropyl etc.), hydroxypropyl starches are the commonly used hydroxyalkyl derivatives in food and industrial applications (Fleche, 1985). Hydroxypropylation has been shown to retard gelling and retrogradation tendency of starch, increase water holding capacity, improve freeze thaw stability of starch based products and reduce the tensile strength and increase elongation of starch films. Hydroxypropyl starch is produced by treating an aqueous starch suspension with propylene oxide at 38-40°C, in the presence of NaOH as a catalyst. The reactive nature of propylene oxide is due to its highly strained epoxide ring (Tuschhoff, 1986). In the formation of hydroxypropyl starch derivatives, part of the hydroxyl groups of the glucose units are converted into -O-(2-hydroxypropyl) group. The extent of hydroxypropylation is expressed in terms of molar substitution, which is defined as the number of moles of substituent per anhydroglucose unit (Fig. 2.29). In early stages, MS was determined by colourimetric method (Johnson, 1969). In this method, the development of colour is based on the reaction of ninhydrin with allyl alcohol and enol form of propanol, which is released during acid digestion. Recently, use of new techniques such as FTIR, ^{1}H NMR (Forrest, 1992; de Graaf *et al.*, 1995) has been employed for the rapid acquisition of results.

Several researchers postulated that hydroxypropyl substitution reaction occurs primarily in the amorphous regions of the starch granule composed of

Fig. 2.29 Glucose units substituted with hydroxypropyl groups (X) showing different molar substitution (MS)levels.



MS = 1



MS = 3

amylose and also in the intercrystalline areas of amylopectin (Hood, 1982; Blanshard, 1987). This is in agreement with the findings of Hood & Mercier (1978) who showed that most of the hydroxypropyl groups in modified tapioca starch is located in the amorphous regions (which contain the majority of α-(1-6) branch points) whereas the more compact crystalline regions would be relatively impervious to the modifying agents, thus less affected. However, the loss of birefringence in chemically modified starch granules suggests that an irreversible change takes place within crystalline regions of the starch granule during modification (Hood & Mercier, 1978). Kim *et al.* (1992) showed [using iodine affinity and Periodic Acid-Schiff's reagent staining] that hydroxypropylation mainly takes place in the central region of the potato starch granule. Furthermore; substitution at C-2 and/or C-3 on glucose units in hydroxypropylated potato starch has been reported to be > 90%, whereas at C-6 it was ~10% (Ostergard *et al.*, 1988). Wootton & Hariyadi (1992) reported that substitution at C-2 and C-6 was 94% and 6%, respectively, but negligible at C-3. The distribution of substituents between C-2 and C-6 was not affected by the level of MS or whether the parent starch was pre-gelatinized or not. However, the substitution at C-6 was affected by the starch type [wheat starch showed a higher substitution (6%) at C-6, than maize, waxy maize or high amylose maize (3%)], but unaffected by the proportion of amylose in these starches (Wootton & Hariyadi, 1992).

Xu & Seib (1997) studied (using ^{1}H NMR) the distribution of hydroxypropyl substituents in alpha limit dextrins of 8 starches. The substitution at C-2, C-3 and C-6 were 67-78%, 15-29% and 3-17%, respectively. The MS of these alpha limit dextrins was in the range of 0.05-0.23, and correlated well with the values determined by the colorimetric procedure of Johnson (1969).

Hydroxypropylation was shown to alter physicochemical properties of starches (Wootton & Manatsathit, 1984; Butler *et al.*, 1986; Seow & Thevamalar, 1993; Yeh & Yeh, 1993; Yeh & Jeng-Yune, 1996). Wootton & Manatsathit (1984) showed (using DSC) that increase in MS of hydroxypropylated maize starches (MS 0-0.27) decreased gelatinization enthalpy (ΔH), onset (T_o) and peak (T_p) temperatures, whereas the gelatinization temperature range ($T_c - T_o$) remained unaffected. Furthermore, a broadening of the endotherm was observed at MS > 0.1 (Wootton & Manatsathit, 1984). At 1:1 starch : water ratio, hydroxypropylated rice starch showed a progressive shift of a biphasic gelatinization endotherm to lower temperatures and a decrease in ΔH with increase in MS (0.0 to 0.1) [Seow & Thevamalar, 1993]. The above authors postulated that hydroxypropyl groups attached to starch molecules are primarily in the and behave as flexible side chains. The motion of these side chains creates a large amount of free volume, which may be considered as increasing internal plasticization and destabilization of amorphous regions of the granule. This destabilization of amorphous region lowers the glass transition and crystallite melting temperatures. At high MS levels, gelatinization temperature

range increases, because derivatization has a greater effect on the amorphous region, and also because of the increased inhomogeneity within both amorphous and crystalline regions of the granule. The decrease in ΔH was attributed to the increased disruption of local order in the amorphous regions (Seow & Thevamalar, 1993). Decreased transition temperatures and ΔH (compared to native starch) were also observed for hydroxypropylated rice starch by Yeh & Yeh (1993) and Yeh & Jeng-Yune (1996).

Butler *et al.* (1986) reported that swelling power (SP) of buffalo gourd root starch was unaltered on hydroxypropylation (MS 0.003 - 0.06). In hydroxypropylated maize starches (MS 0 - 0.27), SP increased at MS > 0.12 (Wootton & Manatsathit, 1983), whereas water binding capacity increased (MS > 0.1) following an initial decrease (MS 0.0 - 0.1). The above authors suggested that the initial decrease in water binding capacity was due to the blocking of water binding sites by substituent groups, whereas further increase in water binding capacity was due the tendency of granule to swell at higher MS (Butler *et al.*, 1986).

Hydroxypropylation (MS 0-0.12) of field pea starch exhibited reduced PT and increased viscosity at 95°C and 50°C, with increasing MS levels (Hoover *et al.*, 1988). Increased viscosity at 50°C implies reduced susceptibility to retrogradation (Butler *et al.*, 1986). Similar results have been reported for acetylated legume starches (Hoover & Sosulski, 1985), hydroxypropylated buffalo gourd root starch (MS 0.00-0.06) and hydroxypropylated rice starch

(Yeh & Yeh, 1993). However, hydroxypropylation did not improve heat and shear resistance in rice starch (Yeh & Yeh, 1993). Kim *et al.* (1992) observed altered pasting properties in hydroxypropylated potato starch and the changes were attributed to the decrease in associative forces within the starch granule. Increased paste clarity in hydroxypropyl starches with increasing MS has been noted by several researchers (Butler *et al* ., 1986; Hoover *et al* ., 1988; Rege & Pai, 1996) and Rege & Pai (1996) attributed the increased paste clarity to the increased amount of water bound to starch molecules.

Several researchers observed the decreased α-amylolysis of raw starches with increase in MS (Leegwater & Luten, 1971; Wootton & Chaudhry, 1981; Mohd Azemi & Wootton, 1984; Hoover *et al.*, 1988). Mohd Azemi & Wootton (1984), reported that susceptibility of raw waxy maize starch to α-amylase attack showed a continuous drop with increase in MS, whereas normal maize and high amylose maize starches showed an initial decrease in hydrolysis followed by an increase in hydrolysis at higher substitution levels. Hydrolysis of gelatinized wheat [MS 0 - 0.17] (Wootton & Chaudhry, 1981), normal maize [MS 0- 0.12], waxy maize [MS 0-0.13] and high amylose maize [MS 0-0.08] (Mohd Azemi & Wootton, 1984) starches decreased continuously with increase in MS. The decrease in hydrolysis in gelatinized starch and the initial decrease in hydrolysis in raw starch at low substitution levels was attributed to the presence of bulky hydroxypropyl groups on C-2, which sterically hinder the action of catalytic carboxylate ion on the glycosidic bond (Mohd

Azemi & Wootton, 1984; 1995; Hoover *et al.*, 1988), while the subsequent increase in hydrolysis in raw starch at higher MS (MS 0.12) was attributed to an increase in swelling power of the amorphous regions of the starch granules (Wootton & Chaudhry, 1981; Mohd Azemi & Wootton, 1985; Hoover *et al.*, 1988) and also to granule disruption (Mohd Azemi & Wootton, 1984).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Potato tubers (*Solanum tuberosum* cv Russett Burbank) were purchased from the local market. Crystalline porcine pancreatic α-amylase (EC 3211) type 1A was obtained from Sigma Chemical Co (St. Louis, MO, USA). Other chemicals and solvents were analytical grade. Solvents were distilled from glass before use.

3.2 Methods

3.2.1 Starch isolation and purification

Potato tubers were divided into two lots representing the whole sample. Each lot was further subdivided into two parts and the starch was isolated according to the method of Hoover & Hadziyev (1981) as follows :

The tubers were washed, diced, dipped in ice-cold water containing 100 ppm $NaHSO_3$ and homogenized at low speed in a Waring blender. The slurry was squeezed through a 100-mesh polyester sieve cloth and the filtrate centrifuged at 700 x g for 15 min. The supernatant and the amber-brown layer of protein atop the starch layer was removed. Further purification was achieved by repeated suspension in water, centrifugation and removal of contaminating proteins and cell debris. The purified starch was dried overnight at 30°C in a vacuum oven to a moisture content of ~ 10%.

3.2.2 Chemical composition of starch

3.2.2.1 Moisture content

Quantitative estimation of moisture was performed according to standard AACC (1984) procedures. Preweighed (3-5g, db) samples of starch were dried in a forced air oven (Isotemp 614G, Fisher Scientific, Fair Lawn, NJ, USA) at 130°C for 1 h. The samples were then removed and cooled in a desiccator. The moisture content was calculated as the percentage weight loss of the sample.

3.2.2.2 Nitrogen content

The nitrogen content was determined according to Micro Kjeldahl method. The samples (0.3 g db) were weighed on nitrogen-free papers and placed in the digestion tubes of a Buchi 430 (Buchi Laboratoriums-Technik AG, Flawill / Schweiz) digester. The catalyst [2 Kjeltabs M pellets (Fisher Scientific, Fair Lawn, NJ, USA)] and 20 ml of concentrated H_2SO_4 acid were added and the samples were digested in the Buchi 430 digester until a clear yellow solution was obtained.

The digested samples were then cooled, diluted with 50 ml of distilled water, 100 ml of 40% (w/w) NaOH were added, and the released ammonia was steam distilled into 50 ml of 4% H_3BO_3 containing 12 drops of end point indicator (N-point indicator, Sigma Chemical Co, St. Louis, MO, USA) using a Buchi 321 distillation unit until 150 ml of distillate was collected. The amount of ammonia in the distillate was determined by titrating it against 0.05N H_2SO_4 (AACC, 1984).

$$\%N = \frac{(\text{volume of acid - blank}) \times \text{Normality of acid} \times 14.0067 \times 100}{\text{sample weight (mg)}}$$

3.2.2.3 Ash content

Preweighed samples (3-5 g, db) were transferred into clean, dry porcelain crucibles, charred using a flame and then placed in a pre-heated (550°C) muffle furnace (Lab Heat, Blue Island, IL, USA) and left overnight until a gray ash was obtained. The samples were then cooled in a desiccator and weighed. The ash content was calculated as percentage weight of the remaining material (AACC, 1984).

3.2.2.4 Lipid content

Surface lipids were extracted at ambient temperature (25-27°C) by mixing starch (5 g, db) with 100 ml 2:1(v/v) chloroform / methanol under vigorous agitation in a wrist action shaker for 1 h. Bound lipids were extracted using the residue left from surface lipid extraction. The residue was refluxed with 3:1 (v/v) n-propanol water in a soxhlet apparatus at 90-100°C for 7 h. Total starch lipid was determined by hydrolysing starch (2 g, db) with 25 ml of 24% HCl at 70-80°C for 30 min and extracting the hydrolysate 3 times with n-hexane (Vasanthan & Hoover, 1992b).

The crude lipid extracts from above extractions were purified by chloroform / methanol / water (1:2:0.8, v/v/v) and forming a biphasic system [chloroform / methanol / water (1:1:0.9 v/v/v)] by addition of chloroform and water

at room temperature (Bligh & Dyer, 1959). The chloroform layer was then diluted with benzene and evaporated to dryness in a rotary evaporator (Rotavapor R 110, Brinkmann Instruments, Westbury, NJ, USA).

3.2.2.5 Amylose content

The apparent amylose content of native starch was determined by the method of Chrastil (1987). Starch samples (20 mg, db) were fully dispersed in 10 ml of 0.5N KOH in cornical flasks. The dispersions were transferred into volumetric flasks and diluted with distilled water up to 100 ml. Aliquots (10 ml) were neutralized with 5 ml of 0.1 N HCl and diluted to 50 ml with distilled water. The sample preparation for determination of total (true) amylose content of starch samples was carried out following the same procedure, as above, after defatting starch with 3:1(v/v) n-propanol water for 7 h.

3.2.2.5.1 Chrastil's method of determination of amylose content

Aliquots (0.1 ml) of the neutralized and diluted solution were transferred into screw cap tubes containing 5 ml of 0.5% trichloroacetic acid and then 0.05 ml of 0.01N I_2-KI solution was added. The tubes were allowed to stand for 30 min at room temperature for colour development. The absorbance of the blue colour was measured in a spectrophotometer (Novaspec Model 4049, LKB Biochrom, Cambridge, UK) at 620 nm. The absorbance of the reaction blank with water was zero. The amylose content was determined using the formula :

Absorbance x 32.5 = mg amylose / litre in cuvette, and expressed as mg amylose per 100 mg starch.

3.2.2.6 Estimation of starch damage

The starch damage was estimated following the standard AACC (1984) procedure. Starch samples (1 g, db) were digested with fungal α-amylase from *Aspergillus oryzae* (12500 Sigma units) having specific activity of 50-100 units/mg, in a water bath (30°C) for 15 min. At the end of incubation, the enzyme action was terminated by adding 3.68N H_2SO_4 (3 ml) and 12% $Na_2WO_4.2.H_2O$ (2 ml), 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 using the method of Bruner (1964). Aliquots (1 ml) of the filtrate were mixed with 2 ml of chilled 3,5- dinitrosalicylic acid and diluted to 4 ml with distilled water. The diluted samples were heated in a boiling water bath for 5 min. The reaction mixture was chilled, diluted with 8 ml distilled water and the absorbance was measured at 540 and 590 nm. A reagent blank was determined by the same procedure, but without starch.

The percentage starch damage was calculated as follows:

% starch damage = [M x 1.64] / [W x 1.05] x 100

where M = mg maltose equivalants in the digest; W = mg starch (db); 1.05 = molecular weight conversion of starch to maltose and 1.64 = the reciprocal of the mean percentage maltose yield from gelatinized starch. The latter is an empirical

factor which assumes that under the conditions of the experiment, the maximum hydrolysis is 61%.

3.2.3 Starch modification

3.2.3.1 Preparation of defatted potato starch

Defatted starch was prepared by soxhlet extraction with 75% aqueous n-propanol for 7 h. The solvent was removed by vacuum evaporation and the starch was air dried to a moisture content of ~ 10%.

3.2.3.2 Preparation of heat- moisture treated potato starch

The heat-moisture treatment was essentially that of Sair (1964). Starch (15 g, db) was weighed into glass containers. Starch moisture content was brought to 30%. The sealed samples (in glass jars) were heated in a forced air oven (Isotemp 615G, Fisher Scientific, Fair Lawn, NJ, USA) at 100°C for 16 h. After cooling, the jars were opened and the starch samples were air-dried to a moisture content of ~10%.

3.2.3.3 Preparation of hydroxypropylated potato starches

Native, defatted and heat-moisture treated potato starch samples were converted into a range of hydroxypropyl derivatives according to the procedure of Leegwater & Luten (1971). Samples (200 g, db) from each of the above starches were weighed into 600 ml screw cap jars. Into each jar, a solution of

NaOH (2.6 g) and Na_2SO_4 (30 g) in distilled water (240 ml) was added at room temperature. The jars with samples were placed in a water bath at 40°C and propylene oxide (0, 4, 10, 20, 30 and 50 ml) was added and the suspensions thoroughly mixed and the jars closed. The reaction was continued at 40°C for 24 h with shaking. The starch suspensions were then adjusted to pH 5.5 with dilute H_2SO_4 (1M). The starch cakes were washed with distilled water until negative to sulphate ions when tested with $BaCl_2$. All hydroxypropylated suspensions were freeze dried until the moisture content was reduced to 10-12%. Control potato starch was prepared by treatment of native, defatted and heat-moisture treated starches (200g, db) with distilled water containing NaOH (2.6 g) and Na_2SO_4 (30 g) but without addition of propylene oxide, according to the procedure for preparation of hydroxypropylated potato starch as described above.

3.2.3.3.1 Determination of molar substitution

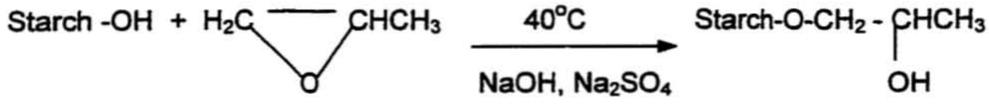
The hydroxypropyl content was determined by the spectrophotometric method of Johnson (1969). Modified starch samples (0.09-0.1 g db) were weighed into volumetric flasks, 25 ml of 1N H_2SO_4 were added and heated in a boiling water bath until the samples dissolved. The samples were then cooled and diluted to 100 ml with distilled water. Aliquots (1 ml) of diluted samples were transferred into 25 ml test tubes which were immersed in cold water, 8 ml of concentrated H_2SO_4 was added, mixed and heated in a boiling water bath for 3 min. After the incubation, tubes chilled, 0.6 ml of ninhydrin reagent [3% ninhydrin

(1,2,3 triketohydrin) in 5% $Na_2S_2O_5$] was added and the tubes were placed in a water bath at 25°C for 100 min. The solutions were then transferred into 25 ml volumetric flasks, made up to the mark with concentrated H_2SO_4 and allowed to stand for 5 min. The absorbance was measured at 595 nm using the solution without starch as the reference. A calibration curve was made using standard aqueous solutions containing 0-100 µg propylene glycol / ml. The hydroxypropyl content was calculated and expressed in terms of molar substitution.

$$\% \text{ of hydroxypropyl groups} = \frac{\mu g \text{ propylene glycol} \times 0.7763 \times 100 \times 100}{10^6 \times \text{weight of starch (g, db)}}$$

$$\text{Molar substitution} = \frac{\% \text{ hydroxypropyl groups} \times 162.14}{59.08 \times (100 - \% \text{ hydroxypropyl groups})}$$

A factor of 0.7763 was used to convert µg of propylene glycol to µg of hydroxypropyl groups (Johnson, 1969). 59.08 = molecular weight of hydroxypropyl group, 162.14 = molecular weight of anhydroglucose unit.



3.2.4 Determination of physicochemical properties

3.2.4.1 Scanning electron microscopy (SEM)

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

3.2.4.2 X-ray diffraction

X-ray diffractograms of starches were obtained with a Rigaku RU 200R X-ray diffractometer (Rigaku-Denki Co., Tokyo, Japan) with a chart speed of 20 mm/min. The starch powder was scanned through the 2θ range of 3-35°. Diffractograms were obtained using Cu-Kα radiation detector with a nickel filter and a scintillation counter operating under the following conditions : 40 kV, 50 mA, 1°/1° divergence silt / scattering slit, 0.30 mm receiving slit, 1s time constant and scanning rate of 3°/ min. The results were analyzed using the software Jade (version 2.1).

3.2.4.3 Differential scanning calorimetry (DSC)

DSC measurements on native, defatted and heat-moisture treated starches were carried out using a Perkin-Elmer DSC-2 (Norwalk, CT, USA) Differential scanning calorimeter with a thermal analysis data station. Water (8.0 μl) was added with a microsyringe to starch (2.5 mg) in DSC pans which were then sealed, reweighed and kept overnight at room temperature. The scanning temperature range and heating rate, were 20-120°C and 10°Cmin^{-1}, respectively. The thermogram was recorded with water as a reference.

The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c) of the gelatinization endotherm. Indium was used for calibration. The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and the base line under the peak and expressed as joules per unit weight of dry starch (J/g).

3.2.4.4 Swelling factor (SF)

The SF of the starches when heated to 60-90°C in excess water was determined according to the method of Tester and Morrison (1990a). Starch samples (50 mg, db) were weighed into screw cap tubes, 5 ml water were added and heated in a shaking water bath at the appropriate temperature for 30 min. The tubes were then cooled to 20°C, 0.5 ml of blue dextran (Pharmacia, M_r 2x10^6, 5 mg/ml) was added and mixed the contents by inverting the tubes. The tubes were then centrifuged at 1500xg for 5 min and the absorbance of the

supernatant was read at 620 nm using a spectrophotometer (Novospec Model 4049, LKB Biochrom, Cambridge, UK). The absorbance of the reference which contained no starch was also measured at 620 nm.

Calculation of SF was based on starch weight corrected to 10% moisture, assuming a density of 1.4 g / ml.

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

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

A_r and As represent the absorbance of the reference and sample respectively.

The initial volume of the starch (V_o) of weight W (in mg) is

$V_o = W / 1400$

and the volume of the absorbed intragranular water (V_1) is thus

$V_1 = 5.0 - FW$

Hence, the volume of the swollen starch granules (V_Z) is

$V_Z = V_o + V_1$ and

$SF = V_Z / V_o$

This can also be expressed by the single equation

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

This method measures only intragranular water and hence the true SF at a given temperature. The SF is reported as the ratio of the volume of swollen starch granule to the volume of the dry starch.

3.2.4.5 Extent of amylose leaching

Starch (20 mg, db) was heated in water (10 ml) in volume calibrated sealed tubes (50-90°C) for 30 min. The tubes were then cooled to 25°C and centrifuged at 2000g for 10 min. The supernatant liquid (0.1 ml) was withdrawn and its amylose content determined by the method of Chrastil (1987). Percentage amylose leaching was expressed as mg amylose leached per 100 g starch.

3.2.4.6 Brabender viscosity measurements (Pasting properties)

A Brabender viscoamylograph, Model VA-V (C.W.Brabender Instruments, Hackensack, NJ, USA) equipped with a 700 cm.g cartridge was used to study pasting properties of starch slurries at a concentration of 6% (w/v) and pH 5.5. The starch dispersions were stirred at 75 rpm and heated at a rate of $1.5°Cmin^{-1}$ to 95°C, kept at this temperature for 30 min, and cooled to 50°C. The viscosity was expressed in terms of Brabender units (BU) and the pasting temperature was defined as the temperature at which the viscosity showed an increase in 10 BU in the heating cycle.

3.2.5 Starch digestibility

3.2.5.1 Preparation of defatted potato starch for enzyme hydrolysis

Defatted starch was prepared by soxhlet extraction with 75% aqueous n-propanol for 1, 2 ,4, 7, 9 and 12 h. The solvent was removed by vacuum evaporation and the starch was air dried to a moisture content of ~ 10%.

3.2.5.2 Preparation of heat-moisture treated potato starch for enzyme hydrolysis

The heat-moisture treatment was essentially that of Sair (1964). Starch (15 g dry basis) was weighed into glass containers. Starch moisture content was brought to 30%. The sealed samples (in glass jars) were heated in a forced air oven (Isotemp 614G, Fisher Scientific, Fair Lawn, NJ, USA) at 100°C for 1, 3, 6, 8, 10, 16 and 30 h. After cooling the jars were opened and the starch samples air-dried to a moisture content ~10%.

3.2.5.3 Scanning electron microscopy of enzyme hydrolyzed starches

Granule morphology of native, defatted (75% n-propanol for 7 h) and heat-moisture treated (30% moisture, 100°C, 16 h) starches [before and after α-amylase hydrolysis (72 h)] were studied by using a Hitachi (S570) scanning electron microscope (Nissei Sangyo Inc., Rexdale, ON, Canada) under the operating conditions mentioned before.

3.2.5.4 Enzymatic hydrolysis

The extent of hydrolysis was determined using a crystalline suspension of porcine pancreatic α-amylase in 2.9 M saturated sodium chloride containing 3 mM calcium chloride in which the concentration of α- amylase was 30 mg/ ml and the specific activity was 790 units per mg of protein. One unit was defined as the α-amylase activity which liberates 1 mg maltose in 3 min at 20°C at pH 6.9. The procedure was essentially that of Knutson *et al.* (1982). Starch (100 mg, db) was suspended in distilled water (25 ml) and 5 ml aliquots were placed in a constant temperature water bath at 37°C. Then 4.0 ml of 0.1 M phosphate buffer (pH 6.9) containing 0.006 M NaCl were added to the slurry . The mixture was gently stirred before adding α-amylase suspension (12 units / mg starch). The reaction mixtures were shaken by hand in constant time periods to resuspend the deposited granules. Then 1 ml aliquots were removed at specific time intervals, pipetted into 0.2 ml of 95% ethanol, and centrifuged (3000g). Aliquots of the supernatant were analyzed for soluble carbohydrate (Bruner, 1964). Percentage hydrolysis was calculated as the amount (mg) of maltose released per 100 mg of dry starch. Controls without enzyme, but subjected to the above experimental conditions, were run concurrently.

3.2.6 Starch retrogradation

3.2.6.1 Sample preparation for Turbidity measurements

A 2% aqueous suspension of potato starch (native, defatted and heat-moisture treated), near neutral pH, was heated in a boiling water bath for 1 h under constant stirring. After the suspension was cooled for 1 h at 25°C, the turbidity was determined by measuring absorbance at 640 nm against a water blank with a Shimadzu UV-visible spectrophotometer (UV-260, Shimudzu Corporation, Kyoto, Japan). The development of turbidity was monitored by storing samples for 1 day at 4 °C followed by 2-35 days 40°C. This sequential incubation at 4°C and 40°C was applied to obtain extensive retrogradation in a short time by favouring nucleation (formation of crystal nuclei) at 4°C and propagation (growth of crystallites from the nuclei formed) of starch crystallites at 40°C (Wunderlich, 1976).

3.2.6.2 Gel preparation for X-ray diffraction

Gels were prepared (with minor modifications) as described by Krusi & Neukom (1984). A 3% (w/v) potato starch gel was prepared by heating the suspension under gentle stirring for 15 min in a boiling water bath. After cooling to 30°C, sufficient starch was added to obtain suspension with 40% (w/v) dry matter. These suspensions were then homogenized for 2 min at 8000 rpm and then heated in a forced air oven (Isotemp 614G, Fisher Scientific, Fair Lawn, NJ,

USA) at 110°C for 2 h. After cooling, the gels formed were stored at 4°C for 1 day followed by 29 days at 40°C.

3.2.6.2.1 Gel powder preparation for X-ray diffraction

The procedure (with minor modifications) of Routlet *et al.* (1988) was used to convert freshly gelatinized and stored gels to a powder prior to examination by X-ray diffraction. The gels were rinsed with water, cut into small pieces and mixed with 100 ml acetone. After homogenization using a polytron (T25 S-1, IKA works Inc., Cincinnati, OH, USA) the mixture was left to settle (for 5 min) and then decanted. The liquid was discarded and the rest was transferred to screw cap tubes. Acetone was again added, the mixture centrifuged (3000g) and the supernatant discarded. The procedure was repeated three times and the remaining mass was then freeze dried.

X-ray diffractograms of gel powders were obtained with a Rigaku RU 200R X-ray diffractometer under the same conditions mentioned previously.

3.2.6.3 Scanning electron microscopy of retrograded starch gels

The specimen preparation of freshly gelatinized and stored (1 day at 4°C) potato starch gels for SEM was carried out as follows : The starches (2%, w/v) were gelatinized under the conditions described for turbidity measurements and then stored for 1 day at 4°C. The gels were then freeze dried and the samples examined and photographed in a Hitachi (S570) scanning electron microscope

(Nissei Sangyo Inc., Rexdale, ON, Canada). The SEM operating conditions were carried out as outlined before.

3.2.6.4 Differential scanning calorimetry of retrograded starches

Thermal transitions of retrograded starches were investigated using a Perkin-Elmer DSC-2 (Norwalk, CT, USA) differential scanning calorimeter equipped with a thermal analysis data station. Water (3 μl) was added, with a microsyringe to starch (3 mg) in DSC pans, which were then sealed, reweighed and kept for 1 h at room temperature. The scanning temperature range and the heating rate were 20-120°C and 10°C/min, respectively. The heated pans were then cooled to room temperature and stored for 1 day at 4°C followed by 2-7 days at 40°C. After this time period, the pans were left to equilibrate for 1 h at room temperature and then scanned under the same previous conditions. In all measurements an empty pan was used as reference and experiments were repeated at least thrice.

The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c) of the retrogradation endotherm. The enthalpy of retrogradation (ΔH_R) was estimated by integrating the area between the thermogram and the baseline under the peak and expressed as joules per unit weight of dry starch.

3.2.6.5 Enzymatic hydrolysis of retrograded starches

The reactivity of porcine pancreatic α-amylase towards freshly gelatinized and retrograded potato starches was determined as follows : starch samples were dispersed in distilled water to make a 2% suspension. The dispersions were shaken while heated at 100°C for 1 h. The gelatinized starches were cooled to 30°C and then stored at 4°C for 1 day, followed by at 40°C for 14 days. At the end of the storage period, the retrograded gels were freeze dried and converted to powders prior to enzyme hydrolysis.

The extent of hydrolysis of the freshly gelatinized and retrograded starches was determined using a crystalline suspension of porcine pancreatic α-amylase in 2.9 M saturated sodium chloride containing 3 mM calcium chloride in which the concentration of α- amylase was 30 mg/ ml and the specific activity was 790 units/mg of protein. One unit was defined as the α-amylase activity which liberates 1 mg maltose in 3 min at 20°C at pH 6.9. The details of the procedure have been outlined before.

3.2.7 Statistical analysis

All experiments were done in triplicate. Analysis of variance was performed using Minitab statistical package (Minitab Inc., 1991). Duncan's new multiple range test was utilized for comparison among means.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 CHEMICAL COMPOSITION OF NATIVE POTATO STARCH

Data on the composition of isolated potato starch are presented in Table 4.1. The chemical composition showed that the starch contained 0.37% ash and 0.01% nitrogen. The nitrogen in isolated starch may come from the internal proteins (residual material from lipid-protein membranes of the original amyloplast or of membrane-bound starch synthesizing systems employed during development) [Galliard,1983], phospholipids containing ethanolamine, choline or endosperm storage proteins (Morrison, 1981). The purity of the starch was judged on the basis of composition and microscopic observations. The low values of nitrogen and ash indicated that the isolated potato starch is of high purity. Total lipids (obtained by acid hydrolysis) in potato starch (0.12%) represent free and bound starch lipids. The free lipids (obtained by extraction with $CHCl_3$-CH_3OH) amounted to 0.03%, while the corresponding values for bound lipids (obtained by extraction of the $CHCl_3$-CH_3OH residue with n-propanol water) was 0.09%. These values are in agreement with those reported by Vasanthan & Hoover (1992b) for potato starch obtained from Sigma Co (St. Louis, MO, USA). The difference between total and apparent amylose contents indicates the amount of amylose complexed with lipid. According to the results obtained, the total amylose content was 25.6% of which 16.8% was complexed by native lipids (Table 4.1).

Table 4.1 Proximate composition of native potato starch

Characteristics	Composition[1] (%)
Moisture	13.4±0.0
Ash	0.37±0.01
Nitrogen	0.01±0.01
Lipid	
Solvent extracted	
chloroform-methanol[2]	0.03±0.01
n-propanol-water[3]	0.09±0.00
Acid hydrolysed[4]	0.12±0.01
Amylose content	
total[5]	25.6±0.9
apparent[6]	21.3±0.6
Amylose complexed by lipids[7]	16.8±0.6
Starch damage	0.3±0.0

[1] All data reported on dry basis and represent the mean of 3 determinations.

[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] $\frac{\text{Total amylose - apparent amylose}}{\text{Total amylose}} \times 100$

4.2 EFFECT OF DEFATTING AND HEAT-MOISTURE TREATMENT ON THE STRUCTURE AND PHYSICOCHEMICAL PREOPERTIES OF NATIVE POTATO STARCH

4.2.1 Morphological granular characteristics

Native potato starch granules were mainly oval to elliptical in shape. The surfaces appeared to be smooth (devoid of cracks or other damages) when viewed under the scanning electron microscope (Fig. 4.1a). Neither defatting (Fig. 4.1b) nor heat-moisture treatment (Fig. 4.1c) altered the shapes or the surface characteristics of the starch granules.

4.2.2 X-ray diffraction

The X- ray patterns and X-ray intensities of native, defatted and heat-moisture treated potato starches are presented in Fig. 4.2. and Table 4.2. Native potato starch (Fig. 4.2a) exhibited the characteristic 'B' type X-ray pattern of tuber starches with peaks centered at 15.8, 5.2, 4.5, 3.9 and 3.8 Å. Defatting (Fig. 4.2b) resulted in an increase in intensity of the peak centred at 3.9 Å, a decrease in intensity of the peaks at 15.8 and 5.2 Å, elimination of the peak at 4.5 Å and in the appearance of four additional peaks at 2.9, 3.3, 3.8 and 4.6 Å (Table 4.2). Furthermore, defatting changed the X-ray pattern from 'B' to 'A+B' (the 'A' pattern is characteristic of cereal starches) with intensities at d-spacings of 5.8, 5.2 and 3.8 Å (Fig. 4.2). Heat-moisture treatment at 100°C, 30% moisture

Fig. 4.1 Scanning electron micrographs of native (A), defatted (B) and heat-moisture treated (C) potato starch granules.

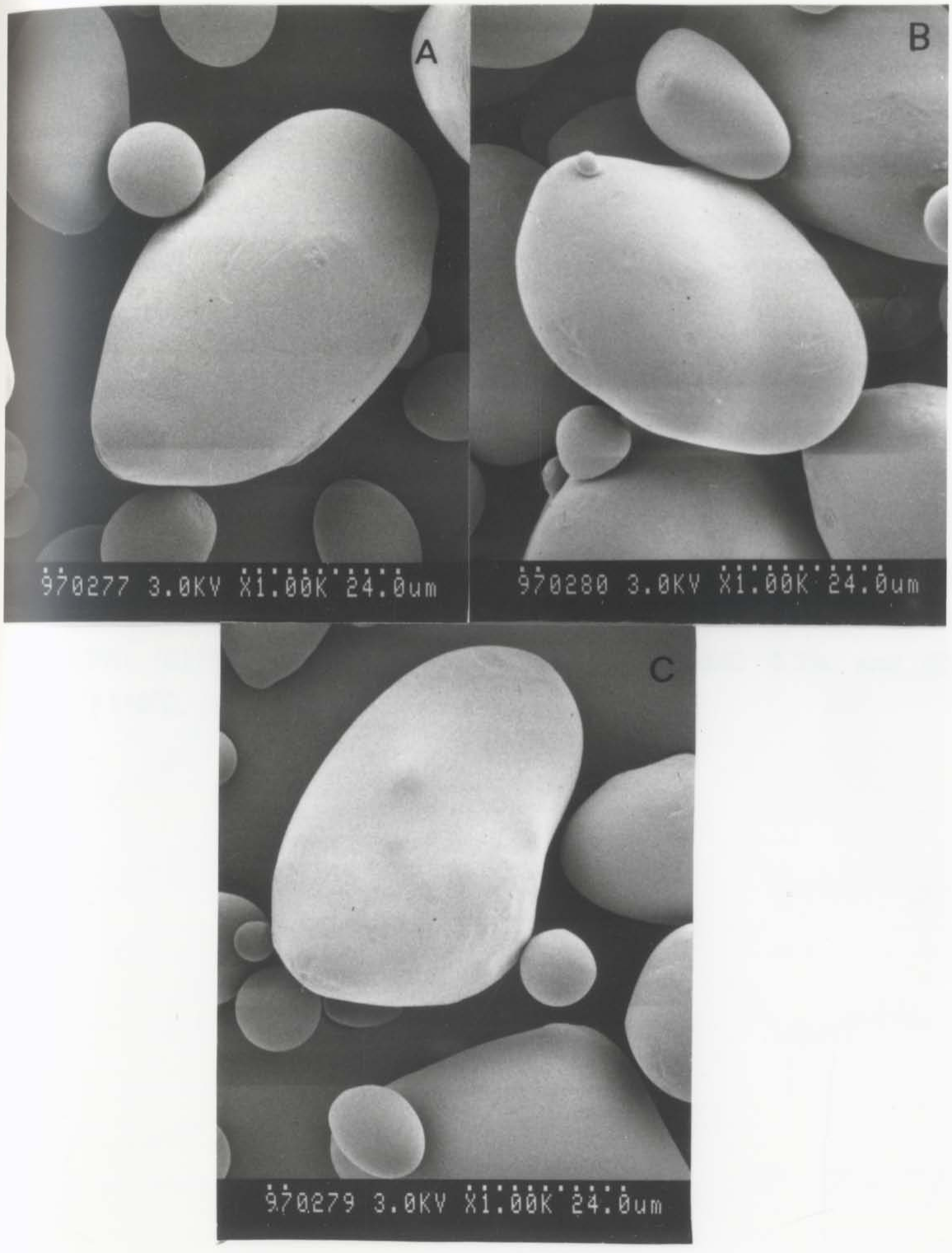

A
970277 3.0KV X1.00K 24.0um
B
970280 3.0KV X1.00K 24.0um
C
970279 3.0KV X1.00K 24.0um

Fig. 4.2 X-ray diffraction patterns of native, defatted and heat-moisture treated (HMT) potato starches. (A) native [moisture content (MC) 9.5%], (B) defatted (MC 9.3%), (C) HMT [100°C, 30% moisture, 16 h] (MC 9.3%) and (D) HMT [110°C, 30% moisture, 16 h] (MC 9.3%).

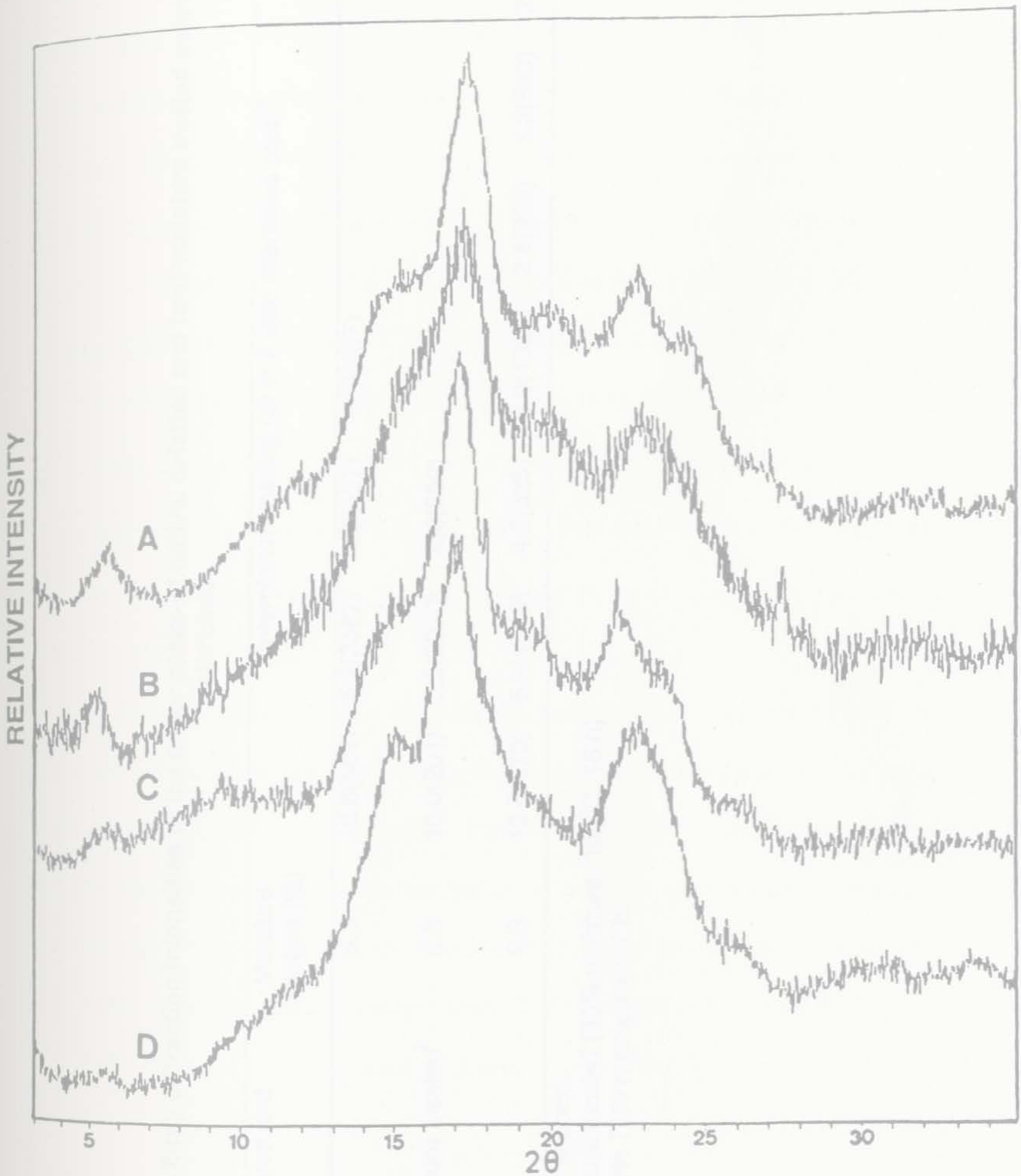

RELATIVE INTENSITY
A
B
C
D
5
10
15
20
25
30
2θ

Table 4.2 X-ray diffraction intensities of the major peaks of native, defatted and heat-moisture treated potato starches

Starch source and treatment	Moisture content (%)	Interplanar spacing (d) in Å with intensities (cps)[a]						
Native	9.5	15.8(581)	5.2(2362)	4.5(327)	3.9(867)	-	-	-
Heat-moisture treated[b]	9.3	16.0(201)	5.2(2020)	4.0(850)	-	-	-	-
Defatted[c]	9.3	15.0(570)	5.2(1979)	4.6(525)	3.9(1022)	3.8(935)	3.3(590)	2.9(491)

[a]Counts per second.
[b]Heat-moisture treated (30% moisture, 100°C, 16 h).
[c]Defatted with 75% n-propanol, 7 h.

for 16 h (Fig. 4.2c) decreased the intensity (Table 4.2) of the peaks at 15.8 (the extent of this decrease was higher than that on defatting) and 5.2 Å (similar to that observed on defatting) and increased the intensity (Table 4.2) of the peak at 4.5 Å. A change in X-ray pattern 'B' → 'A+B' was also observed on heat-moisture treatment. However, the resemblance to the 'A' X-ray pattern was more marked in heat-moisture treated (Fig. 4.2c) than in defatted potato starch (Fig. 4.2b). Heat-moisture treatment at 110°C at 30% moisture for 16 h, (Fig. 4.2d) resulted in the elimination of the peak at 15.8 Å (this peak is characteristic of 'B' type starches), an increase in intensity of the peak at 5.2 Å, and in the appearance of two new peaks centered at 5.9 and 3.8 Å. The X-ray pattern (Fig. 4.2d) after heat-moisture treatment (at 110°C) resembled those of cereal starches (Hoover & Vasanthan, 1994a).

The decrease in X-ray intensity on heat-moisture treatment (Table 4.2) can be attributed to crystallite disruption and/or to reorientation of the double helices forming the crystalline array. Imberty *et al.* (1988) and Imberty & Perez (1988) have shown that double helices of 'A' and 'B' type starches are packed in a pseudohexagonal array. The lattices of 'B' starch have a large void in which 36 water molecules can be accommodated. This void is not present in 'A' starch. In both 'A' and 'B' arrangements there is a pairing of double helices that corresponds to 1.1 nm distance between the axes of the two double helices. A transformation from 'B' to 'A' starch occurs by rearrangement of a pair of double

helices (Imberty *et al.*, 1988). The extent of formation of 'A' type unit cells would therefore depend on the kinetic energy of the double helices involved in this arrangement. This seems plausible, since more 'A' type unit cells are formed when the temperature of heat-moisture treatment is increased from 100 to 110°C (Figs. 4.2c, d). Le Bail *et al.* (1993) also demonstrated the development of 'A+B' pattern from 'B' type short chain amylose (35% moisture) when heated to 112°C, whereas perfect 'A' type was obtained at 152°C.

The increase in X-ray intensities on defatting (Table 4.2) suggests that a clustering of the outer 'A' chains of amylopectin may have occurred resulting in the formation of new crystallites (that are perfectly arrayed to diffract X-rays). It is highly unlikely that the increase in X-ray intensity is due to reorientation of existing crystallites since the moisture and thermal energy (82°C) during defatting is too low to impart the required kinetic energy for reorientation.

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

The SF and AML at different temperatures (50-90°C) are presented in Tables 4.3 and 4.4. The SF and AML of native, defatted and heat-moisture treated starches increased with rise in temperature (native > defatted > heat-moisture treated). Both defatting and heat-moisture treatment decreased SF and AML (Tables 4.3 & 4.4), which is in agreement with the resulted by Stute (1992), Hoover & Vasanthan (1994a) and Hoover & Manuel (1996a) .

Table 4.3 Swelling factor of native, defatted and heat-moisture treated potato starches

Starch source and treatment	Swelling factor Temperature (°C)			
	60	70	80	90
Native	21.5±1.2[a]	34.8±1.0[a]	56.7±0.9[a]	77.8±1.4[a]
Heat-moisture treated[1]	5.6±0.3[c]	10.0±0.5[c]	12.6±0.4[c]	20.1±1.1[c]
Defatted[2]	6.7±0.5[b]	12.6±0.8[b]	15.8±0.9[b]	27.4±1.0[b]

[1]Heat-moisture treated (30% moisture, 100°C, 16 h).
[2]Defatted with 75% n-propanol, 7 h.
[a-c]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

Table 4.4 Amylose leaching of native, defatted and heat-moisture treated potato starches.

Starch source and treatment	Amylose leaching (%)				
	Temperature °C				
	50	60	70	80	90
Native	4.1±0.3[a]	7.3±0.4[a]	13.6±0.4[a]	16.1±0.6[a]	22.7±1.4[a]
Heat-moisture treated[1]	0.7±0.2[b]	1.4±0.6[c]	3.5±0.4[c]	6.1±0.5[c]	8.3±0.6[c]
Defatted[2]	1.5±0.7[b]	3.2±0.4[b]	6.6±0.3[b]	8.4±0.4[b]	13.4±0.7[b]

[1]Heat-moisture treated (30% moisture,100°C, 16 h).
[2]Defatted with 75% n-propanol, 7 h.
[a-c]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

The decrease in AML, suggests interaction between amylose-amylose (AM-AM) and/or amylose-amylopectin (AM-AMP) chains. These interactions decrease the number of hydroxyl groups that can potentially bind to water molecules. This would then partially explain the decrease in SF on defatting and heat-moisture treatment. Tester & Morrison (1990a,b) have shown, by comparative studies on non waxy and waxy maize starches, that swelling is primarily a property of amylopectin and that amylose is a diluent. Furthermore, Cooke & Gidley (1992) have shown that the forces holding the granule together are mainly at the double helical level. The large decrease in SF on heat-moisture treatment implies that some of the double helices (free and/or present in crystalline lattices) may have unraveled on heat-moisture treatment. This seems plausible, since X-ray diffraction intensities decrease on heat-moisture treatment (Fig. 4.2). Thus, both starch chain interactions and loss of double helical order contribute to the decrease in SF on heat-moisture treatment. The decrease in SF is less pronounced on defatting due to the interplay of two factors : 1) AM-AM and/or AM-AMP interactions which tend to decrease the SF and 2) the increase in crystallinity (Fig. 4.2) (which suggests an increase in double helical order) which tends to increase the SF.

4.2.4 Differential Scanning Calorimetry

The influence of defatting and heat-moisture treatment on gelatinization temperatures [onset (T_o), mid point (T_p) and conclusion (T_c)] and gelatinization

enthalpy (ΔH) are presented in Table 4.5. T_o, T_p and T_c increased on defatting and heat-moisture treatment (heat-moisture treatment > defatting). Gelatinization enthalpy decreased by 5.3 J/g on heat-moisture treatment, and slightly increased on defatting. The gelatinization temperature range (T_c-T_o) increased by 2.5 and 6.0°C, respectively, on defatting and heat-moisture treatment. Similar observations have also been reported on heat-moisture treatment and defatting of cereal, legume and tuber starches (Lorenz & Kulp 1982; Donovan *et al.*, 1983; Stute, 1992; Hoover & Vasanthan, 1994a, Hoover & Manuel, 1996a,b).

The gelatinization of starch is considered to be a solvent and heat induced melting of crystallites. When starch is heated in excess water, the water penetrates into the more accessible amorphous region of the starch granule, resulting in hydration and limited swelling. The swelling of the amorphous region imparts a stress on the crystalline region and thereby disrupts the polymer chains in the starch crystallites (Donovan, 1979). Therefore, any starch attributes that suppressed swelling would delay gelatinization and thus lead to a high T_o, T_p and T_c. Cooke & Gidley (1992) have shown that both crystalline and double helical order are lost concomitantly during gelatinization. Hoover & Manuel (1996a) have shown that the increase in T_o, T_p and T_c on heat-moisture treatment of maize starches follows the order : amylomaize V (65.5 % amylose) > normal maize (29.9% amylose) > waxy maize (1.2 % amylose). Furthermore, T_c-T_o remains unchanged in waxy maize, but increases respectively, by 6 and

Table 4.5 Gelatinization parameters of native, defatted and heat-moisture treated starches

Starch source and treatment	Transition temperatures (°C)				Enthalpy
	T_o[1]	T_p[1]	T_c[1]	T_c-T_o[2]	ΔH (J/g)[3]
Native	54.0±0.9[c]	61.0±0.6[c]	65.5±0.8[c]	11.5±0.3[c]	16.2±0.4[b]
Heat-moisture treated[5]	63.5±0.4[a]	73.5±0.5[a]	81.0±0.3[a]	17.5±0.1[a]	10.9±0.8[c]
Defatted[6]	59.0±0.5[b]	65.5±0.4[b]	73.0±0.3[b]	14.0±0.2[b]	17.1±0.2[a]

[1] T_o, T_p, & T_c indicate respectively, the temperature of onset, mid point, and end of gelatinization.

[2] Gelatinization temperature range.

[3] Enthalpy of gelatinization.

[4] Parent starch treated with NaOH and Na_2SO_4 at 40°C, but without addition of propylene oxide.

[5] Heat-moisture treated (30% moisture, 100°C, 16 h).

[6] Defatted with 75% n-propanol, 7 h.

[a-c] Means within a column with different superscripts are significantly different ($p \leq 0.05$).

8°C, on heat-moisture treatment of normal maize and amylomaize V starches. This indicates that the increase in T_o, T_p and T_c (Table 4.5) reflect melting of crystallites that were formed solely due to interaction between AM-AM and AM-AMP chains during heat-moisture treatment. As discussed earlier, interaction between AM-AM and/or AM-AMP chains are stronger on heat-moisture treatment. Therefore, suppression of granule swelling would be greater on heat-moisture treatment than on defatting (Table 4.3). Consequently, the destabilization effect of the amorphous regions on crystallite melting would be less pronounced in heat-moisture treated than in defatted granules. This would then explain the higher increases in T_o, T_p and T_c on heat-moisture treatment (Table 4.5). The increase in T_c-T_o on defatting and heat-moisture treatment reflects the melting of crystallites (that were formed by interaction between AM-AM and/or AM-AMP chains) of different stability. The decrease in ΔH on heat-moisture treatment suggests that double helices present in crystalline and non-crystalline arrays may have disrupted under the conditions prevailing during heat-moisture treatment. The increase in ΔH on defatting (Table 4.5) suggests that additional double helices may have formed under the conditions prevailing during defatting.

4.2.5 Brabender viscosities (Pasting characteristics)

The pasting characteristics of the starches at a concentration of 6% (w/v) and pH 5.5 were investigated with the Brabender viscoamylograph and the

results are presented in Table 4.6. Heat-moisture treatment and defatting increased the pasting temperature, by 27.7 and 24.7°C, respectively. The viscosity at 95°C decreased by 1140 BU (Brabender Units) and 560 BU, respectively, on heat-moisture treatment and defatting. The viscosity during the holding cycle (at 95°C) decreased by 670 BU in native potato starch, whereas it increased by 170 BU and 440 BU, respectively, in heat-moisture treated and defatted potato starches. All three starches showed an increase in viscosity during the cooling cycle (defatted > heat-moisture treated > native) [Table 4.6]. Similar observation have been reported for heat-moisture treated and defatted legume (Hoover *et al.*, 1993; Hoover & Vasanthan, 1994a) and tuber (Stute, 1992; Hoover & Vasanthan, 1994a) starches.

The increased pasting temperature and the decreased viscosity at 95°C on heat-moisture treatment (Table 4.6) reflects, to a large extent, the decrease in granular crystallinity (which decrease both granular rigidity and the volume fraction occupied by the swollen granules) and the interaction between AM-AM and/or AM-AMP chains (which decreases granular swelling). The pasting curve of defatted potato starch reflects the interplay between the increase in granule crystallinity (which increases both granular rigidity and the volume fraction occupied by the swollen granules) and the interaction between starch chains (which decreases granular swelling). This would then explain the more pronounced changes in the pasting properties on heat-moisture treatment. In native potato starch, the breakdown in viscosity during the holding (Table 4.6)

Table 4.6 Brabender viscosities (Pasting properties) of native, defatted and heat-moisture treated potato starches

Starch source and treatment	Pasting temperature (°C)	Viscosity at 95°C (BU[1])	Viscosity after 30 min at 95°C (BU[1])	Viscosity at 50°C (BU[1])
Native	62.3±0.5[c]	1190±10[a]	520±10[b]	715±10[b]
Heat-moisture treated[2]	90.0±0.5[a]	50±5[c]	220±5[c]	350±5[c]
Defatted[3]	87.0±1.0[b]	630±5[b]	1070±15[a]	1500±15[a]

[1]Brabender units.
[2]Heat-moisture treated (30% moisture, 100°C, 16 h).
[3]Defatted with 75% n-propanol, 7 h.
[a-c]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

cycle (at 95°C) can be attributed to weak associative bonding forces within the granule interior. However, the additional interactions that occur between AM-AM and AM-AMP chains on defatting and heat-moisture treatment impart shear and thermal stability during the holding cycle.

4.3 THE EFFECT OF ALKALINE TREATMENT ON THE STRUCTURE AND PROPERTIES OF NATIVE, DEFATTED AND HEAT-MOISTURE TREATED STARCHES

Sodium hydroxide and sodium sulphate are used in the preparation of hydroxypropylated starches to ensure good reaction efficiency and to depress granular swelling and gelatinization. Therefore, it was deemed necessary to investigate the influence of above reagents (under the conditions prevailing during hydroxypropylation, but in the absence of propylene oxide) on the structure and properties of native, defatted and heat-moisture treated starches.

4.3.1 Morphological granular characteristics

The scanning electron micrographs showed that alkali treatment did not alter the shape (oval to elliptical) and appearence of native, defatted and heat-moisture treated starch granules.

4.3.2 X-ray diffraction of alkali treated starches

The X-ray intensities and X-ray patterns of the control starches are presented respectively in Table 4.7 and Fig. 4.3. The X-ray intensities and X-ray patterns of native and defatted starches were drastically altered after alkaline treatment. The altered X-ray patterns (neither 'A', 'B' or 'C') were identical in both starches (Figs. 4.3 a,c). However, for heat-moisture treated starch, X-ray intensities decreased only marginally (Table 4.7) and the X-ray pattern remained unchanged after alkaline treatment (Fig. 4.3b).

4.3.3 Swelling factor (SF) and amylose leaching (AML) of alkali treated starches

In native, defatted and heat-moisture treated starches the SF at all temperatures (60 - 90°C) increased after alkaline treatment (Table 4.8). The increase in SF followed the order : native > defatted > heat-moisture treated. However, AML (at 90°C) in all three starches remained unaffected (Table 4.9) after alkaline treatment.

4.3.4 Differential Scanning Calorimetry of alkali treated starches

In all three starches (native, defatted and heat-moisture treated starches with no added propylene oxide), the gelatinization transition temperatures and the gelatinization transition temperature range (T_c-T_o) remained unaltered after

Table 4.7 X-ray diffraction intensities of the major peaks of native, defatted and heat-moisture treated potato starches before and after alkaline treatment

Starch source and treatment	Moisture content (%)	Interplanar spacing (d) in Å with intensities (cps)[1]						
Native								
before treatment	9.5	15.8(581)	5.2(2362)	4.5(327)	3.9(867)	-	-	-
after treatment[2]	9.3	-	5.3(1212)	4.0(576)	-	-	-	-
Heat-moisture treated[3]								
before treatment	9.3	16.0(201)	5.2(2020)	4.0(850)	-	-	-	-
after treatment[2]	9.4	15.8(369)	5.2(1949)	3.8(1069)	-	-	-	-
Defatted[4]								
before treatment	9.3	15.0(570)	5.2(1979)	4.6(525)	3.9(1022)	3.8(935)	3.3(590)	2.9(491)
after treatment[2]	9.5	-	5.4(1426)	-	3.9(698)	-	-	-

[1]Counts per second.
[2]Parent starch treated with NaOH and Na_2SO_4 at 40°C.
[3]Heat-moisture treated (30% moisture, 100°C, 16 h).
[4]Defatted with 75% n-propanol, 7 h.

Fig. 4.3 X-ray diffraction patterns of native, heat-moisture treated (HMT) [30% moisture, 100°C, 16 h] and defatted (75% n-propanol, 7 h) potato starches after alkaline treatment (NaOH and Na_2SO_4) at 40°C. (A) native after alkaline treatment (MC% 9.3), (B) HMT after alkaline treatment (MC 9.4%), (C) defatted after alkaline treatment (MC 9.5%).

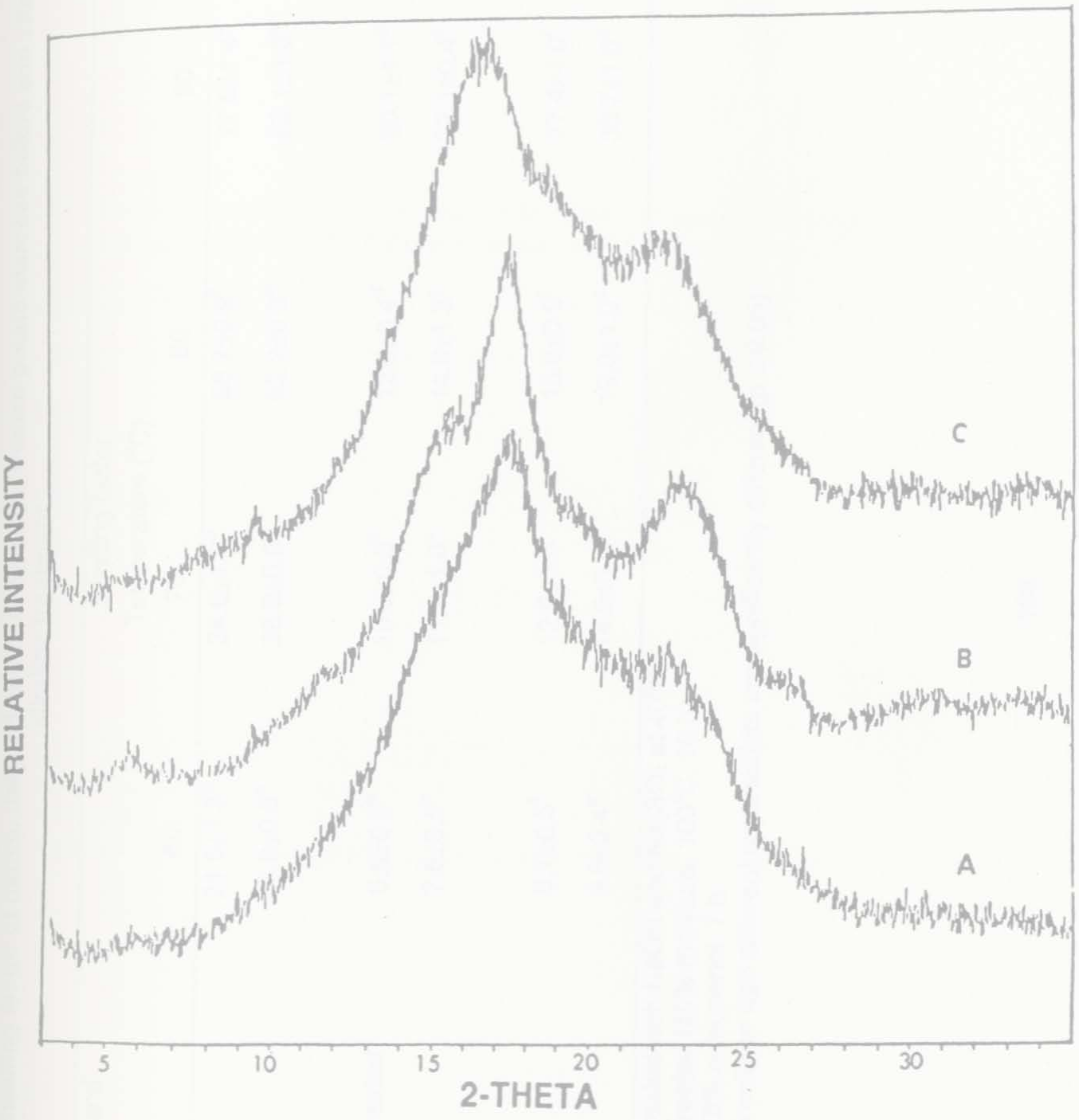

RELATIVE INTENSITY
C
B
A
5
10
15
20
25
30
2-THETA

Table 4.8 Swelling factor of native, defatted and heat-moisture treated potato starches before and after alkaline treatment

Starch source and treatment	Swelling factor Temperature (°C)			
	60	70	80	90
Native	21.5±1.2[b]	34.8±1.0[b]	56.7±0.9[b]	77.8±1.4[b]
Alkali treated[1]	25.9±0.8[a]	38.9±0.9[a]	62.2±0.3[a]	82.1±1.3[a]
Heat-moisture treated[2]	5.6±0.3[d]	10.0±0.5[d]	12.6±0.4[d]	20.1±1.1[d]
Alkali treated[1]	7.6±0.5[c]	11.9±1.0[c]	14.8±1.2[c]	22.1±0.4[c]
Defatted[3]	6.7±0.5[f]	12.6±0.8[f]	15.8±0.9[f]	27.4±1.0[f]
Alkali treated[1]	9.5±0.4[e]	14.5±0.5[e]	19.0±1.0[e]	30.7±1.0[e]

[1]Parent starch treated with NaOH and Na_2SO_4 at 40°C.
[2]Heat-moisture treated (30% moisture, 100°C, 16 h).
[3]Defatted with 75% n-propanol, 7 h.
[a-f]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

Table 4.9 Amylose leaching (at 90°C) of native, defatted and heat-moisture treated potato starches before and after alkaline treatment

Starch source and treatment	Amylose leaching (%)
Native	22.7±1.4[a]
Alkali treated[1]	21.9±1.0[a]
Heat-moisture treated[2]	8.3±0.6[c]
Alkali treated[1]	8.5±0.9[c]
Defatted[3]	13.4±0.7[b]
Alkali treated[1]	13.8±0.5[b]

[1]Parent starch treated with NaOH and Na_2SO_4 at 40°C.
[2]Heat-moisture treated (30% moisture, 100°C, 16 h).
[3]Defatted with 75% n-propanol, 7 h.
[a-c]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

treatment. However, ΔH decreased by 5.3, 1.3 and 1.2 J/ g, respectively, in native, heat-moisture treated and defatted potato starches after alkali treatment (Table 4.10).

4.3.5 Brabender viscosities (pasting characteristics) of alkali treated starches

In all three (native, defatted and heat-moisture treated) starches, the pasting temperature changed only marginally on alkaline treatment (Table 4.11). However, the viscosity at 95°C decreased by 440, 20 and 300 BU, respectively, in native, heat-moisture treated and defatted starches (Table 4.11). Alkaline treatment improved the thermal stability (during the holding period at 95°C) of all three starches.

The principal sequence of events during gelatinization is postulated to be disordering of crystalline clusters, then dissociation of double helices to give loosely ordered (semi random) chains (Tester & Morrison, 1990a). The gelatinization endotherm is given by clusters of double helices (formed from the comparatively short free ends of A and B chains in amylopectin) rather than by separated double helices (Tester & Morrison, 1990a). The ΔH values mainly reflect the loss of double helical order rather than loss of crystalline register (Cook & Gidley, 1992; Whittam *et al.*, 1990, 1991). This means that the forces

Table 4.10 Gelatinization parameters of native, defatted and heat-moisture treated starches before and after alkaline treatment

Starch source and treatment	Transition temperatures (°C)				Enthalpy
	T_o[1]	T_p[1]	T_c[1]	T_c-T_o[2]	ΔH (J/g)[3]
Native	54.0±0.9[a]	61.0±0.6[a]	65.5±0.8[a]	11.5±0.3[a]	16.2±0.4[a]
Alkali treated[4]	54.5±0.2[a]	61.4±0.1[a]	66.4±0.6[a]	11.9±0.2[a]	10.9±0.2[b]
Heat-moisture treated[5]	63.5±0.4[c]	73.5±0.5[c]	81.0±0.3[c]	17.5±0.1[c]	10.9±0.8[c]
Alkali treated[4]	63.0±0.0[c]	73.0±0.2[c]	80.0±0.1[d]	17.0±0.4[c]	9.6±0.1[d]
Defatted[6]	59.0±0.5[e]	65.5±0.4[e]	73.0±0.3[e]	14.0±0.2[e]	17.1±0.2[e]
Alkali treated[4]	58.0±0.4[e]	65.0±0.1[e]	72.5±0.3[e]	14.5±0.2[e]	15.9±0.3[f]

[1]T_o, T_p, & T_c indicate respectively, the temperature of onset, mid point, and end of gelatinization.

[2]Gelatinization temperature range.

[3]Enthalpy of gelatinization.

[4]Parent starch treated with NaOH and Na_2SO_4 at 40°C, but without addition of propylene oxide.

[5]Heat-moisture treated (30% moisture, 100°C, 16 h).

[6]Defatted with 75% n-propanol, 7 h.

[a-o] Means within a column with different superscripts are significantly different ($p \leq 0.05$).

Table 4.11 Brabender viscosities (Pasting properties) of native, defatted and heat-moisture treated potato starches before and after alkaline treatment

Starch source & treatment	Pasting temperature (°C)	Viscosity at 95°C (BU[1])	Viscosity after 30 min at 95°C (BU[1])	Viscosity at 50°C (BU[1])
Native	62.3±0.5[b]	1190±10[a]	520±10[b]	715±10[b]
Alkali treated[2]	64.5±0.5[a]	750±10[b]	870±10[a]	1140±20[a]
Heat-moisture treated[3]	90.0±0.5[d]	50±5[c]	220±5[d]	350±5[c]
Alkali treated[2]	91.5±0.5[c]	30±5[d]	130±10[c]	230±5[d]
Defatted[4]	87.0±1.0[e]	630±5[e]	1070±15[e]	1500±15[e]
Alkali treated[2]	87.5±1.0[e]	330±5[f]	940±5[f]	1460±10[f]

[1]Brabender units.
[2]Parent starch treated with NaOH and Na_2SO_4 at 40°C.
[3]Heat-moisture treated (30% moisture, 100°C, 16 h).
[4]Defatted with 75% n-propanol, 7 h.
[a-f]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

responsible for structural stability of starch granules are largely at the double helical level, and that chain packing energy contributions are insignificant.

The lack of influence of the alkaline treatment on T_o, T_p and T_c, (Table 4.10) suggests that double helices comprising the crystalline clusters are not disrupted during alkaline treatment. Therefore, the extent of decrease in ΔH, suggests that the alkaline treatment disrupts double helices (native > defatted > heat-moisture treated) present within the amorphous regions of the granule. The smaller decrease in ΔH for alkali treated defatted and heat-moisture treated starches, suggest that less double helices are available within the amorphous regions of these starches for disruption by alkali. This indicates, that during the process of defatting and heat-moisture treatment the thermal energy imparted to the starch chains (heat-moisture treatment > defatting) may have caused some disruption of double helices within the amorphous domains of the granule. Double helices disrupt to a greater extent on heat-moisture treatment than on defatting due to the higher thermal energy imparts to the starch chains during heat-moisture treatment (100°C *vs* 82°C during defatting).

The extent of increase in SF (Table 4.8) on alkaline treatment (native > defatted > heat-moisture treated) also suggests that double helices within amorphous regions disrupt (exposes more hydroxyl groups for water interaction) on alkaline treatment.

The decrease in X-ray intensities and the change in the X-ray patterns (Fig. 4.3) of alkali treated native and defatted starches are more likely due to a

change in crystallite orientation rather than crystallite disruption (crystallite disruption would have altered the gelatinization transition temperatures).

It is necessary at this stage to give a brief description of the 'A' and 'B' type X-ray patterns of starches, which will allow a subsequent discussion of the changes in X-ray patterns on alkaline treatment. The 'A' X-ray pattern which is typical of cereal starches, consists of chains which are crystallized in a monoclinic lattice unit having the maltotriose as a repeating unit and 4 water molecules per unit cell. The hexagonal sub cell of the 'B' pattern of tuber starches has a more 'open' packing of double helices, and a maltose moiety as an asymmetric unit, and 36 water molecules (at ~27% w/w hydration) per unit cell. Fifty percent of the water is tightly bound to the chains and the other half is only connected to the other water (Imberty & Perez, 1988; Imberty *et al.*, 1988). This indicates that the number of water molecules within the unit cell of the starches used in this study follow the order : native ('B' type unit cell) > defatted ('A'+'B' type unit cell) > heat-moisture treated (mainly 'A' type unit cells). Crystallite reorientation on alkaline treatment (native > defatted) can be attributed to a decrease (native > defatted) in the number of water molecules (due to the dehydrating action of Na_2SO_4) within the unit cells of native and defatted starches. Crystallite reorientation does not occur to any significant extent in heat-moisture treated potato starch, since the sulphate ions are unable to access the water molecules within the compactly packed 'A' type unit cells.

The decrease in viscosity 95°C (native > defatted > heat-moisture treated), and the increase in granular resistance towards shear during the holding cycle at 95°C (native > defatted > heat-moisture treated) on alkaline treatment (Table 4.11) suggest an increase in granular rigidity. Amylose leaching (Table 4.9) and DSC (Table 4.10) studies have shown that interactions do not occur between starch chains on alkaline treatment. Therefore, the increase in granular rigidity may have been solely due to crystallite reorientation.

4.5 EFFECT OF DEFATTING AND HEAT-MOISTURE TREATMENT ON THE HYDROXYPROPYLATION OF NATIVE POTATO STARCH

The extent of hydroxypropylation was quantified and expressed in terms of Molar substitution (MS). The MS of native, defatted and heat-moisture treated potato starches by hydroxypropyl groups at different levels of propylene oxide (2-25% v/w) are presented in Fig. 4.4. There were no significant difference ($p > 0.05$) in MS between the starches up to a level of 5% propylene oxide. Thereafter, the extent of MS followed the order : heat-moisture treated > native > defatted.

The extent of MS (at 10% propylene oxide) increased with the time course of heat-moisture treatment, but decreased during the time course of defatting (Fig. 4.5). As described earlier, the amount of amorphous regions within the starch granule increases on heat-moisture treatment, but decrease on defatting. Thus, the differences in MS among the three starches at the same level of

Fig. 4.4 The level of molar substitution (MS) of native, heat-moisture treated and defatted potato starch by hydroxypropyl groups.

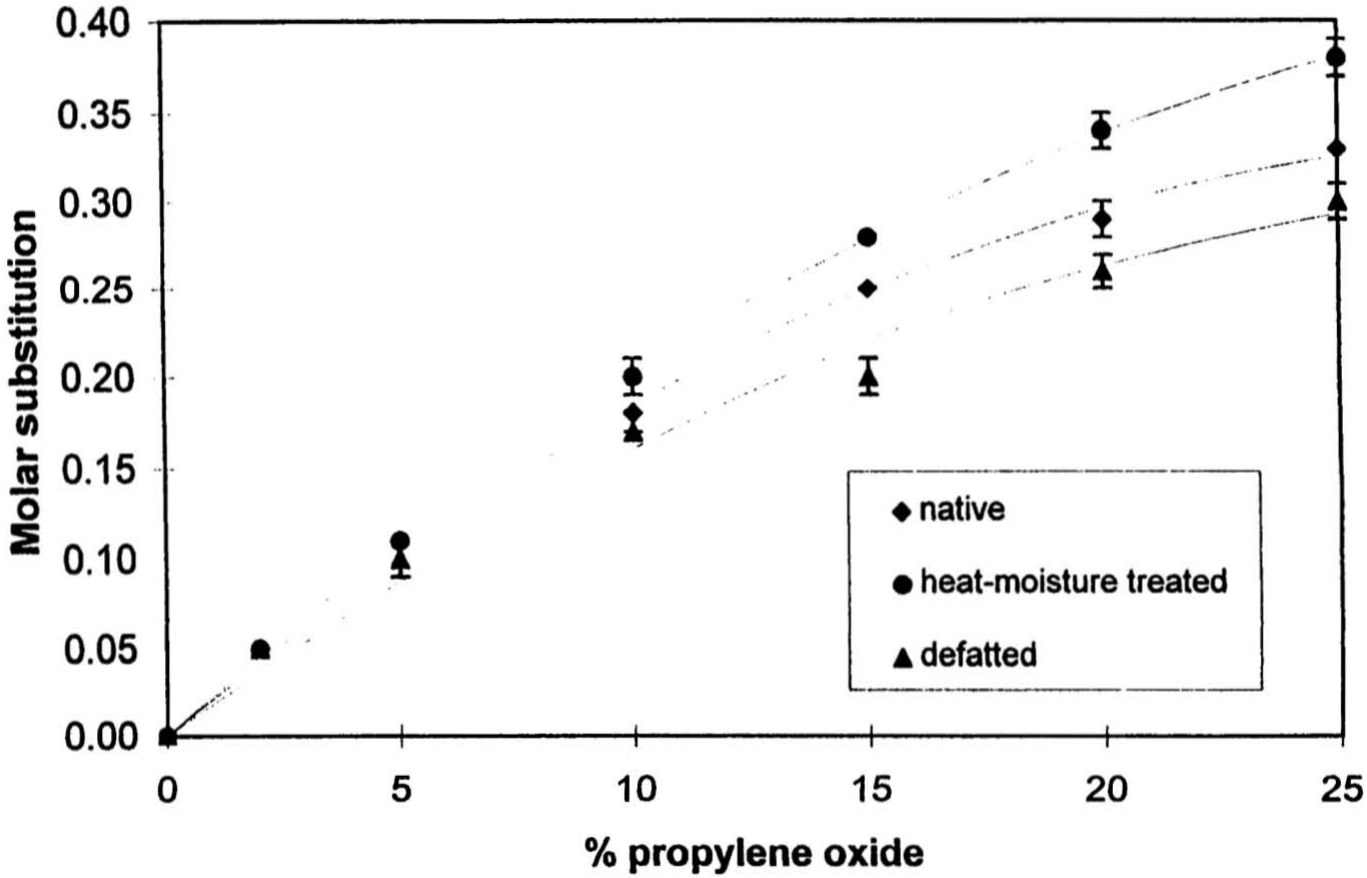
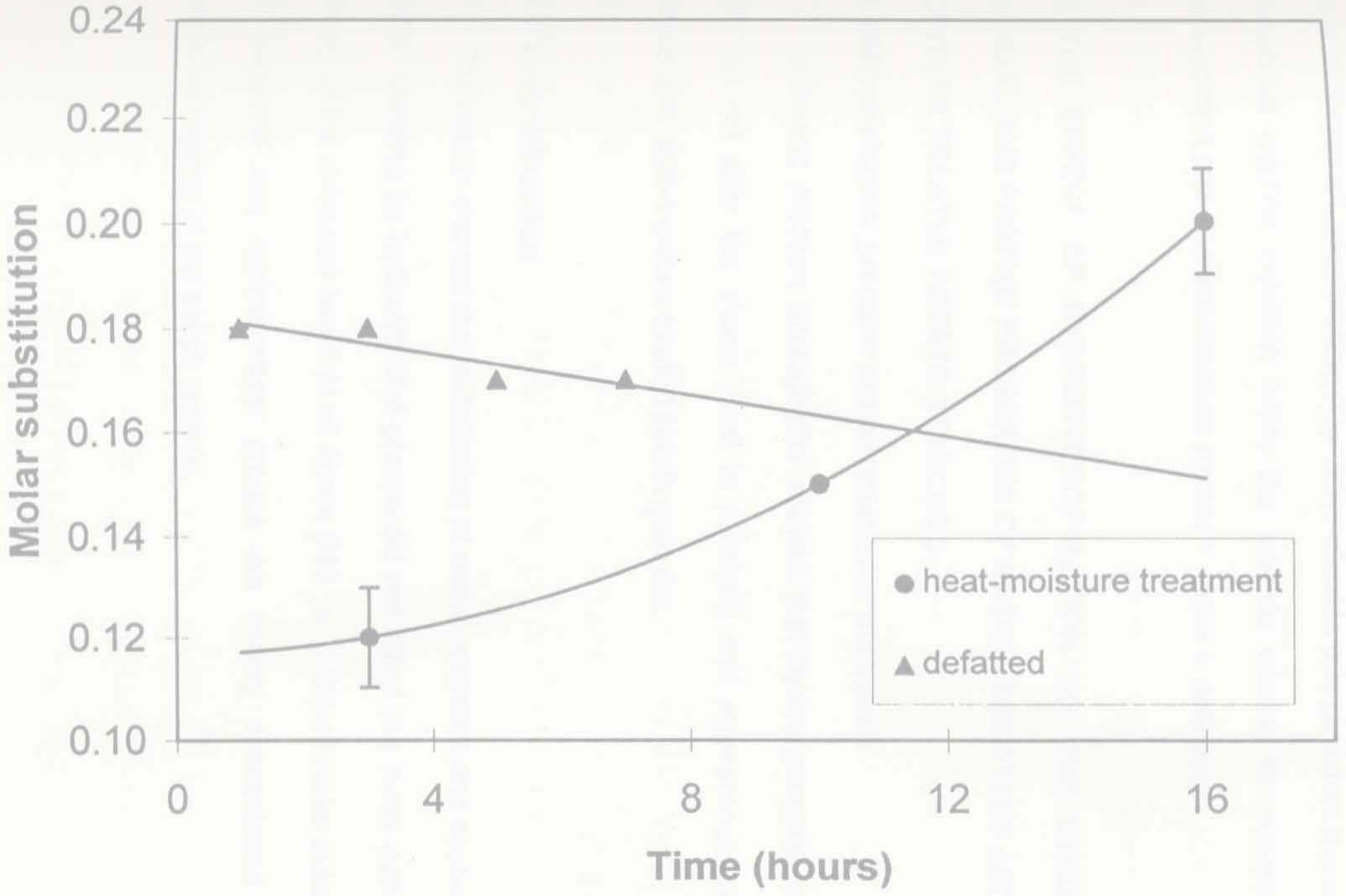

0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Molar substitution
0
5
10
15
20
25
% propylene oxide
native
heat-moisture treated
defatted

Fig. 4.5 Influence of time of defatting (75% n-propanol) and heat-moisture treatment (30% moisture, 100°C) on MS level.


0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
Molar substitution
0
4
8
12
16
Time (hours)
heat-moisture treatment
defatted

propylene oxide and the changes in the level of MS with increase in time of heat-moisture treatment and defatting (Fig. 4.5), reflect to a large extent the amount of amorphous regions available within the granule interior for substitution by hydroxypropyl groups (heat-moisture treated > native > defatted).

4.5 THE EFFECT OF HYDROXYPROPYLATION ON THE STRUCTURE, THERMAL AND PASTING PROPERTIES OF NATIVE, DEFATTED AND HEAT-MOISTURE TREATED POTATO STARCHES

4.5.1 Morphological granular characteristics of the starch

Scanning electron micrographs showed that hydroxypropylation (MS ≤ 0.28) did not alter the shape (oval to elliptical) and appearence of native, defatted and heat-moisture treated starch granules.

4.5.2 X-ray diffraction

The results showed that substitution of native, defatted and heat-moisture treated starches by hydroxypropyl groups did not affect the X-ray pattern and intensity of the diffracted beam at all levels (MS ≤ 0.28) of molar substitution. This suggests that hydroxypropyl groups are mainly concentrated in the amorphous regions of the starch granule.

4.5.3 Swelling factor (SF)

The SF of control and hydroxypropylated potato starches in the temperature range 60-90°C are presented in Table 4.12. At 60, 70, 80 and 90°C, the SF of hydroxypropylated native starch increased (with reference to control starch), by 28.6, 15.4, 14.3 and 9.6%, respectively as the MS increased from 0 to 0.05 (Table 4.12). Thereafter, the SF at 60°C increased only marginally with increase in MS (0.05 → 0.25). However, at 70 and 80°C, the SF began to decrease at MS 0.25. However, at 90°C the decline in SF was seen at MS 0.11. In hydroxypropylated heat-moisture treated starches, the SF at 60, 70, 80 and 90°C increased respectively, by 5.3, 18.5, 40.5 and 45% as the MS increased from 0 to 0.05. Thereafter, the SF at 60, 70 and 80°C increased only marginally with increase in MS (0.05 → 0.28) (Table 4.12). However, at 90°C, the SF began to decrease at MS 0.28. In hydroxypropylated defatted starches, the increase in SF [60°C (6.2%), 70°C (23.0%), 80°C (63.0%) and 90°C (66.4%)] with increase in MS (0 → 0.05), was much higher than that observed for native and heat-moisture treated starches at the same level of MS (0.05). Furthermore, in defatted starches the SF continued to increase with increase in MS (0.05 → 0.20) at all temperatures (Table 4.12).

The introduction of bulky hydroxypropyl groups into the starch molecule may be considered as a reaction which disrupts associative hydrogen bonds within the amorphous regions of the starch granule. Thus, the increase in SF

Table 4.12 Swelling factor of hydroxypropyl native, defatted and heat-moisture treated potato starches

Starch source & treatment	Molar substitution	Swelling factor Temperature (°C)			
		60	70	80	90
Native	0.00[1]	25.9±0.8[d]	38.9±0.9[c]	62.2±0.3[b]	82.1±1.3[b]
	0.05	33.3±0.8[c]	44.9±0.6[b]	71.1±0.8[a]	90.0±0.9[a]
	0.11	36.0±0.6[b]	49.8±0.6[a]	71.1±0.2[a]	82.6±0.4[b]
	0.18	37.6±0.6[a]	50.2±1.3[a]	71.9±0.4[a]	81.5±0.8[b]
	0.25	38.0±0.4[a]	39.6±1.4[c]	58.5±1.0[c]	71.9±0.6[d]
Heat-moisture treated[2]	0.00[1]	7.6±0.5[h]	11.9±1.0[h]	14.8±1.2[h]	22.1±0.4[g]
	0.05	8.0±1.2[h]	14.1±1.3[h]	20.8±0.6[g]	32.1±0.4[f]
	0.11	11.1±0.8[g]	17.2±0.3[g]	22.5±0.7[f]	33.0±1.1[f]
	0.20	12.6±0.4[f]	18.7±0.7[f]	24.6±0.5[e]	35.2±1.0[e]
	0.28	19.5±0.6[e]	21.9±0.7[e]	25.7±0.7[e]	32.0±1.2[f]
Defatted[3]	0.00[1]	9.5±0.4[m]	14.5±0.5[m]	19.0±1.0[m]	30.7±1.0[m]
	0.05	15.4±0.4[l]	17.9±0.3[l]	31.1±0.4[l]	51.1±0.8[l]
	0.10	16.6±0.2[k]	20.5±0.4[k]	38.1±0.8[k]	56.4±0.7[k]
	0.17	20.4±0.5[j]	26.9±1.0[j]	43.1±0.5[j]	60.2±0.6[j]
	0.20	28.0±0.6[i]	38.0±1.1[i]	55.8±1.0[i]	70.6±0.6[i]

[1]Parent starch treated with NaOH and Na_2SO_4 at 40°C.
[2]Heat-moisture treated (30% moisture, 100°C, 16 h).
[3]Defatted with 75% n-propanol, 7 h.
[a-m]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

with increase in MS could be attributed to interaction of water molecules with the released hydroxyl groups of the starch chains (which were previously hydrogen bonded), and with the hydroxyl group of the hydroxypropyl chain. Polysubstitution on hydroxypropyl substituents already present will not increase the amount of hydroxyl groups. Thus, the marginal increase in SF with increase in MS could only be attributed to increased disruption of the associative hydrogen bonds by the hydroxypropyl groups. The decrease in SF observed at 70 and 80°C for native hydroxypropylated potato starch at MS 0.25 could be attributed to disruption (due to increased flexibility of the hydroxypropyl chains at these temperatures) of the associative bonding forces within the amorphous regions of the granule. This disruption of local order would reduce the rigidity of the granule, thereby decreasing its ability to swell. This seems plausible since at 90°C the decrease in SF begins at a much lower MS (0.11).

As discussed earlier, heat-moisture treatment causes additional interactions to occur between starch chains in the amorphous region. This, would then explain why the observed decrease in SF only occurs at higher temperatures (90°C) and at higher levels of MS (0.28) in hydroxypropylated heat-moisture treated starch.

The increase in granular crystallinity that occurs on defatting (Fig. 4.2), increases granular rigidity. Thus, in defatted starch, even though associative bonding forces (within the amorphous regions) are disrupted (by heat and by the motion of the flexible hydroxypropyl groups), the granules do not collapse to the

same extent as in native and heat-moisture treated starches. The increase in SF with increase in MS (for defatted starch) within the temperature range of 60-90°C thus stands explained (Table 4.12).

4.5.4 Amylose leaching (AML)

The extent of amylose leaching at 90°C decreased with increase in MS (Table 4.13). The extent of AML at this temperature was 4.9, 2.1 and 4.6, respectively for native (MS 0.25), heat-moisture treated (MS 0.28) and defatted (MS 0.20) starches. The interaction of the bulky hydroxypropyl groups with amylose chains probably prevents their diffusion out of the granule during thermal treatment.

4.5.5 Differential scanning calorimetry (DSC)

The enthalpies (ΔH) of gelatinization and the gelatinization transition temperatures (T_o, T_p and T_c) of native, defatted and heat-moisture treated hydroxypropylated starches are presented in Table 4.14. In all starches, increase in the level of MS resulted in a decrease in ΔH, T_o, T_p and T_c and a broadening of the gelatinization temperature range (T_c-T_o). DSC results, similar to this study, have been reported for hydroxypropylated pea starch (Hoover *et al.*, 1988), hydroxypropylated rice starch (Seow & Thevamalar, 1993), hydroxypropylated potato starch (Kim & Eliasson, 1993) and hydroxypropylated maize starches

Table 4.13 Amylose leaching of hydroxypropyl native, defatted and heat-moisture treated starches

Starch source and treatment	Molar substitution	Amylose leaching (%)
Native	0.00[1]	21.9±0.7[a]
	0.05	17.1±1.0[b]
	0.11	14.3±0.3[c]
	0.18	7.9±1.0[d]
	0.25	4.9±0.2[e]
Heat-moisture treated[2]	0.00[1]	8.5±0.9[f]
	0.05	7.6±0.6[f]
	0.11	6.1±0.4[g]
	0.20	4.5±0.6[h]
	0.28	2.1±0.7[i]
Defatted[3]	0.00[1]	13.8±0.5[j]
	0.05	11.9±0.4[k]
	0.10	9.8±0.6[l]
	0.17	6.7±0.8[m]
	0.20	4.6±0.5[n]

[1]Parent starch treated with NaOH and Na_2SO_4 at 40°C.
[2]Heat-moisturetreated (16 h, 100°C/ 30% moisture).
[3]Defatted with 75% propanol water.
[a-n]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

Table 4.14 Gelatinization parameters of hydroxypropyl native, defatted and heat-moisture treated starches

Starch source and treatment	Molar substitution	Transition temperatures (°C)				Enthalpy
		T_o[1]	T_p[1]	T_c[1]	T_c-T_o[2]	ΔH (J/g)[3]
Native	0.00[4]	54.5±0.2[a]	61.4±0.1[a]	66.4±0.6[a]	11.9±0.2[d]	10.9±0.2[b]
	0.11	47.1±0.1[b]	56.2±0.4[b]	64.0±0.2[b]	16.9±0.3[c]	9.6±0.2[c]
	0.18	44.0±0.2[c]	53.1±0.2[c]	63.0±0.1[b]	19.0±0.2[b]	8.0±0.1[d]
	0.25	41.0±0.3[d]	52.0±0.2[d]	61.0±0.2[c]	20.0±0.3[a]	7.4±0.2[e]
Heat-moisture treated[5]	0.00[4]	63.0±0.0[e]	73.0±0.2[e]	80.0±0.1[e]	17.0±0.4[g]	9.6±0.1[e]
	0.11	54.0±0.3[f]	61.0±0.1[f]	73.5±0.4[f]	19.5±0.2[f]	4.7±0.1[f]
	0.20	52.0±0.3[g]	60.1±0.4[g]	72.0±0.1[g]	20.0±0.6[f]	2.7±0.1[g]
	0.28	47.3±0.4[h]	59.8±0.2[g]	69.7±0.3[h]	22.4±0.2[e]	2.0±0.3[h]
Defatted[6]	0.00[4]	58.0±0.4[i]	65.0±0.1[i]	72.5±0.3[i]	14.5±0.2[j]	15.9±0.3[i]
	0.10	51.0±0.7[j]	57.0±0.2[j]	68.0±0.2[j]	17.0±0.5[k]	11.1±0.2[j]
	0.18	49.5±0.1[k]	55.0±0.1[k]	69.0±0.2[k]	19.5±0.3[j]	9.2±0.2[k]
	0.20	48.5±0.2[l]	56.0±0.4[l]	69.5±0.2[k]	21.0±0.1[l]	8.4±0.1[l]

[1] T_o, T_p, & T_c indicate respectively, the temperature of onset, mid point, and end of gelatinization.

[2] Gelatinization temperature range.

[3] Enthalpy of gelatinization.

[4] Parent starch treated with NaOH and Na_2SO_4 at 40°C.

[5] Heat-moisture treated (30% moisture, 100°C, 16 h).

[6] Defatted with 75% n-propanol, 7 h.

[a-l] Means within a column with different superscripts are significantly different ($p \leq 0.05$).

(Wootton & Bamunuarachchi, 1979). At excess water content (as used in this study), potato starch develops only one endotherm (G transition). Donovan (1979) postulated that the G endotherm is due to the solvation assisted transition resulting from hydration and swelling of the amorphous region that facilitates the melting of crystallites during heating. The results indicate that the decrease in T_o, T_p and T_c on hydroxypropylation is due to disruption of the hydrogen bonds between starch chains in the amorphous regions by the hydroxypropyl groups. This increases the mobility of the starch chains which decreases the glass transition (Tg) temperature (Seow & Thevamalar, 1993) and indirectly the melting temperature of starch crystallites.

The decrease in ΔH on hydroxypropylation (Table 4.14) suggests that hydroxypropyl groups disrupt double helices (due to rotation of the flexible hydroxypropyl groups) within the amorphous regions of the granule. Consequently, the number of double helices that unravel and melt during gelatinization would be higher in unmodified than in hydroxypropylated starches. Due to crystallite disruption on heat-moisture treatment, the thermal motion of hydroxypropyl groups within the amorphous regions would be of a greater order of magnitude in heat-moisture treated than in native starch. Thus, at the same level of MS (0.11), more double helices would be disrupted in heat-moisture treated than in native starch granule. Consequently, the number of double helices that unravel and melt during gelatinization would be lower (this represents a large decrease in ΔH) in the former.

The extent of decrease in ΔH on hydroxypropylation should have been theoretically lower in defatted than in native starch due to the following reasons : 1) lower degree of molar substitution in defatted starch and 2) increase in crystallinity on deffating (Fig. 4.2). However, as discussed earlier, crystallite reorientation occurs in both native and defatted (defatted > native) starches (Fig. 4.3 and Table 4.7) on treatment (at 40°C) with the alkaline reagents used during hydroxypropylation. Therefore, it can be postulated that after reorientation, the crystallites of defatted starch are not packed closely enough to significantly influence the mobility of the hydroxypropyl chains within the amorphous regions.

4.5.6 Brabender viscosities (Pasting properties)

The data in Table 4.15 summarizes the pasting characteristics of native, defatted and heat-moisture treated starches after hydroxypropylation. The pasting temperature of all starches decreased with increase in MS. Similar observations have been made on hydroxypropylated buffalo gourd (Butler *et al.*, 1986) and pea starch (Hoover *et al.*, 1988). Increases in the level of MS progressively increased the viscosity at 95°C for defatted starch (Table 4.15). However, for native and heat-moisture treated starches, an increase in the level of MS (beyond 0.18 and 0.20, respectively), resulted in a decrease in viscosity at 95°C. The changes in pasting temperature and viscosity with increase in MS levels are a reflection of the decrease in the strength of the associative bonding forces within the micellar network and to increases in SF (Table 4.12). The

Table 4.15 Brabender viscosities (Pasting properties) of hydroxypropyl native, defatted and heat-moisture treated potato starches

Starch source & treatment	Molar substitution	Pasting temperature (°C)	Viscosity at 95°C (BU[1])	Viscosity after 30 min at 95°C (BU[1])	Viscosity at 50°C (BU[1])
Native	0.00[2]	64.5±0.5[a]	750±10[c]	870±10[a]	1140±20[a]
	0.11	57.5±1.0[b]	1050±10[b]	550±5[b]	580±10[c]
	0.18	56.2±0.5[b]	1200±15[a]	540±5[b]	670±5[b]
	0.25	52.0±0.5[c]	760±10[c]	380±5[d]	410±5[d]
Heat-moisture treated[3]	0.00[2]	91.5±0.5[e]	30±5[h]	130±10[h]	230±5[h]
	0.11	79.5±0.5[f]	190±10[g]	250±15[g]	360±10[g]
	0.20	67.5±1.0[g]	430±10[e]	580±10[e]	850±15[e]
	0.28	60.0±1.0[h]	310±5[f]	340±5[f]	480±10[f]
Defatted[4]	0.00[2]	87.5±1.0[i]	330±5[l]	940±5[m]	1460±10[l]
	0.10	74.3±0.5[j]	900±15[k]	1090±10[i]	1300±5[m]
	0.17	66.0±0.5[k]	1025±15[j]	1060±15[j]	1210±10[n]
	0.20	61.5±0.5[l]	1300±10[i]	870±10[n]	940±10[o]

[1]Brabender units.
[2]Parent starch treated with NaOH and Na_2SO_4 at 40°C.
[3]Heat-moisture treated (30% moisture, 100°C, 16 h).
[4]Defatted with 75% n-propanol, 7 h.
[a-o]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

decrease in the viscosity at 95°C seen at MS levels of 0.25 (native) and 0.28 (heat-moisture treated) can be attributed to the decrease in SF (at 90°C) that occurs at the above MS levels for both native and heat-moisture treated starches (Table 4.12).

4.6 STARCH DIGESTIBILITY BY PORCINE PANCREATIC α-AMYLASE

4.6.1 Enzyme hydrolysis of native, defatted and heat-moisture treated potato starches

The extent of hydrolysis of native, defatted and heat-moisture treated potato starches by porcine pancreatic α-amylase at different time intervals are presented in Table 4.16. Both defatting and heat-moisture treatment increased (heat-moisture treatment > defatting) the susceptibility of potato starch granules towards hydrolysis by α-amylase. For instance, hydrolysis (at the end of 72 h) increased by 25.6 and 17.3%, on heat-moisture treatment and defatting (Table 4.16), respectively. It has already been shown that crystalline regions of potato starch granules disrupt on heat-moisture treatment. Consequently, the amount of amorphous regions available for enzyme hydrolysis would increase after heat-moisture treatment. This would then explain the difference in reactivity of native and heat-moisture treated starches towards enzyme hydrolysis. It has also been shown that defatting increases the crystallinity of potato starch granules. On this basis, defatted granules should have been less susceptible than native granules

Table 4.16 Hydrolysis of native, defatted and heat-moisture treated potato starches by porcine pancreatic α-amylase

Starch source and treatment	Hydrolysis (%) Time (h)							
	0.5	1	3	5	8	24	48	72
Native	0.2±0.1[b]	0.5±0.2[b]	3.3±0.5[b]	5.4±0.5[b]	7.7±0.4[b]	14.1±0.8[b]	16.5±0.6[c]	20.1±0.4[c]
Heat-moisture treated[1]	4.1±0.2[a]	5.8±0.1[a]	13.5±0.4[a]	18.1±0.2[a]	26.8±0.7[a]	34.8±1.1[a]	40.5±0.2[a]	45.7±0.5[a]
Defatted[2]	0.4±0.2[b]	1.0±0.4[b]	3.9±0.3[b]	6.1±0.4[b]	8.6±0.5[b]	18.5±1.0[c]	29.5±1.1[b]	37.4±0.3[b]

[1]Heat-moisture treatment (30% moisture, 100°C, 16 h).

[2]Defatted with 75% n-propanol for 7h.

[a-c]Means within a column with dfferent superscripts are significantly different ($p \leq 0.05$).

towards hydrolysis by α-amylase. Vasanthan & Hoover (1992a) have shown that amylose chains entrapped within adjacent chain clusters of amylopectin are released into the amorphous regions on defatting. Thus, more amylose chains would be available for α-amylase action in defatted than in native granules. This would then explain the increase in hydrolysis on defatting.

The mode of attack by α-amylase on native, defatted and heat-moisture treated starches was investigated by scanning electron microscopy (Figs. 4.6a-f). The attack of α-amylase (72 h) on native potato starch manifested itself in only superficial surface erosion of the granules (Figs. 4.6a,b). During the same time period, granules of defatted (Figs4.6c,d) and heat-moisture treated (Figs. 4.6e,f) starches were more extensively attacked by α-amylase than those of native starch (Figs. 4.6a,b). The surfaces of both defatted and heat-moisture treated starches were extensively eroded and were covered with numerous pits of varying size. ranule splitting was more evident in heat-moisture treated than in defatted granules.

The influence of time of heat-moisture treatment and defatting on the susceptibility of potato starch granules towards α-amylase hydrolysis (24 h) are presented in Tables 4.17 and 4.18 respectively. Heat-moisture treatment for 1, 3, 6 and 8 h, respectively, increased hydrolysis by 17.6, 21.8, 24.8 and 29.1%. However, the above trend was not observed in granules which were heat-

Fig. 4.6 Scanning electron micrographs of native (A, B), defatted (C, D), and heat-moisture treated (E, F) potato starch granules hydrolyzed by porcine pancreatic α-amylase for 72 h.

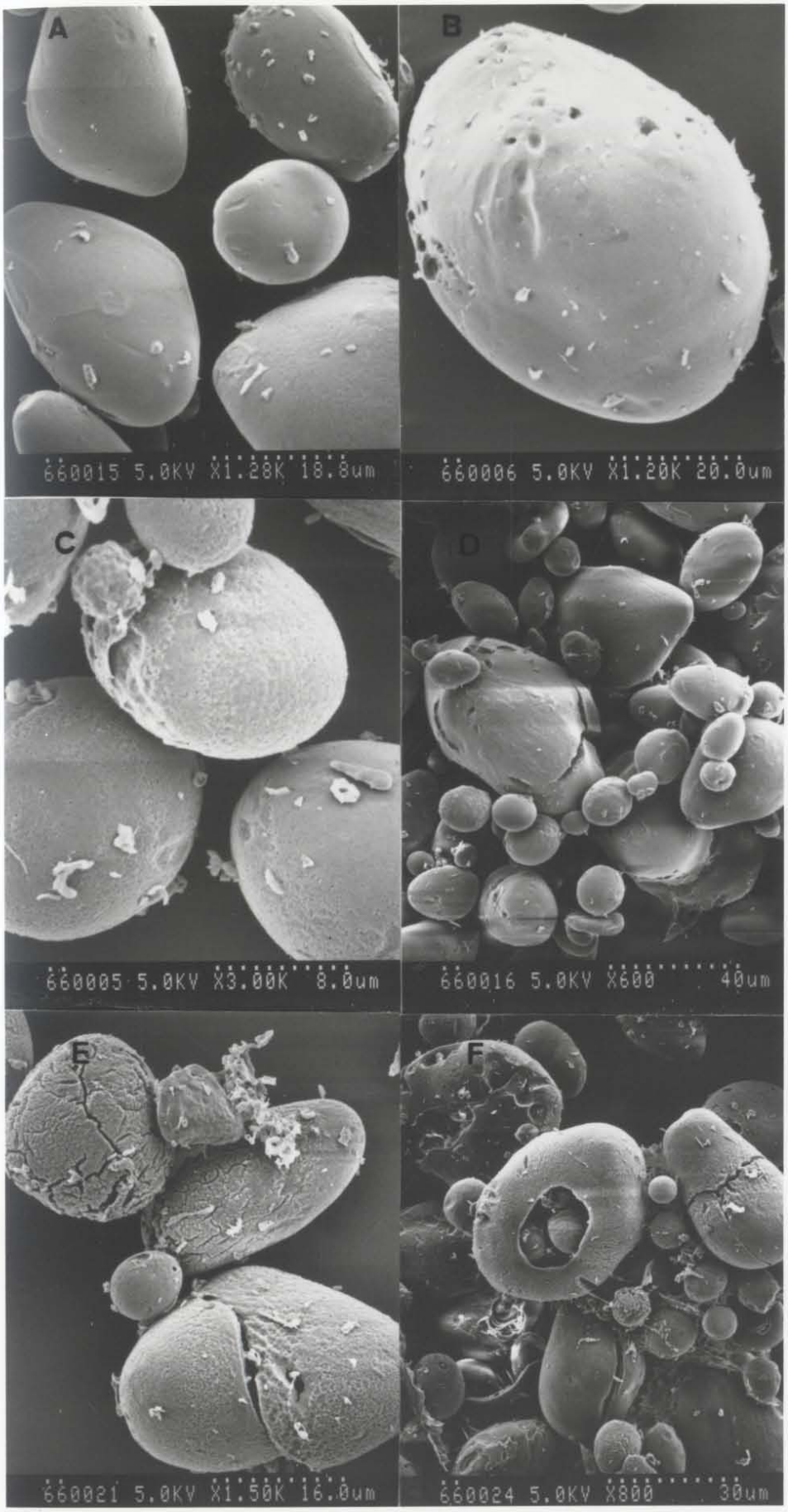

A
660015 5.0KV X1.28K 18.8um
B
660006 5.0KV X1.20K 20.0um
C
660005 5.0KV X3.00K 8.0um
D
660016 5.0KV X600 40um
E
660021 5.0KV X1.50K 16.0um
F
660024 5.0KV X800 30um

Table 4.17 Effect of time of heat-moisture treatment on hydrolysis of potato starch by porcine pancreatic α-amylase

Time of heat-moisture treatment[1] (h)	Hydrolysis[2] (%)
Control (native potato starch)	14.1 ± 0.8^{g}
1	31.7 ± 1.2^{e}
3	35.9 ± 0.5^{cd}
6	38.9 ± 0.8^{b}
8	43.5 ± 1.1^{a}
10	37.5 ± 1.2^{bc}
16	34.8 ± 1.1^{d}
30	28.6 ± 1.0^{f}

[1]Heat-moisture treatment (30% moisture, 100°C).

[2]Hydrolysis with α-amylase (12 units / mg starch, 24 h).

[a-g]eans within the same column with different superscripts are significantly different ($p \leq 0.05$).

Table 4.18 Effect of time of defatting on hydrolysis of potato starch by porcine pancreatic α-amylase

Time of defatting[1] (h)	Hydrolysis[2] (%)
Control (native potato starch)	14.1 ± 0.8^{c}
1	13.4 ± 0.9^{cd}
2	13.6 ± 0.6^{cd}
4	14.8 ± 0.8^{c}
7	18.5 ± 1.0^{b}
9	20.8 ± 0.6^{a}
12	12.6 ± 0.4^{d}

[1]Defatting with 75% n-propanol.

[2]Hydrolysis with α-amylase (12 units / mg starch, 24 h).

[a-d]Means within the same column with different superscripts are significantly different ($p \leq 0.05$).

moisture treated for periods exceeding 8 h. For instance, after 10, 16 and 30 h, the increase in hydrolysis was 23.4, 20.7 and 14.5%, respectively (Table 4.17). Significant differences in hydrolysis between native and defatted starches were observed only after 7 h defatting (Table 4.18). Hydrolysis increased by 5.1 and 6.4%, respectively, in starches defatted for 7 and 9 h (Table 4.18). However, potato starch defatted for 12 h was hydrolyzed to a lesser extent (12.6%) than native starch (14.1%) [Table 4.18].

As discussed earlier, crystallite destruction occurs on heat-moisture treatment. Consequently, more amorphous regions are made available for α-amylase action than in native starch. This would then explain the progressive increase in hydrolysis during the first 8 h of heat-moisture treatment (Table 4.17).

Hoover & Vasanthan (1994a) have shown that in potato starch, AM-AM and AM-AMP interactions occur during heat-moisture treatment (100°C, 30% moisture, 16 h). The difference in enzyme susceptibility between heat-moisture treated starches for periods greater than 8 h, and heat-moisture treated starches for less than 8 h (Table 4.17) suggests that heat-moisture treatment for periods greater than 8 h induces interactions to occur between AM-AM and AM-AMP chains. These interactions lead to the formation of new crystallites which reduce the accessibility of α-amylase to the glycosidic linkages of the starch chains. The data also suggests that crystallite disruption does not occur to any significant extent beyond 8h of heat-moisture treatment.

The X-ray intensity of the peak centred at 5.2 Å (Table 4.19 and Fig. 4.7), also suggests that new crystallites begin to form only after 8 h of heat-moisture treatment. For instance, at 1, 8, 16 and 30 h of heat-moisture treatment, the intensity of the 5.2 Å peak, was 1674, 1575, 2105 and 2809 cps, respectively. The reduction in peak intensity at 8 h, indicates crystallite disruption.

Amylose complexed with lipids has been shown to significantly reduce the availability to α-amylase *in vitro* (Holm *et al.*, 1983; Biliaderis *et al.*, 1985). The results (Table 4.18) indicate that the increase in hydrolysis on defatting could be due to the interplay of two factors : (1) lipid removal and (2) the availability of more free amylose chains for α-amylase action (Vasanthan & Hoover, 1992a). This seems plausible since granule susceptibility to α-amylase continues to increase (Table 4.18) even after complete lipid removal (lipid removal is completed after 7 h of defatting [Fig. 4.8]). Defatting beyond 9 h decreases hydrolysis (Table 4.18) possibly due to AM-AM, AM-AMP and AMP-AMP interactions. The progressive change in X-ray diffraction patterns during defatting (0-12 h) [Fig. 4.9] may also reflect that changes occurred in the crystalline pattern of the starch granule. These interactions probably result in the formation of double helices which are resistant to α-amylase action. Thoma (1968) postulated that the enzyme catalyzed hydrolysis of the α-D (1→4)-glycosidic bond of the starch molecule involves enzyme induced ring distortion of one of the D-glucosyl residues from the $^4C_1$ 'chair' conformation to a 'half chair'

Table 4.19 X-raydiffraction intensities of the 5.2 Å peak with time course of heat-moisture treatment

Time of heat-moisture treatment[1] (h)	X-ray intensities of the peak centered at 5.2 Å (cps)[2]
1	1674
8	1575
16	2105
30	2809

[1]At 100°C, 30% moisture.
[2] Counts per second.

Fig. 4.7 X-ray diffraction patterns of potato starches heat-moisture treated for (A) 0 h, (B) 1 h, (C) 8 h, (D) 16 h and (E) 30h.

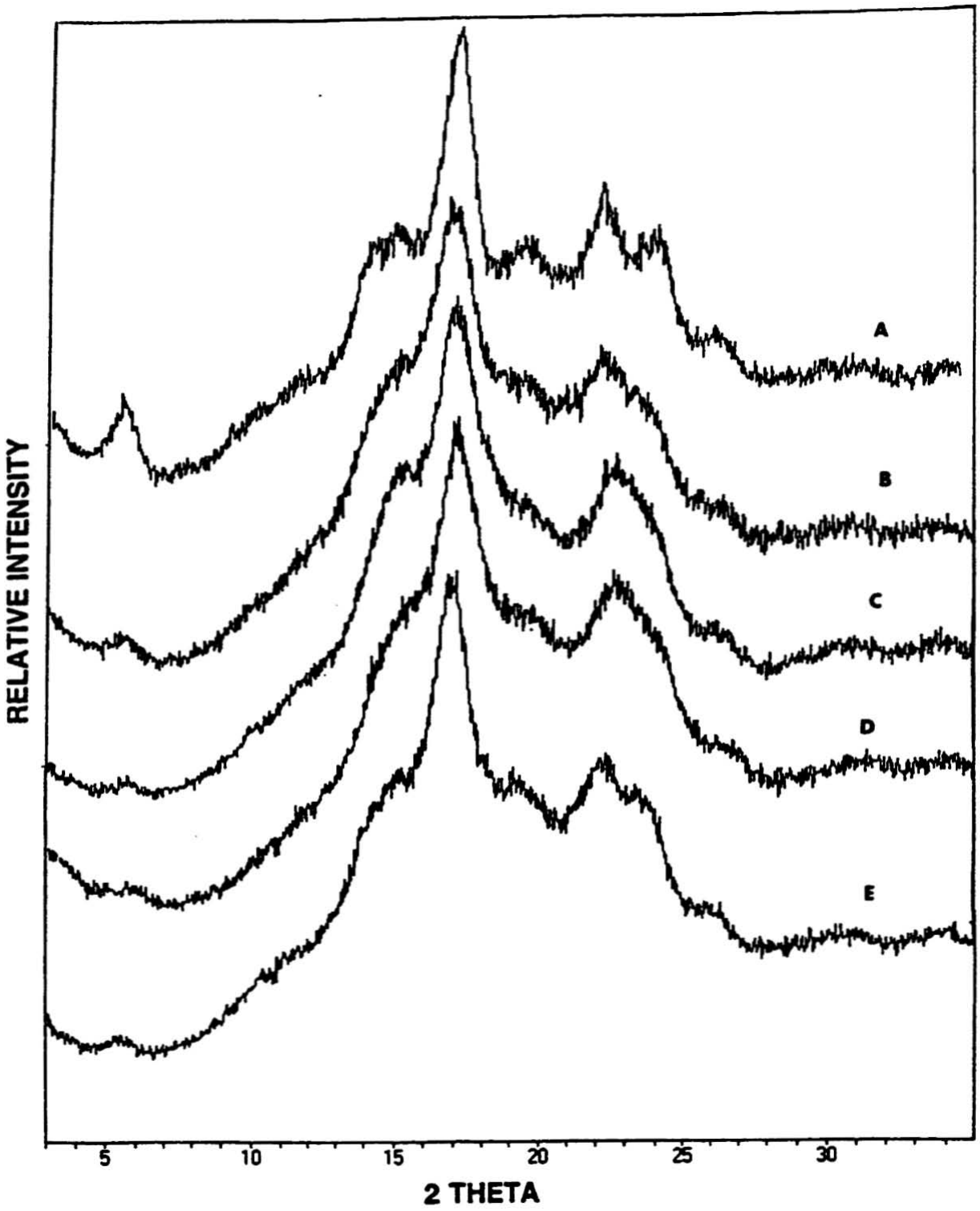

RELATIVE INTENSITY
A
B
C
D
E
5
10
15
20
25
30
2 THETA

Fig. 4.8 The influence of defatting time on the extent of lipid removal from native potato starch.

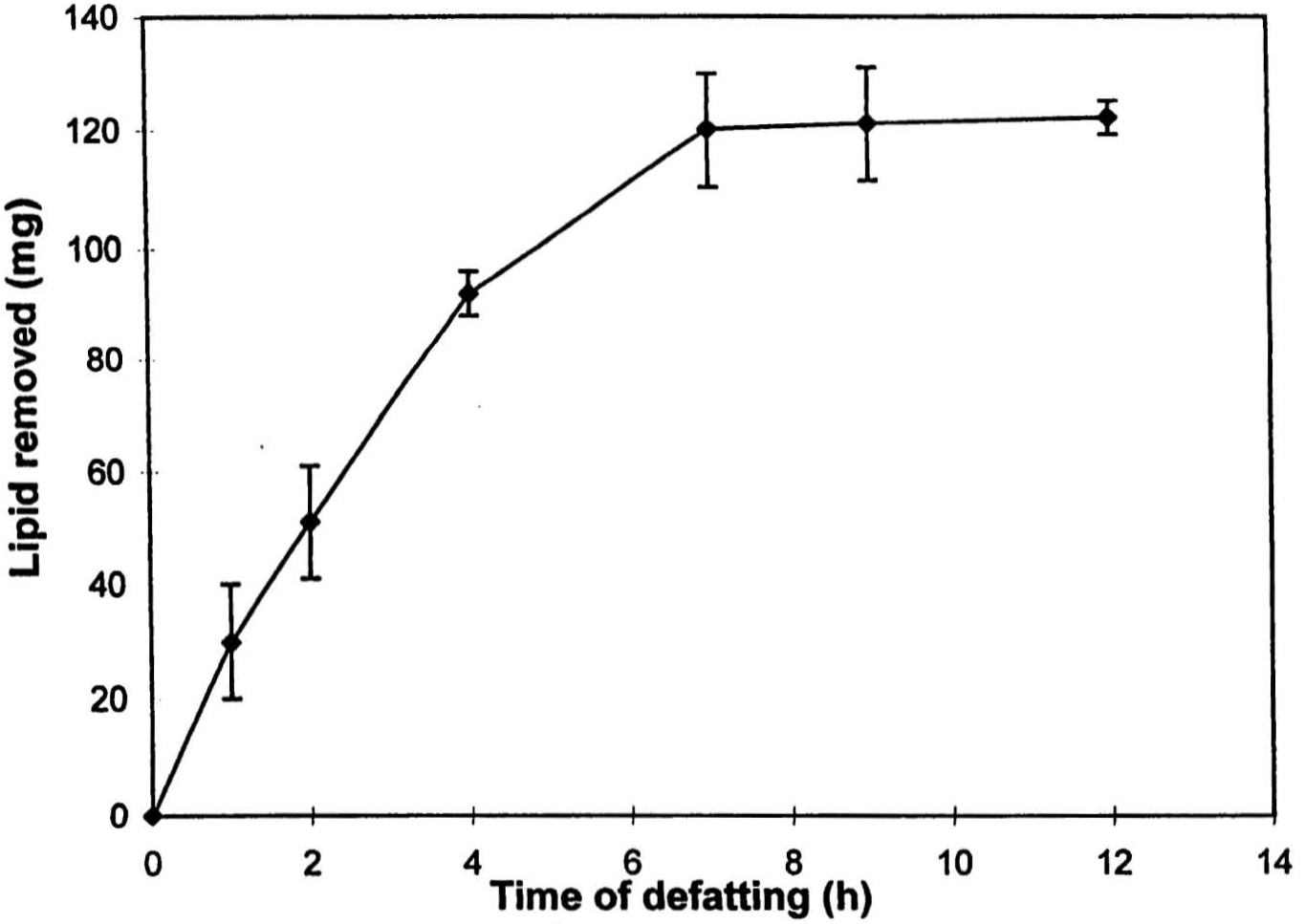
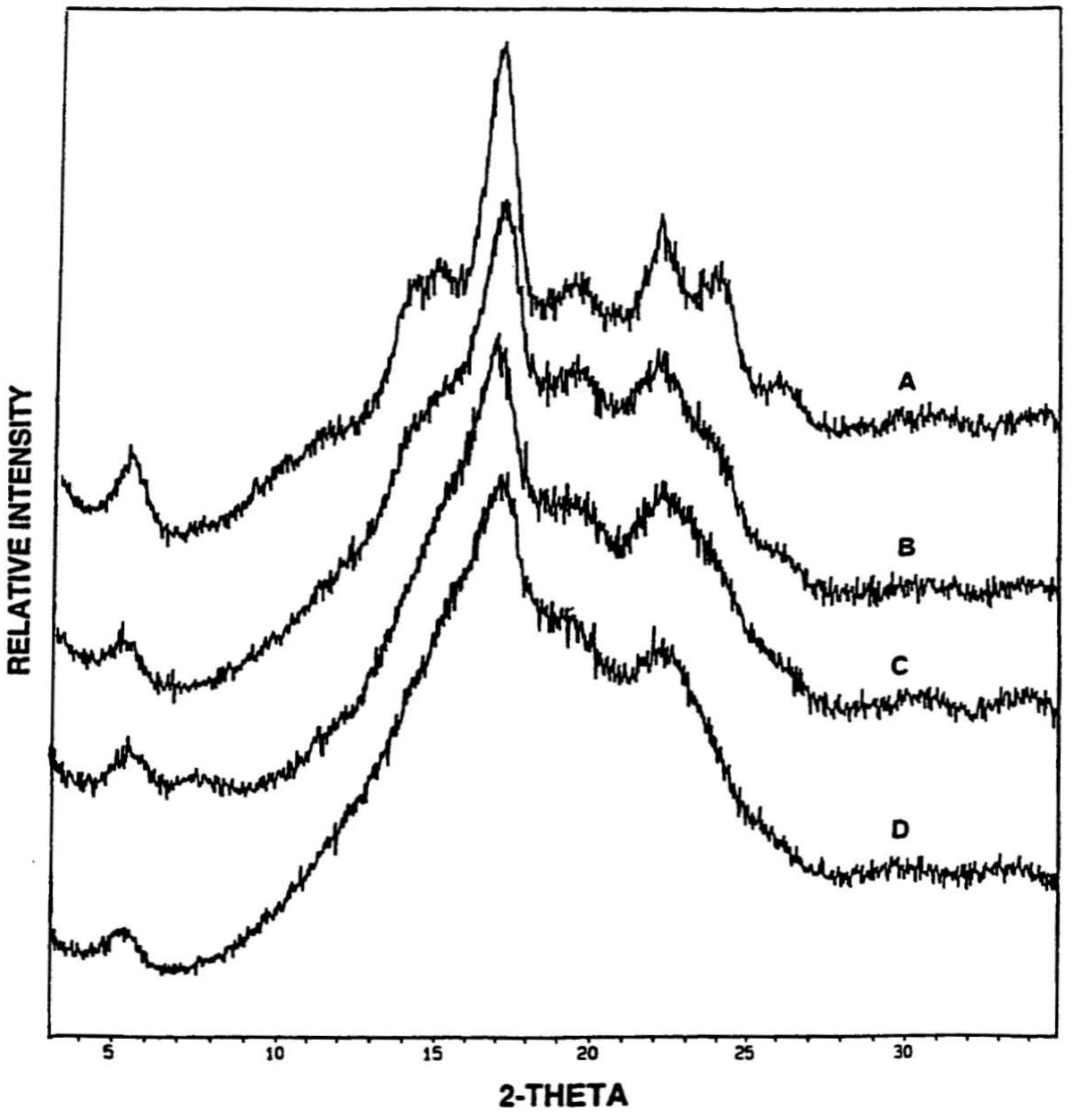

Lipid removed (mg)
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Time of defatting (h)

Fig. 4.9 X-ray diffraction patterns of potato starched defatted for (A) 0 h, (B) 1 h, (C) 7 h, and (D) 12 h.


RELATIVE INTENSITY
A
B
C
D
5
10
15
20
25
30
2-THETA

conformation (Fig 2.28b). This ring distortion decreases the enthalpy of activation and increases the susceptibility of the glucosyl residues to nucleophilic attack by functional groups on the enzyme and water. Laszló *et al.* (1978) have shown that ring distortion or a 'half chair' conformation is involved in the transition state of α-amylase. It is therefore plausible that conformational changes (chair-half chair) during α-amylase hydrolysis may be difficult for those amylose chains that form double helices during defatting. This would then explain the decrease in hydrolysis when defatting is continued beyond 9 h. This decrease in hydrolysis cannot be due to crystallite formation (resulting from aggregation of the newly formed double helices) since X-ray diffraction intensities did not change during the time course of defatting (not shown).

4.6.2 Influence of alkali on enzyme hydrolysis of native, defatted and heat-moisture treated potato starches

The influence of the alkaline conditions used during hydroxypropylation on enzyme digestibility of native, defatted (7 h) and heat-moisture treated (30% moisture, 100°C, 16 h) potato starches are presented in Table 4.20. Alkaline treatment increased the susceptibility of the above starches (native > defatted > heat-moisture treated) towards hydrolysis by α-amylase. For instance after 24 h, hydrolysis was increased by 20.0, 0.7% and 12.2%, respectively, in alkali treated native, heat-moisture treated and defatted starches (Table 4.20).

Table 4.20 Hydrolysis of native, defatted and heat-moisture treated potato starches by porcine pancreatic α-amylase before and after hydroxypropylation

Starch source and treatment	Propylene oxide added (%v/w)	Molar substitution	Hydrolysis[1] (%)
Native	-	-	14.1 ± 0.8^{d}
Native (alkali treated)[2]	0	0.00	34.1 ± 0.3^{c}
	2	0.05	33.5 ± 1.1^{c}
	5	0.11	35.4 ± 1.0^{c}
	10	0.18	39.7 ± 1.1^{b}
	15	0.25	44.5 ± 0.9^{a}
	20	0.29	39.0 ± 0.9^{b}
Heat-moisture treated (HMT)[3]	-	-	34.8 ± 1.1^{h}
HMT (alkali treated)[2]	0	0.00	35.5 ± 1.0^{gh}
	2	0.05	37.5 ± 0.9^{fg}
	5	0.11	37.9 ± 1.1^{f}
	10	0.20	44.4 ± 1.0^{e}
	15	0.28	30.5 ± 0.4^{i}
	20	0.34	26.2 ± 1.0^{j}
Defatted[4]	-	-	18.5 ± 1.0^{o}
Defatted (alkali treated)[2]	0	0.00	30.7 ± 0.3^{n}
	2	0.05	38.2 ± 0.7^{j}
	5	0.10	39.7 ± 0.5^{i}
	10	0.17	42.3 ± 0.7^{k}
	15	0.20	43.1 ± 0.9^{k}
	20	0.26	35.5 ± 0.6^{m}

[1]Hydrolysis with α-amylase for 24 h/12units/mg starch.

[2]Parent starch treated with NaOH and Na_2SO_4 at 40°C.

[3]Heat-moisture treatment (16 h / 100°C /30% moisture).

[4]Defatted with 75% propanol for 7 h.

[a-o]Means within a column with different superscripts are significantly different ($p \leq 0.05$).

The increase in hydrolysis on alkaline treatment can be attributed to the interplay of three factors : 1) disruption of double helices (within the amorphous regions) in the presence of the alkaline reagents. (This would provide α-amylase greater accessibility to the glycosidic linkages); 2) Ionization of the hydroxyl groups on adjacent starch chains which results in a partial separation of protons leaving the core of the starch negatively charged (repulsion between negatively charged starch chains would increase hydrolysis, by increasing the degree of accessibility to α-amylase); and 3) an increase in granular solubility resulting from aggregation of water dipoles around the negatively charged centres. Double helices within the amorphous regions have been shown to disrupt during heat-moisture treatment. Consequently, fewer double helices would be present in heat-moisture treated starch than in native and defatted starches. Thus, the number of double helices that disrupt during alkali treatment would be of a lower order of magnitude in heat-moisture treated than in native and defatted starch granules. This would then explain the smaller increase in α-amylase hydrolysis after alkaline treatment of heat-moisture treated starch. The different responses shown by alkali treated native and defatted starches towards hydrolysis by α-amylase can de attributed to the greater degree of crystallinity (a higher degree of crystallinity would hinder the penetration of the alkaline reagents into the granule interior) inherent within granules of defatted starch (Vasanthan & Hoover, 1992a). This would then explain the observed increase in hydrolysis

(native > defatted > heat-moisture treated) after alkaline treatment. The influence of factors 2 and 3 on hydrolysis is probably of the same order of magnitude in all three starches.

4.6.3 The Influence of the level of hydroxypropylation on enzyme hydrolysis of native, defatted and heat-moisture treated starches

The susceptibility of native, heat-moisture treated and defatted potato starches towards hydrolysis by α-amylase increased with increase in MS levels. However, granular susceptibility began to decrease at MS levels of 0.29 (native), 0.28 (heat-moisture treated) and 0.26 (defatted) [Table 4.20].

Studies on the effect of varying MS levels on hydrolysis of hydroxypropylated starches have been conducted on wheat starch (Wootton & Chaudhry, 1981), maize, waxy maize and high amylose maize starches (Mohd Azemi & Wootton, 1985; 1995; Hahn & Hood, 1980), potato starch (Leegwater & Luten ,1971) and pea starch (Hoover *et al.*, 1988). Wootton & Chaudhry (1981) showed that an increase in MS (0.03→ 0.17) increased the digestibility of raw wheat starch by pancreatic α-amylase. However, a similar increase in MS for gelatinized wheat starch decreased its digestibility. The above authors postulated that the increased digestibility of raw starches with higher MS is probably due to the greater tendency of more highly substituted starches to swell in cold water (This would allow better access of the enzyme to the substrate).

However, the reduction in digestibility of gelatinized starch with increase in MS was attributed to greater reduction of enzyme attack by increasing number of substituent groups. Mohd Azemi & Wootton (1985) showed that hydrolysis of gelatinized normal, waxy and high amylose maize starches decreased with increase in the level of MS (0.05→0.13). A similar increase in MS levels for raw normal and high amylose maize starches, resulted in decrease in digestibility at lower MS levels, followed by increases at higher MS levels (> 0.07). However, the digestibility of raw waxy maize starch showed a continuing drop as MS increased. Hahn & Hood (1980) showed that dimethyl sulphoxide solubilized hydroxypropylated waxy maize starch was hydrolyzed to a lesser extent than its unmodified counterpart. However, the extent of hydrolysis was not influenced by increases in MS. Leegwater & Luten (1971) showed that the digestibility of gelatinized potato starch by large amounts of pancreatin decreased exponentially with increase in the degree of substitution (0.02→0.45). Hoover *et al.* (1988) showed that the digestibility of raw field pea starch decreased with increase in MS up to a level of 0.08. Further increases in MS caused an increase in digestibility which was higher than that observed for native starch.

4.7 RETROGRADATION STUDIES ON POTATO STARCHES

4.7.1 Turbidimetric and microstructural analysis

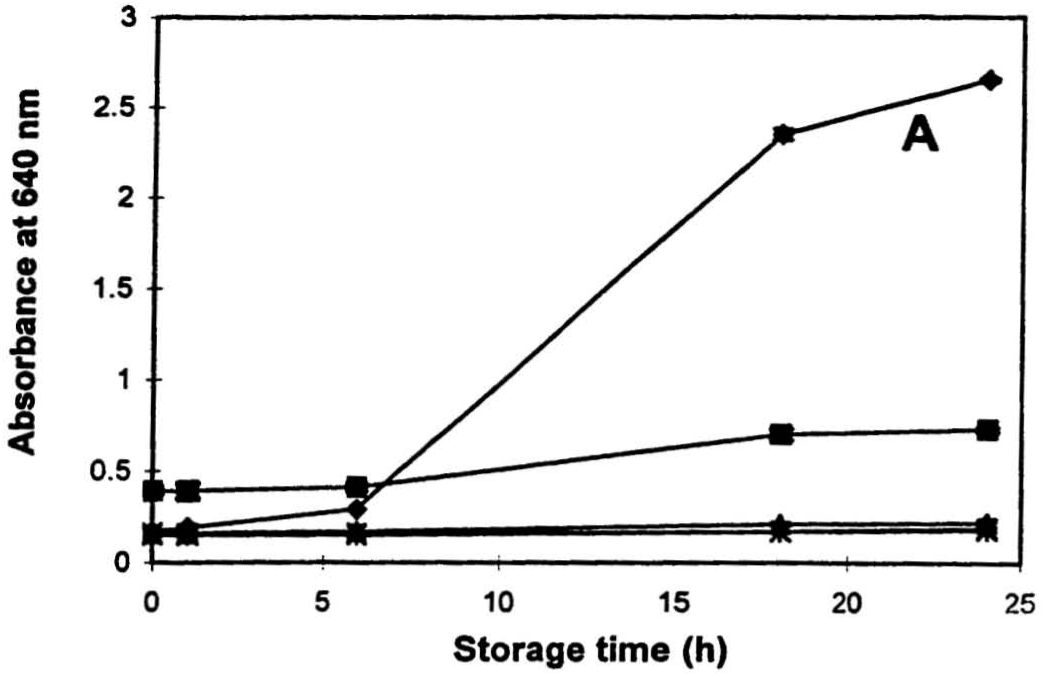
4.7.1.1 Initial turbidity and scanning electron microscopy of fresh pastes of native, defatted and heat-moisture treated potato starch pastes

Turbidity development in native, defatted and heat-moisture treated potato starch pastes during 24 h of storage at 4°C is described in Figs. 4.10a, 4.11a and 4.12a. The initial turbidity of the gelatinized starch pastes followed the order : heat-moisture treated (Fig. 4.12a) > defatted (Fig. 4.11a) > native (Fig. 4.10a). The pellet of freshly gelatinized native potato starch paste was devoid of granule remnants and consisted of large sheets of leached amylose and amylopectin (Fig 4.13a) [Jacobson *et al.*, 1997]. However, fresh pastes of defatted (Fig 4.13c) and heat-moisture treated (Fig 4.13e) starches consisted of granule remnants (heat-moisture treated > defatted) connected by a matrix of leached amylose and amylopectin.

Turbidity effects have their origin in refractive index fluctuation over a distance scale comparable to the wavelength of observation. In a polymer-solvent system this is caused by density fluctuations over the same distance scale and is most likely due to extensive polymer-polymer aggregation (Gidley & Bulpin, 1989). Gidley & Bulpin (1989) have shown, on the basis of their studies of amylose aggregation in aqueous systems, that even at the onset of detectable turbidity, highly aggregated polymer structures are present. Craig *et al.* (1989) have classified starch pastes into three categories depending on the behaviour in

Fig. 4.10 Time course of turbidity development in native potato starch stored at 4°C (A) and 40°C (B).

◆ native; ■ alkali treated native (MS 0.00); ▲ hydroxypropylated native (MS 0.11); X hydroxypropylated native (MS 0.18); ✳ hydroxypropylated native (MS 0.25).


A
Absorbance at 640 nm
Storage time (h)
0
0.5
1
1.5
2
2.5
3
0
5
10
15
20
25

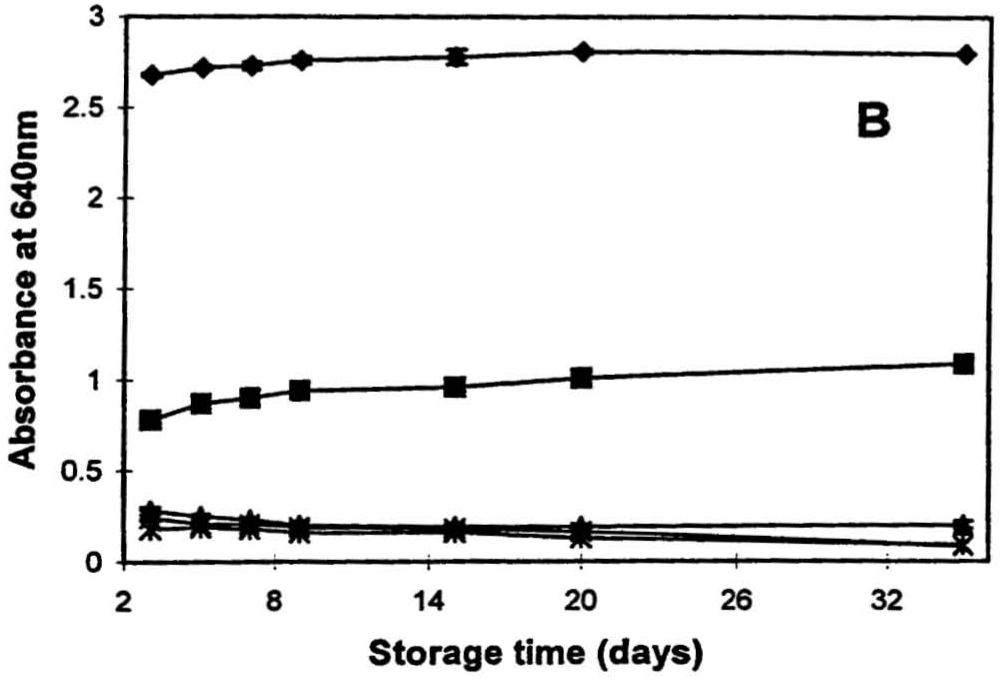
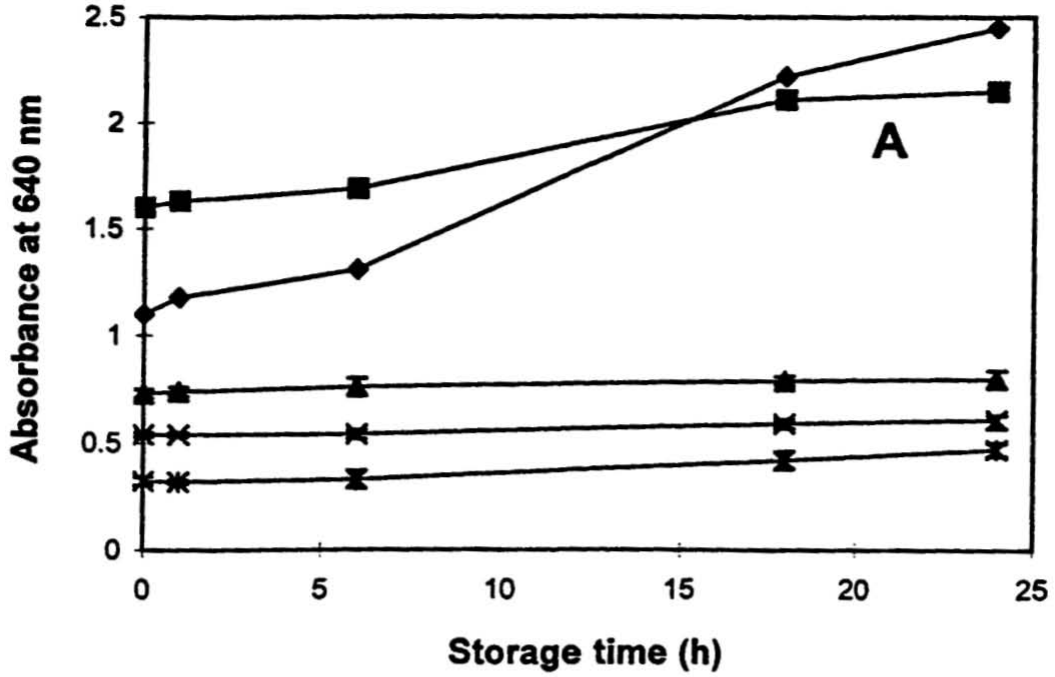

B
Absorbance at 640nm
Storage time (days)
0
0.5
1
1.5
2
2.5
3
2
8
14
20
26
32

Fig. 4.11 Time course of turbidity development in defatted potato starch stored at 4°C (A) and 40°C (B).

◆ defatted; ■ alkali treated defatted (MS 0.00); ▲ hydroxypropylated defatted (MS 0.10), X hydroxypropylated defatted (MS 0.17), ✳ hydroxypropylated defatted (MS 0.20).


Absorbance at 640 nm
2.5
2
1.5
1
0.5
0
A
0
5
10
15
20
25
Storage time (h)

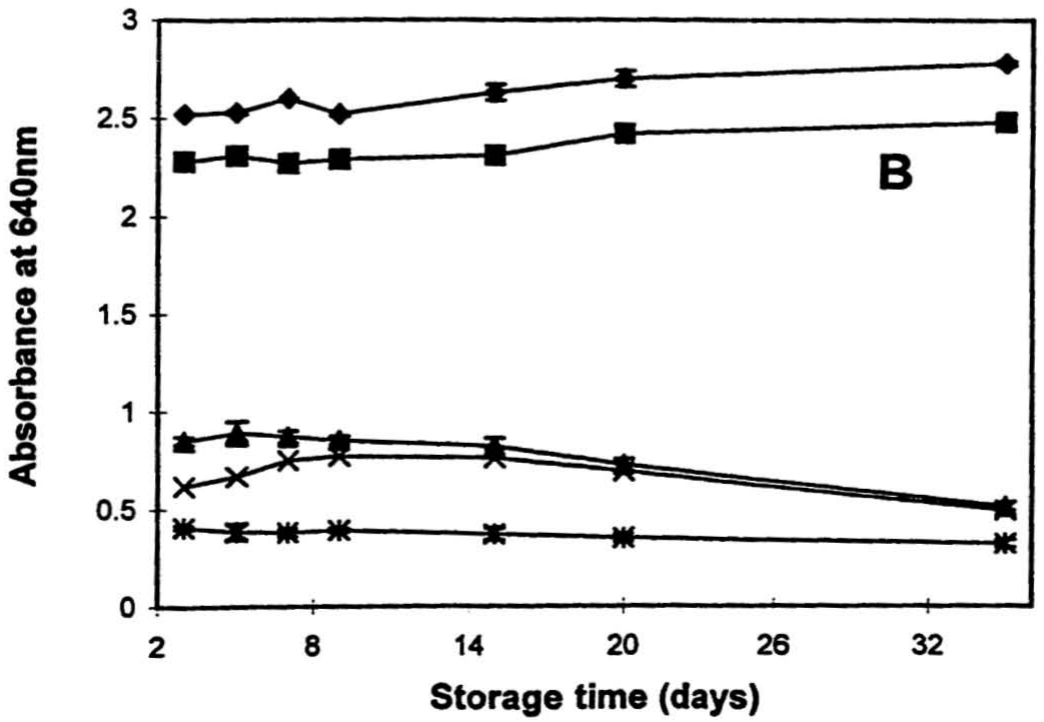
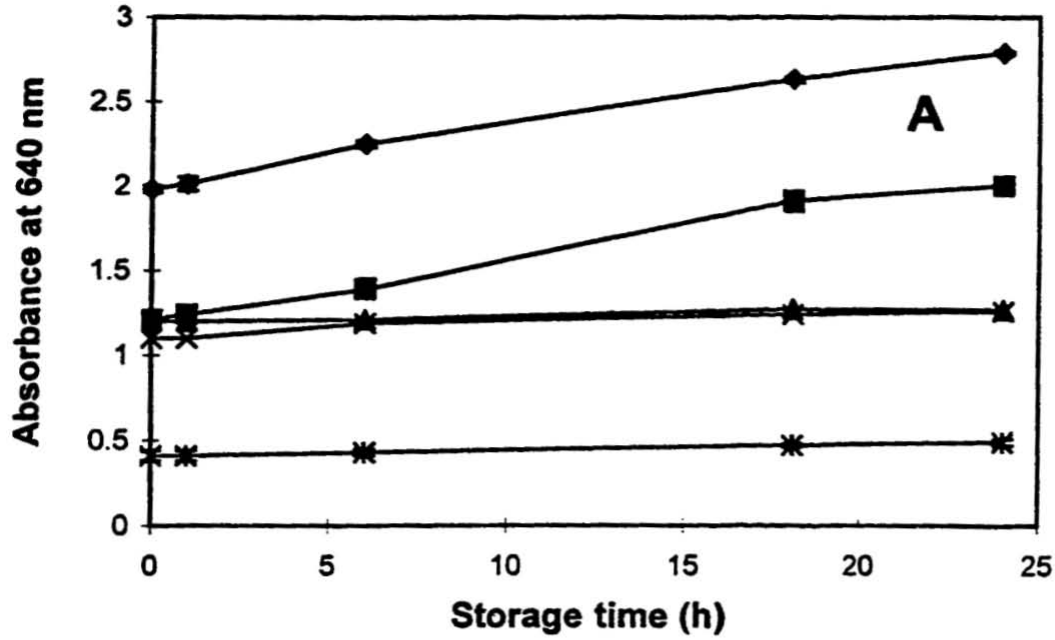

Absorbance at 640nm
3
2.5
2
1.5
1
0.5
0
B
2
8
14
20
26
32
Storage time (days)

Fig. 4.12 Time course of turbidity development in heat-moisture treated potato starch stored at 4°C (A) and 40°C (B).

◆ heat-moisture treated; ■ alkali treated heat-moisture treated (MS 0.00); ▲ hydroxypropylated heat-moisture treated (MS 0.11); X hydroxypropylated heat-moisture treated (MS 0.20); ✳ hydroxypropylated heat-moisture treated (MS 0.28).


A
Absorbance at 640 nm
Storage time (h)
0
0.5
1
1.5
2
2.5
3
0
5
10
15
20
25

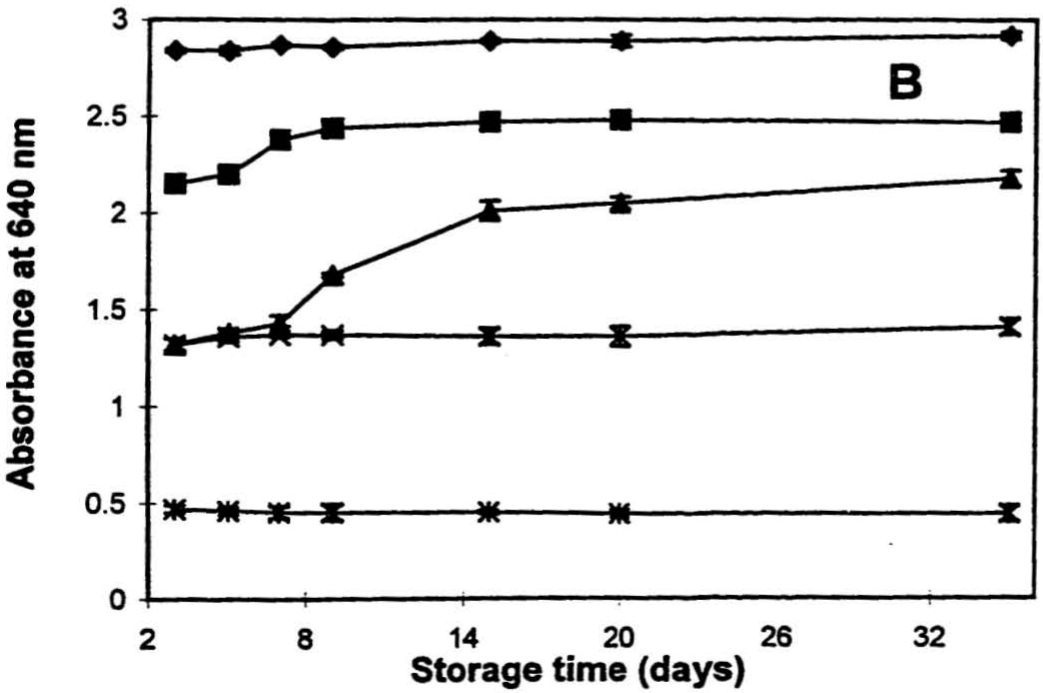

B
Absorbance at 640 nm
Storage time (days)
0
0.5
1
1.5
2
2.5
3
2
8
14
20
26
32

Fig. 4.13 Scanning electron micrographs of freshly gelatinized and stored (24 h at 4°C) potato starch pastes : (A) fresh native potato stash, (B) stored native potato starch, (C) fresh defatted potato starch, (D) stored defatted potato starch, (E) fresh heat-moisture treated potato starch, (F) stored heat-moisture treated potato starch.

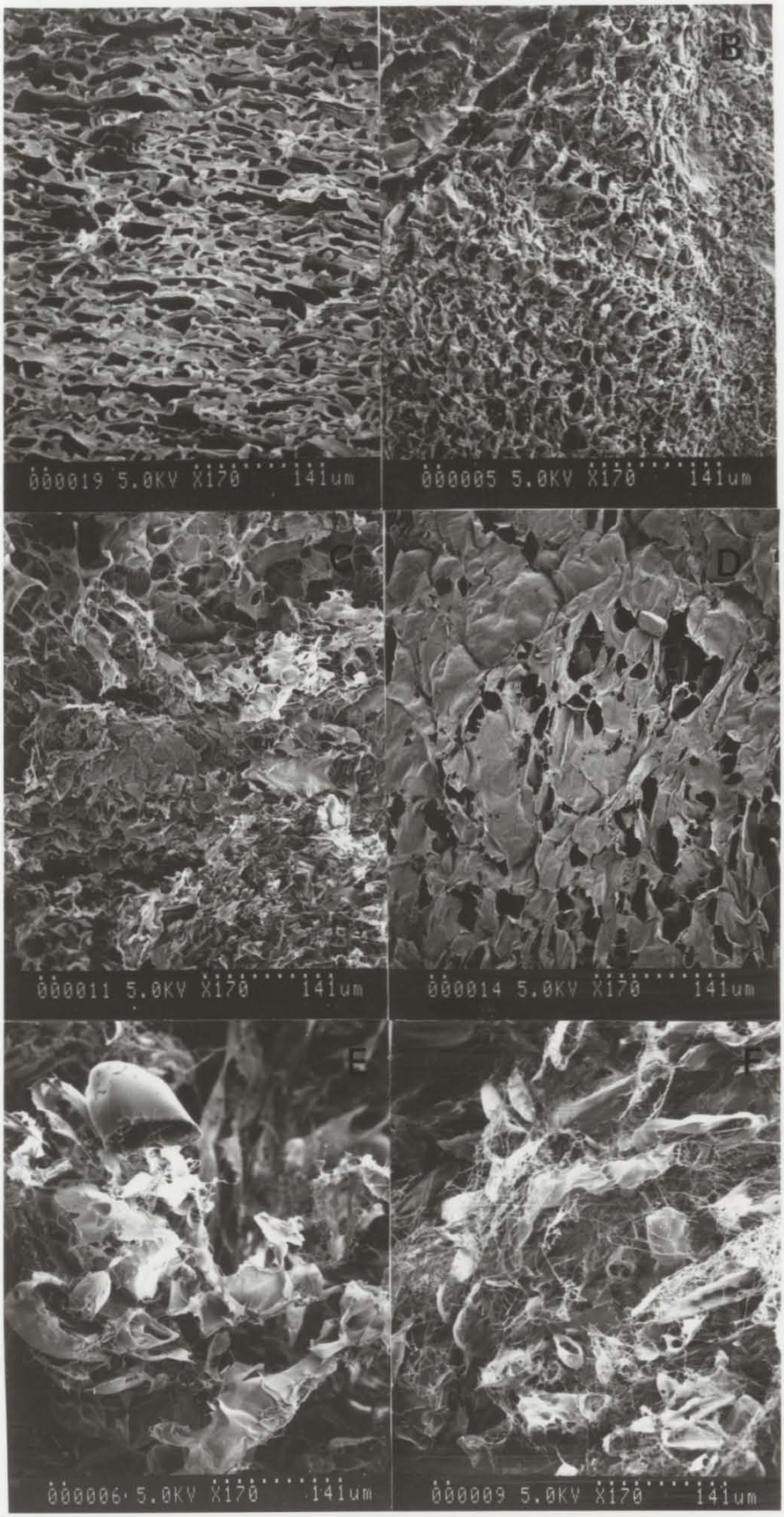

A
000019 5.0KV X170 141um
B
000005 5.0KV X170 141um
C
000011 5.0KV X170 141um
D
000014 5.0KV X170 141um
E
000006 5.0KV X170 141um
F
000009 5.0KV X170 141um

Fig. 4.14 Scanning electron micrographs of gelatinized and stored (24 h at 4°C) alkali treated native potato starch pastes. (A) & (B) fresh paste; (C) & (D) stored paste.

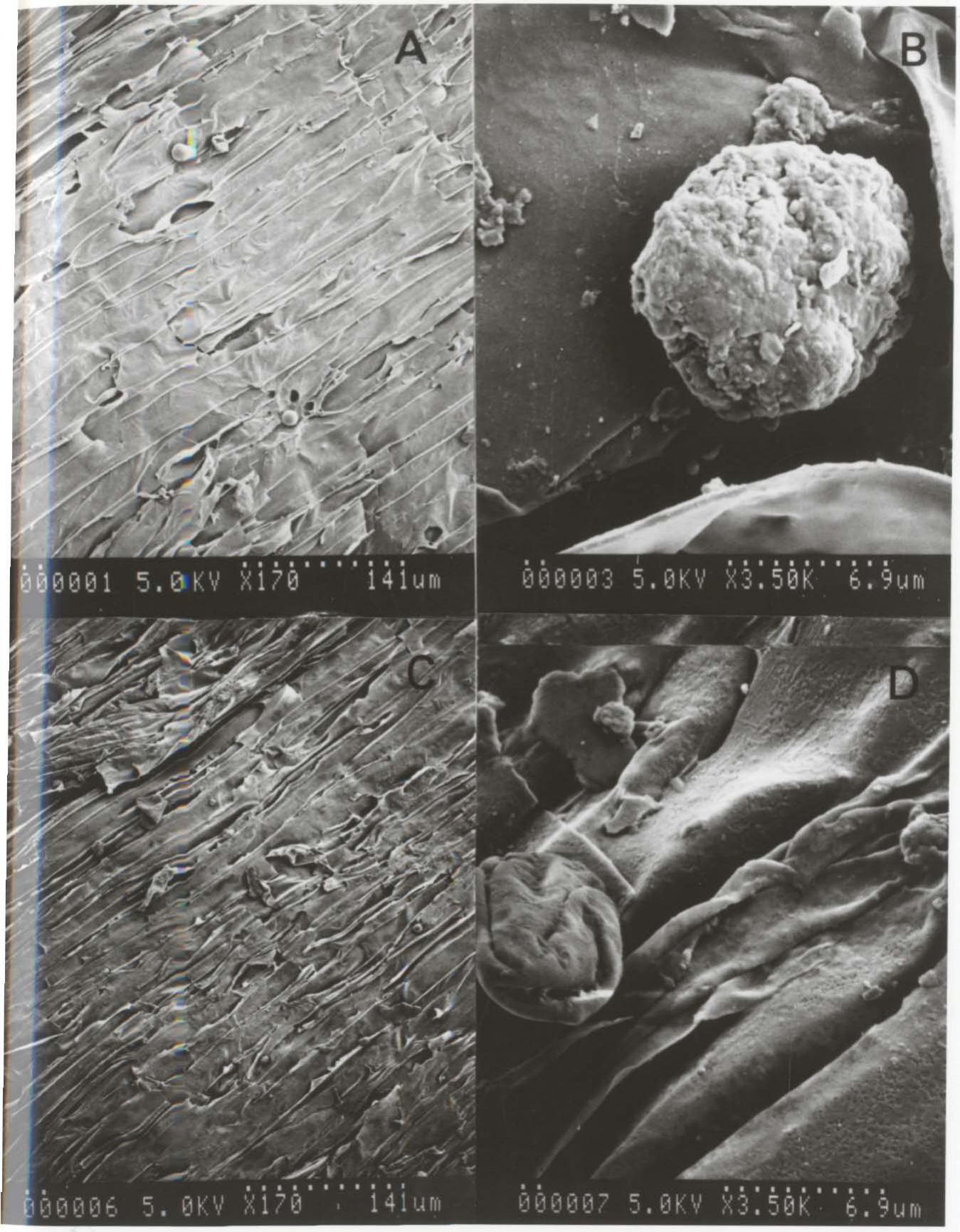

A
B
C
D
000001 5.0KV X170 141um
000003 5.0KV X3.50K 6.9um
000006 5.0KV X170 141um
000007 5.0KV X3.50K 6.9um

Fig. 4.15 Scanning electron micrographs of freshly gelatinized and stored (24 h at 40°C) pastes of alkali treated defatted potato starch : (A) & (B) freshly gelatinized defatted starch, (C) & (D) stored defatted starch.

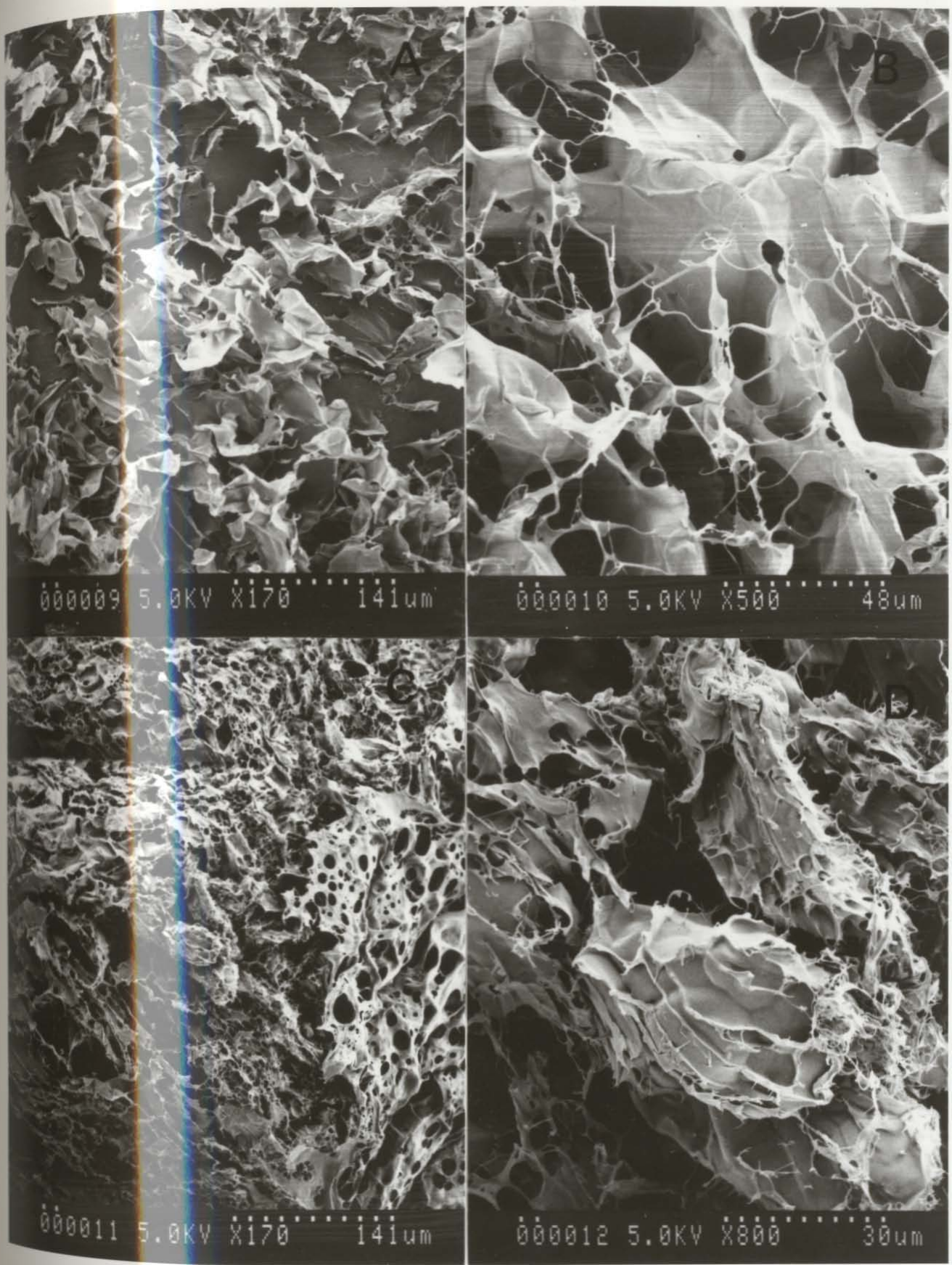
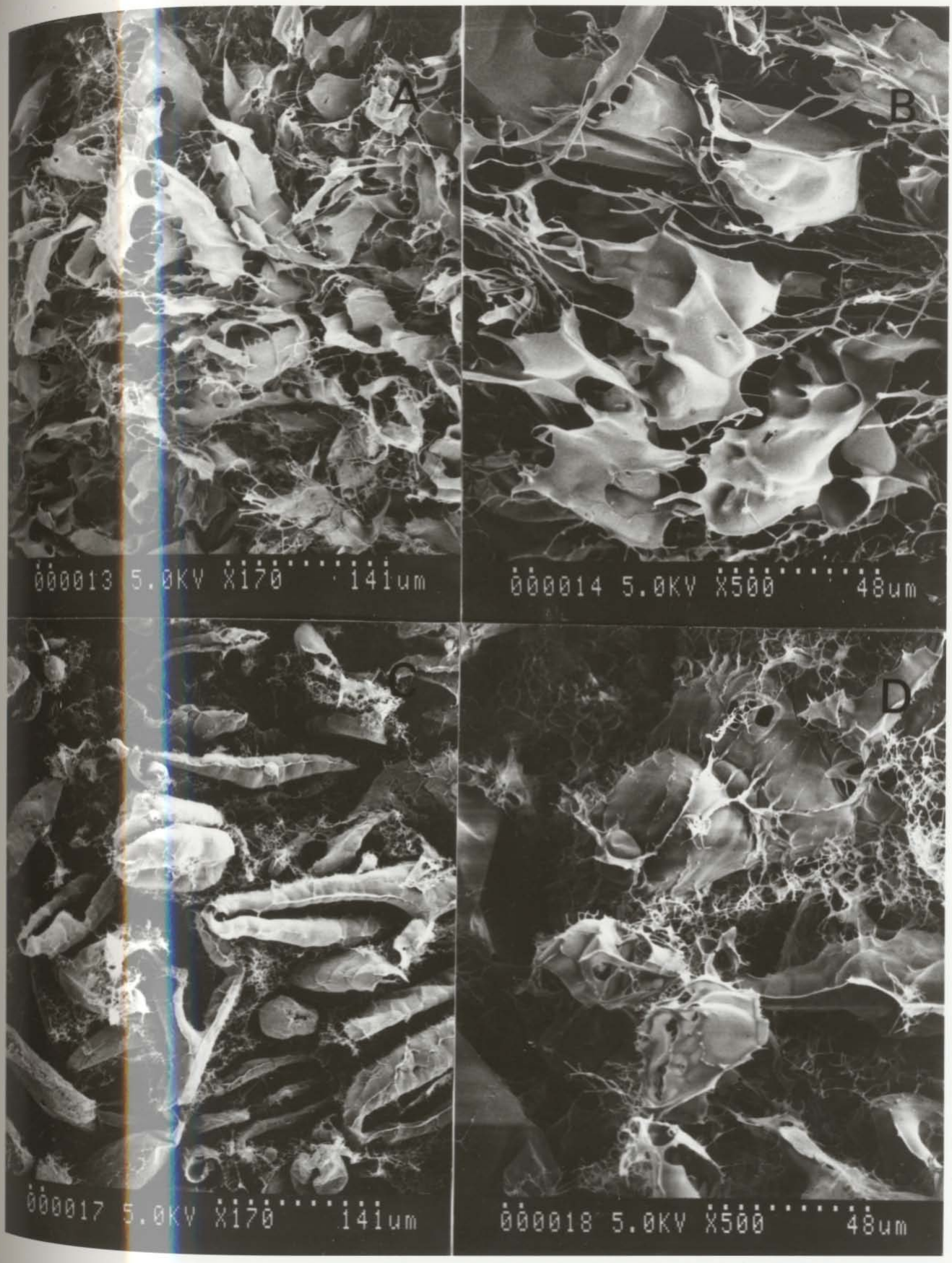

A
B
C
D
000009 5.0KV X170 141um
000010 5.0KV X500 48um
000011 5.0KV X170 141um
000012 5.0KV X800 30um

Fig. 4.16 Scanning electron micrographs of freshly gelatinized and stored (24 h at 4°C) starch pastes of alkali treated heat-moisture treated potato starch : (A) & (B) freshly gelatinized heat-moisture treated starch, (C) & (D) stored heat-moisture treated starch.


A
000013 5.0KV X170 141um
B
000014 5.0KV X500 48um
C
000017 5.0KV X170 141um
D
000018 5.0KV X500 48um

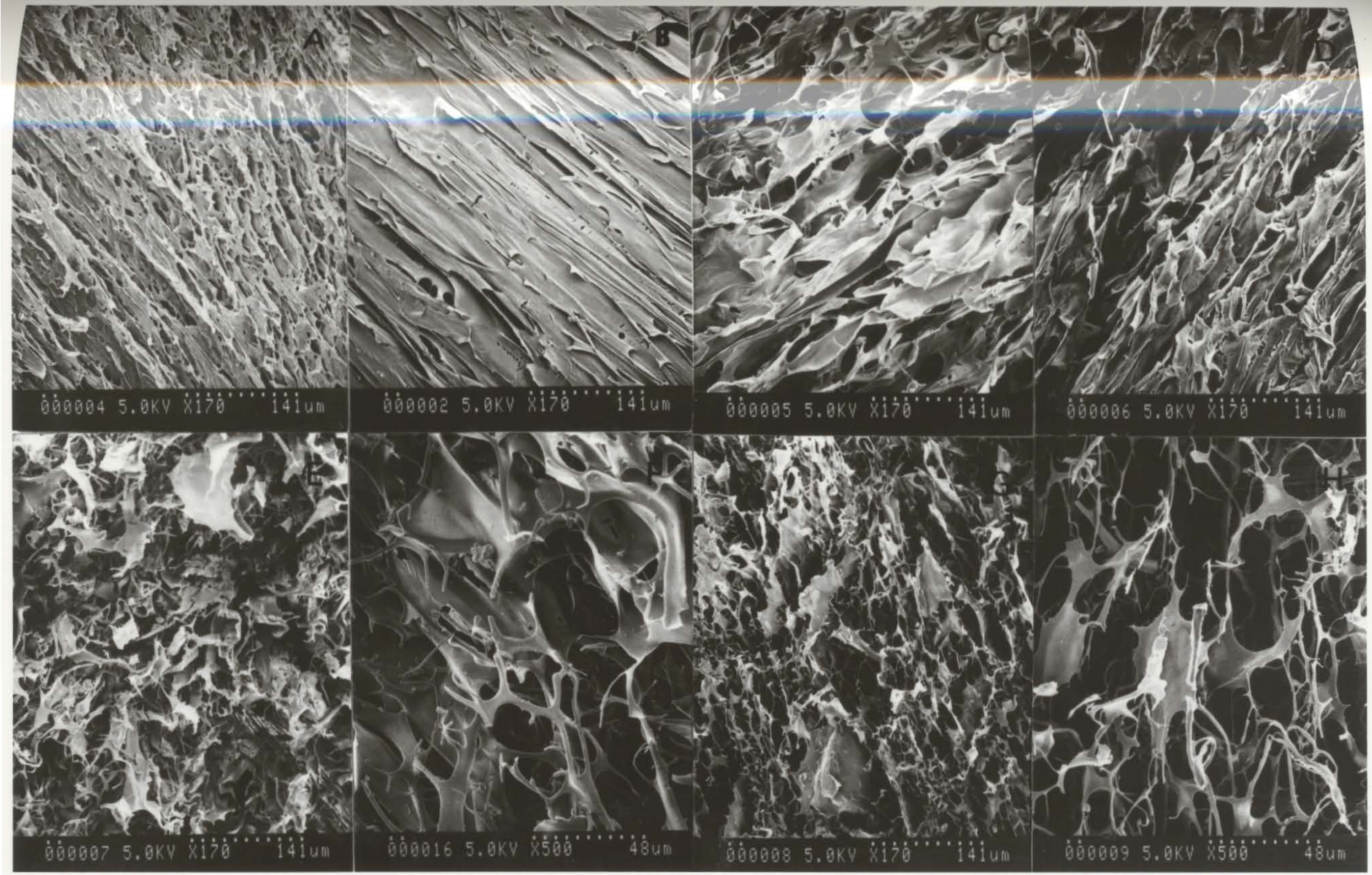
decrease in light absorbance (in spite of an increase in the amount of granule remnants) on alkaline treatment of heat-moisture treated starch (Fig. 4.12a) is probably due to decreased interaction between leached amylose and/or amylopectin chains (compare freshly gelatinized heat-moisture treated gel [Fig. 4.13e] and freshly gelatinized alkali treated heat-moisture treated gel [Figs. 4.16a,b]).

4.7.1.3 Initial turbidity and scanning electron micrographs of hydroxypropylated native, defatted and heat-moisture treated starches

The initial absorption of alkali treated starches decreased on hydroxypropylation (Figs. 4.10a, 4.11a, 4.12a). In alkali treated native starch, absorption decreased by 58.9% at MS levels of 0.11 and 0.18, whereas, at MS 0.25 the corresponding value was 61.2% (Fig 4.10a). In alkali treated defatted starch, absorption decreased by 54.3, 66.2 and 80%, respectively, at MS 0.10, 0.17 and 0.20 (Fig. 4.11a). Whereas, in alkali treated heat-moisture treated starch, absorption decreased by 2.4, 10.0, 66.6%, respectively, at MS 0.11, 0.20 and 0.28 (Fig. 4.12a).

Scanning electron micrographs of freshly gelatinized pastes of native, defatted and heat-moisture treated starches after hydroxypropylation (under alkaline conditions) are presented in Fig. 4.17. Granules of all starches were disrupted on hydroxypropylation. The disrupted granules of hydroxypropylated (MS 0.11) native starch (Fig. 4.17a) and hydroxypropylated (MS 0.10) defatted

Fig. 4.17 Scanning electron micrographs of freshly gelatinized and stored (24 h at 4 °C) pastes of hydroxypropylated potato starches : (A) fresh hydroxypropylated (MS 0.11) native starch; (B) stored hydroxypropylated (MS 0.11) native starch; (C) fresh hydroxypropylated (MS 0.10) defatted starch; (D) stored hydroxypropylated (MS 0.10) defatted starch; (E) &(F) fresh hydroxypropylated (MS 0.11) heat-moisture treated starch; (G) & (H) stored hydroxypropylated (MS 0.11) heat-moisture treated starch.


A
B
C
D
000004 5.0KV X170 141um
000002 5.0KV X170 141um
000005 5.0KV X170 141um
000006 5.0KV X170 141um
000007 5.0KV X170 141um
000016 5.0KV X500 48um
000008 5.0KV X170 141um
000009 5.0KV X500 48um

starch (Fig. 4.17c) were in the form of elongated sheets connected by a network of amylose and amylopectin exudates. The morphology of the granule remnants of both freshly gelatinized native (Fig. 4.17a) and defatted (Fig. 4.17c) starches after hydroxypropylation were different from those of their unmodified counterparts [Fig. 4.13a (native), Fig. 4.13c (defatted)]. In comparison with hydroxypropylated native and defatted starches, the extent of granule disruption was less marked in hydroxypropylated (MS 0.11) heat-moisture treated starch (Fig. 4.17e,f). Furthermore, there were many small intact granules (Fig. 4.17e) which seemed to have escaped disruption during hydroxypropylation.

The hydroxypropyl groups introduced into the starch chains are said to be capable of destroying inter- and intramolecular hydrogen bonds, thereby weakening the granular structure (Wootton & Manatsathit, 1984). Kim *et al.* (1992) have shown, using light microscopy, that the central region of the hydroxypropylated potato starch granules become more and more disrupted with an increase in MS.

The results on hydroxypropylated native starch (Fig. 4.10a, Fig. 4.17a) suggests that although granule stability increases after alkaline treatment (Fig. 4.14a,b), the extent of this increase is not large enough to prevent granule breakdown (Fig. 4.17a) when hydroxypropylated native starch (MS 0.11) is heated under the conditions used for turbidity measurements. Thus, the decrease in initial absorbance at MS 0.11 is due to a sharp decrease in the amount of granule structure present in the starch paste. This would also explain

the lack of influence of increasing MS (0.11-0.25) on light absorbance (Fig. 4.10a).

The progressive decrease in absorbance with increase in MS for defatted (Fig. 4.11a) and heat-moisture treated (Fig. 4.12a) starches suggests that granule remnants of these starches are better able to resist (heat-moisture treated > defatted) the disruptive action of hydroxypropyl groups on granule structure than those of native starch. This can be attributed to the interactions that occur between starch chains on defatting (Vasanthan & Hoover, 1992a) and heat-moisture treatment (Hoover & Vasanthan, 1994a). At similar MS levels (0.20), the absorption decrease is higher in defatted (66%) [Fig. 4.11a] than in heat-moisture treated (10%) starch [Fig. 4.12a] due to greater stability of heat-moisture treated granules. The steep decrease in absorbance (66.2%) at MS 0.28 for heat-moisture treated starch (Fig. 4.12a) suggests that at this MS level granules undergo extensive disruption into small fragments.

4.7.1.4 Turbidity development and scanning electron micrographs of native, defatted and heat-moisture treated starch pastes during storage

The development of turbidity during storage of the above starches at 4°C (1 day) followed by storage at 40°C for 34 days are presented in Figs. 4.10, 4.11 and 4.12. In all starches absorbance increased with storage time. The extent of this increase was more pronounced (native > defatted > heat-moisture treated) during the first day of storage (Figs. 4.10a, 4.11a, 4.12a). The absorbance

increase between 1st and 35th day of storage followed the order : defatted (Fig. 4.11b) > native (Fig. 4.10b) > heat-moisture treated (Fig. 4.12b).

Scanning electron micrographs of the starch pastes after storage for 24 h (at 4°C) are presented in Fig. 4.13b,d,f. The microstructure of the stored pastes of native starch consisted of a dense network formed by leached amylose and amylopectin chains (Fig. 4.13b), whereas, that of defatted starch (Fig. 4.13d) consisted of swollen granule remnants enmeshed and connected together by thick strands of the leached exudate. Heat-moisture treated granule remnants were enmeshed and held together by a network formed by thin strands of the leached exudate (Fig. 4.13f). Many researchers (Craig *et al.*, 1989; Bello-perez & Paredes-Lopez, 1996; Jacobson *et al.*, 1997) have shown that turbidity development during storage is influenced by factors such as granule swelling, granule remnants, leached amylose and amylopectin , amylose and amylopectin chain lengths, intra or intermolecular bonding, lipids, sucrose, cross-linking and substitution.

The first stage of retrogradation has been shown to involve mainly amylose aggregation and crystallization which is completed within the first few hours of storage. However, the second stage that occurs over a longer periods (days) involves mainly amylopectin aggregation and crystallization (Miles *et al.*, 1985, Goodfellow & Wilson, 1990; Silverio *et al.*, 1996).

The rapid increase in turbidity during the first 24 h of storage was thus, mainly due to continued interaction (*via* hydrogen bonding) between leached

amylose chains and to a lesser extent short range ordering of amylopectin side chains. These interactions would lead to the development of junction zones, which would reflect or scatter a significant amount of light.

As shown before, the extent of amylose leaching during gelatinization is more pronounced in native than in defatted or heat-moisture treated starches (defatted > heat-moisture treated). Thus, the magnitude of interaction between leached amylose chains during storage would follow the above trend. This would then partially explain the extent of increase in absorbance [native (Fig. 4.10a) > defatted (Fig. 4.11a) > heat-moisture treated (Fig. 4.12a)] during the first 24 h of storage. It is likely that the magnitude of the increase in absorbance during the 24 h storage period is also influenced by the presence of granule remnants in starch pastes of defatted (Fig. 4.13c) and heat-moisture treated (Fig. 4.13e) starches (heat-moisture treated > defatted). These remnants would hinder chain aggregation during storage. The slow increase in turbidity beyond 24 h storage (Figs. 4.10b, 4.11b, 4.12b) can be attributed to slow interaction between the outer branches of amylopectin chains (Ring *et al.*, 1987) of native, defatted and heat-moisture treated starches. Vasanthan & Hoover (1992a) have shown that defatting increases crystallinity, whereas heat-moisture treatment disrupts crystallinity in potato starch. Consequently, the degree of separation (after gelatinization) between the outer branches of amylopectin chains would be much greater in heat-moisture treated than in defatted starch. Thus, during storage, the formation and lateral association of double helices involving amylopectin

chains would be easier and much stronger in defatted than in heat-moisture treated or native starch (native > heat-moisture treated). This would then explain the observed order of increase in absorbance [defatted (Fig. 4.11b) > native (Fig. 4.10b) > heat-moisture treated (Fig. 4.12b)] between the 1st and 35th day of storage.

4.7.1.5 Turbidity development and scanning electron micrographs of alkaline treated native, defatted and heat-moisture treated starches during storage

In all starches, the extent of absorbance increase during the first 24 h of storage was less pronounced after alkaline treatment (Fig. 4.10a, 4.11a, 4.12a). For instance, during the above time period absorbance increased by only 0.34, 0.55 and 0.77, respectively, in alkali treated native (Fig. 4.10a), defatted (Fig. 4.11a) and heat-moisture treated (Fig. 4.12a) starches. The corresponding values for untreated starches were 2.48 (native), 1.35 (defatted) and 0.81 (heat-moisture treated), respectively. However, the extent of absorbance increase between the 1st and 35th day of storage was more pronounced after alkaline treatment. Scanning electron micrographs of fresh (Fig. 4.14a) and stored (24 h at 4°C [Fig. 4.14c]) pastes of alkali treated native starches were similar. In fresh pastes of alkali treated defatted (Fig. 4.15a) and heat-moisture treated (Fig. 4.16a) starches, granule remnants were scattered throughout the matrix. The size of these remnants was larger in heat-moisture treated starch (Fig. 4.16a).

However, in stored (24 h at 4°C) pastes, granule remnants of both alkali treated defatted (Fig. 4.15c,d) and heat-moisture treated (Fig. 4.16c,d) starches were enmeshed in the amylose exudate. The above results have shown that the alkaline conditions used during hydroxypropylation influence starch chain aggregation during storage. In native starch, the magnitude of the increase in absorbance during 24 h of storage (at 4°C) decreased drastically (Fig. 4.10a) after alkaline treatment. However, in stored pastes of alkali treated defatted (Fig. 4.15c,d) and heat-moisture treated (Fig. 4.16c,d) starches, starch chain aggregation was hindered by aggregated granule remnants (larger in heat-moisture treated). This would explain, differences in absorbance increase (during the 24 h storage period at 4°C) between untreated and alkali treated starches [native (Fig. 4.10a) > defatted (Fig. 4.11a) > heat-moisture treated (Fig. 4.12a)].

4.7.1.6 Turbidity development and scanning electron microscopy of hydroxypropylated native, defatted and heat-moisture treated starch pastes during storage

In hydroxypropylated native starches, absorbance increased with storage time, reaching a maximum value (after 3 days) of 0.28 and 0.24, respectively, at MS 0.11 and 0.18. Thereafter, absorbance decreased gradually reaching a value of 0.20 and 0.16, respectively (after 35 days), at MS 0.11 and 0.18 (Fig. 4.10b). However, at MS 0.25, absorbance remained constant (~ 0.15) until the

20th day (Fig. 4.10b). Thereafter, absorbance decreased gradually reaching a value of 0.09 at the end of the storage period (35th day). In hydroxypropylated defatted starch, absorbance continued to increase with storage time reaching a maximum value of 0.89 (MS 0.10), 0.77 (MS 0.17) and 0.47 (MS 0.20), respectively, after 5 days, 9 days and 1 day (Fig. 4.11b). Thereafter, absorption remained constant until 15th day at MS 0.10 and 0.17, and then decreased steeply reaching a value of 0.51 and 0.49, respectively (after 35 days), at MS 0.10 and 0.17 (Fig. 4.11b). However, at MS 0.20 absorption continued to decrease gradually beyond day 1, reaching a value of 0.32 after 35 days (Fig. 4.11b).

In hydroxypropylated heat-moisture treated starch, absorption continued to increase throughout the storage period at MS 0.11 and 0.20 (Fig. 4.12a,b). The extent of this increase was more pronounced at MS 0.11. However, at MS 0.28 absorption increased only until the 18th h of storage (Fig. 4.12a). Thereafter, it remained unchanged throughout the storage period (Fig. 4.12a,b).

Scanning electron micrographs of stored (1 day) hydroxypropylated native (MS 0.11), defatted (MS 0.10) and heat-moisture treated (MS (0.11) starches are presented in Fig. 4.17. In hydroxypropylated stored native starch paste (MS 0.11), the leached amylose chains and granule remnants were fused together to form a compactly packed structure (Fig. 4.17b). The morphology of the paste was nearly similar to that of stored alkali treated native starch (Fig 4.14c). Granule remnants, leached amylose and amylopectin chains of stored

hydroxypropylated defatted starch (MS 0.10) were loosely packed in the matrix (Fig. 4.17d). Furthermore, the morphology of this paste was totally different from that of stored alkali treated defatted starch (Fig. 4.15c,d). In stored hydroxypropylated heat-moisture treated starch (MS 0.11), granule remnants, leached amylose and amylopectin chains were more loosely packed (Figs. 4.17 g,h) than in stored pastes of hydroxypropylated (MS 0.10) defatted starch (Fig. 4.17c,d).

The results on hydroxypropylation indicate that changes in absorbance during the entire storage period (35 days) is influenced by the interplay of 3 factors : 1) steric effects imposed by bulky hydroxypropyl groups (this decreases the magnitude and rate of increase in absorbance by hindering starch chain alignment during retrogradation); 2) presence of small granule remnants which gradually aggregate during storage (this would increase the intensity of scattered light); and 3) settling of large aggregated granule remnants below the path of the spectrophotometric beam (this would increase light transmittance).

In hydroxypropylated (at MS 0.11 and 0.18) native starch, the gradual increase in absorbance (Fig. 4.10a,b) during the first 3 days of storage is mainly due to slow aggregation of starch chains. The gradual decrease in absorbance beyond day 3 (Fig. 4.10b) at these MS levels, is mainly influenced by factor 2. At MS 0.25, absorbance remained unchanged until day 20 due to factor 1 (which prevents chain aggregation). The decrease in absorbance beyond day 20 at MS 0.25 (Fig. 4.10b) is mainly influenced by factor 2.

In defatted starch, the steep decrease (Fig. 4.11b) in absorbance (after the 15th day) at MS 0.10 and 0.17 is mainly due to factor 3. This decrease does not occur at MS 0.20 since granule remnants are probably disrupted [due to the large increase in granular swelling that occur at this MS level (Table 4.12)]. Consequently, at MS 0.20 the changes in absorbance during storage are mainly influenced by starch chain aggregation.

As described earlier, granule crystallinity is destroyed on heat-moisture treatment. Consequently, heat-moisture treated granules would be more susceptible than defatted granules to disruption by the bulky hydroxypropyl groups. This would explain the absence of large granule remnants (Fig. 4.17e,f) after hydroxypropylation (MS 0.11). The extent of increase in absorbance during storage (35 days) of heat-moisture treated starches at the different MS levels (MS 0.11 > MS 0.20 > MS 0.28) is due to the interplay of factors 1 and 2. The results indicate that all granule remnants are not disrupted by hydroxypropyl groups at MS 0.11 (Fig. 4.17e,f). The undisrupted remnants probably aggregate slowly during storage. This would then explain the steep increase in absorption after 7th day of storage at MS 0.11. However, at MS 0.20 and 0.25 the extent of absorption increase is mainly influenced by factor 1.

4.7.2 Enzyme digestibility

4.7.2.1 Enzyme digestibility of freshly gelatinized and stored pastes of native, defatted and heat-moisture treated starches

Table 4.21 shows the enzyme digestibility of gelatinized and stored native, defatted and heat-moisture treated starch pastes. Freshly gelatinized pastes of native, defatted and heat-moisture treated starches were hydrolyzed nearly to the same extent by porcine pancreatic α-amylase. Storage of gels at 4°C for 24 h decreased hydrolysis of native, defatted and heat-moisture treated starch gels by 5.6, 2.1 and 1.6%, respectively (Table 4.21). However, storage beyond 24 h caused marginal decrease in hydrolysis in the above starches (Table 4.21).

Colonna *et al.* (1992) have shown that α-amylase hydrolysis occurs in three successive steps : diffusion of α-amylase towards its substrate, absorption of the enzyme on the substrate and the catalytic event. Many researchers have shown that enzyme susceptibility of a starch gel decreases with an increase in retrogradation (Ring *et al.*, 1988; Eerlingen *et al.*, 1994; Wang *et al.*, 1995; Cui & Oates, 1997). The following factors have been shown to be responsible for the above observations : 1) an increase in the entanglement of the molecules in the gel network; 2) an increase in molecular order in the short range (double-helix formation); and 3) an increase in the long range (crystallite formation) order. Wang *et al.* (1995) and Cui & Oates (1997) have shown with sago starch gels, that the decrease in enzyme susceptibility during the first 6 h of storage at 5°C, is mainly due to amylose retrogradation.

Table 4.21 α-amylase hydrolysis of freshly gelatinized and stored pastes of native, defatted and heat-moisture treated starches

Starch source and treatment	Hydrolysis (%)[1] Storage time (days)		
	0[2]	1[3]	15[4]
Native	77.2±0.8[a]	71.6±0.7[b]	69.0±1.1[b]
Heat-moisture treated[5]	75.3±0.9[a]	73.7±1.0[ab]	71.2±0.5[b]
Defatted[6]	76.2±1.0[a]	74.1±0.9[a]	74.1±0.4[a]

[1]Hydrolysis for 3 h at 37°C.
[2]Freshly gelatinized.
[3]Storage at 4°C for 24 h.
[4]Storage at 4°C for 24 h and at 40°C for 14 days .
[5]Heat-moisture treatment (16 h / 100°C /30% moisture).
[6]Defatted with 75% propanol for 7 h.
[a-b]Means within a row with different superscripts are significantly different ($p \leq 0.05$).

When an aqueous starch suspension is heated above the gelatinization temperature, swelling is accompanied by the solubilization of amylose. Even on heating to 100°C, there is little evidence for the release of substantial quantities of amylopectin (Ring *et al.*,1987; Morris, 1990). Thus, heating results in a medium composed of gelatinized swollen granules with an apparent amylopectin skeleton embedded in a hot amylose solution. As shown before, the extent of amylose leaching during gelatinization (at 90°C) of native, defatted and heat-moisture treated potato starches were 22.7, 13.4 and 8.3%, respectively (Table 4.4). Leached amylose chains would aggregate faster than those within gelatinized granules (due to steric effects imposed by branched amylopectin chains). Furthermore, aggregation between leached amylose chains would be much faster and stronger in pastes of gelatinized native starch (due to more leached amylose) than in the other starches. Thus, the observed decrease in enzyme hydrolysis during the first 24 h of storage (Table 4.21) [native > defatted > heat-moisture treated] reflects mainly the rate and extent of aggregation between leached amylose chains.

4.7.2.2 Enzyme hydrolysis in freshly gelatinized and stored pastes of alkali treated native, defatted and heat-moisture treated starches

Enzyme digestibility of alkali ttreated starch pastes before and after storage is shown in Table 4.22. Alkaline treatment increased the extent of hydrolysis of freshly gelatinized pastes of native, defatted and heat-moisture

Table 4.22 α-amylase hydrolysis of freshly gelatinized and stored pastes of alkali treated native, defatted and heat-moisture treated starches

Starch source and treatment	Hydrolysis (%)[1] Storage time (days)		
	0[2]	1[3]	15[4]
Native	77.2±0.8[a]	71.6±0.7[b]	69.0±1.1[b]
Alkali treated[5]	93.4±1.2[a]	87.0±0.4[b]	85.5±1.0[b]
Heat-moisture treated[6]	75.3±0.9[a]	73.7±1.0[ab]	71.2±0.5[b]
Alkali treated [5]	81.6±.0.8[a]	76.2±0.9[b]	74.1±1.0[b]
Defatted[7]	76.2±1.0[a]	74.1±0.9[a]	74.1±0.4[a]
Alkali treated[5]	83.5±0.9[a]	80.1±1.2[b]	78.3±0.4[c]

[1]Hydrolysis for 3 h at 37°C.
[2]Freshly gelatinized.
[3]Storage at 4°C for 24 h.
[4]Storage at 4°C for 24 h and at 40°C for 14 days .
[5]Parent starch treated with NaOH and Na_2SO_4 at 40°C.
[6]Heat-moisture treatment (16 h / 100°C /30% moisture).
[7]Defatted with 75% propanol for 7 h.
[a-c]Means within a row with different superscripts are significantly different ($p \leq 0.05$).

treated starches by 16.0, 7.3 and 6.0%, respectively (Table 4.22). The increased hydrolysis on alkaline treatment can be attributed to increased accessibility of α-amylase into the amorphous regions of the granule. This is made possible by repulsion between adjacent amylose chains (carrying negatively charged oxygens) which hinder rapid aggregation of amylose chains immediately after gelatinization. This increase is higher in alkali treated native starch due to more extensive amylose leaching. Storage of alkali treated starches at 4°C for 24 h decreased hydrolysis in native, defatted and heat-moisture treated starches by 4.6, 3.4 and 4.9%, respectively (Table 4.22). In all starches, hydrolysis did not change significantly when storage was continued beyond 24 h (Table 4.22).

4.7.2.3 Enzyme hydrolysis of freshly gelatinized and stored pastes of hydroxypropyl native, defatted and heat-moisture treated starches

Enzyme hydrolysis results of hydroxypropylated native, defatted and heat-moisture treated starch pastes before and after storage are given in Table 4.23. In all starches, hydrolysis decreased on hydroxypropylation (Table 4.23). The extent of hydrolysis of freshly gelatinized native starch decreased by 11.2, 15.6 and 21.5%, respectively, at MS 0.11, 0.18 and 0.25 (Table 4.23). The corresponding values for defatted starch were 14.0, 29.4 and 30.2%, respectively, at MS 0.10, 0.17 and 0.20 (Table 4.23). For heat-moisture treated starch, these values were 5.5, 26.3 and 38.4%, respectively, at MS 0.11, 0.20 and 0.28 (Table 4.23). However, hydrolysis decreased only marginally on

Table 4.23 α-amylase hydrolysis of freshly gelatinized and stored pastes of hydroxypropyl native, defatted and heat-moisture treated starches

Starch source and treatment	Molar substitution	Hydrolysis (%)[1] Storage time (days)		
		0[2]	1[3]	15[4]
Native	0.00[5]	93.4±1.2[a]	87.0±0.4[b]	85.5±1.0[b]
	0.11	66.0±1.0[a]	64.2±1.0[ab]	63.0±0.6[b]
	0.18	61.6±0.7[a]	60.4±1.0[ab]	59.1±0.8[b]
	0.25	55.7±0.3[a]	55.5±0.9[a]	55.0±0.7[a]
Heat-moisture treated[6]	0.00[5]	81.6±.0.8[a]	76.2±0.9[b]	74.1±1.0[b]
	0.11	69.8±0.7[a]	68.0±0.6[ab]	67.4±0.9[b]
	0.20	49.0±1.1[a]	47.8±0.9[ab]	46.4±0.5[b]
	0.28	36.9±0.6[a]	36.1±0.4[a]	36.5±0.9[a]
Defatted[7]	0.00[5]	83.5±0.9[a]	80.1±1.2[b]	78.3±0.4[c]
	0.10	62.2±0.7[a]	61.7±0.8[a]	59.3±1.0[b]
	0.17	46.8±0.8[a]	45.0±1.0[a]	44.1±0.7[b]
	0.20	46.4±0.9[a]	45.6±0.8[a]	45.4±1.1[a]

[1]Hydrolysis for 3 h at 37°C.
[2]Freshly gelatinized.
[3]Storage at 4°C for 24 h.
[4]Storage at 4°C for 24 h and at 40°C for 14 days .
[5]Parent starch treated with NaOH and Na_2SO_4 at 40°C (alkali treated).
[6]Heat-moisture treatment (16 h / 100°C /30% moisture).
[7]Defatted with 75% propanol for 7 h.
[a-c]Means within a row with different superscripts are significantly different ($p \leq 0.05$).

storage of hydroxypropylated starches (Table 4.23). For instance, at a nearly comparable MS level (~0.10-0.11), the decrease in hydrolysis after a 15 day storage period was only 3.0, 2.9 and 3.4%, respectively, in native (MS 0.11), defatted (MS 0.10) and heat-moisture treated (MS 0.11) starches [Table 4.23].

The decrease in hydrolysis on hydroxypropylation is in agreement with Leegwater & Luten (1971), Wootton & Chaudhry (1981) and Hoover *et al.* (1988). A study of the catalytic groups of α-amylase has shown that carboxylate anion acts as the nucleophile and imidazolium cation as the electrophile (Robyt, 1984). It is possible that the decrease in hydrolysis with increase in MS may be due to bulky hydroxypropyl groups on C-2 sterically hindering the action of the catalytic carboxylate ion on the glycosidic bond. The marginal change in hydrolysis on storage (Table 4.23) suggests that hydroxypropylation is very effective in hindering starch chain aggregation within the amorphous domain of the granule.

4.7.3 X-ray diffraction

4.7.3.1 X-ray diffraction of fresh and stored gels of native, defatted and heat-moisture treated starches

The X-ray diffraction patterns of freshly gelatinized and stored (1 day at 4°C and for 29 days at 40°C) pastes of native, defatted and heat-moisture treated starches are presented in Figs. 4.18, 4.19 and 4.20. No significant peak could be distinguished in freshly gelatinized native (Fig. 4.18a), defatted (Fig.

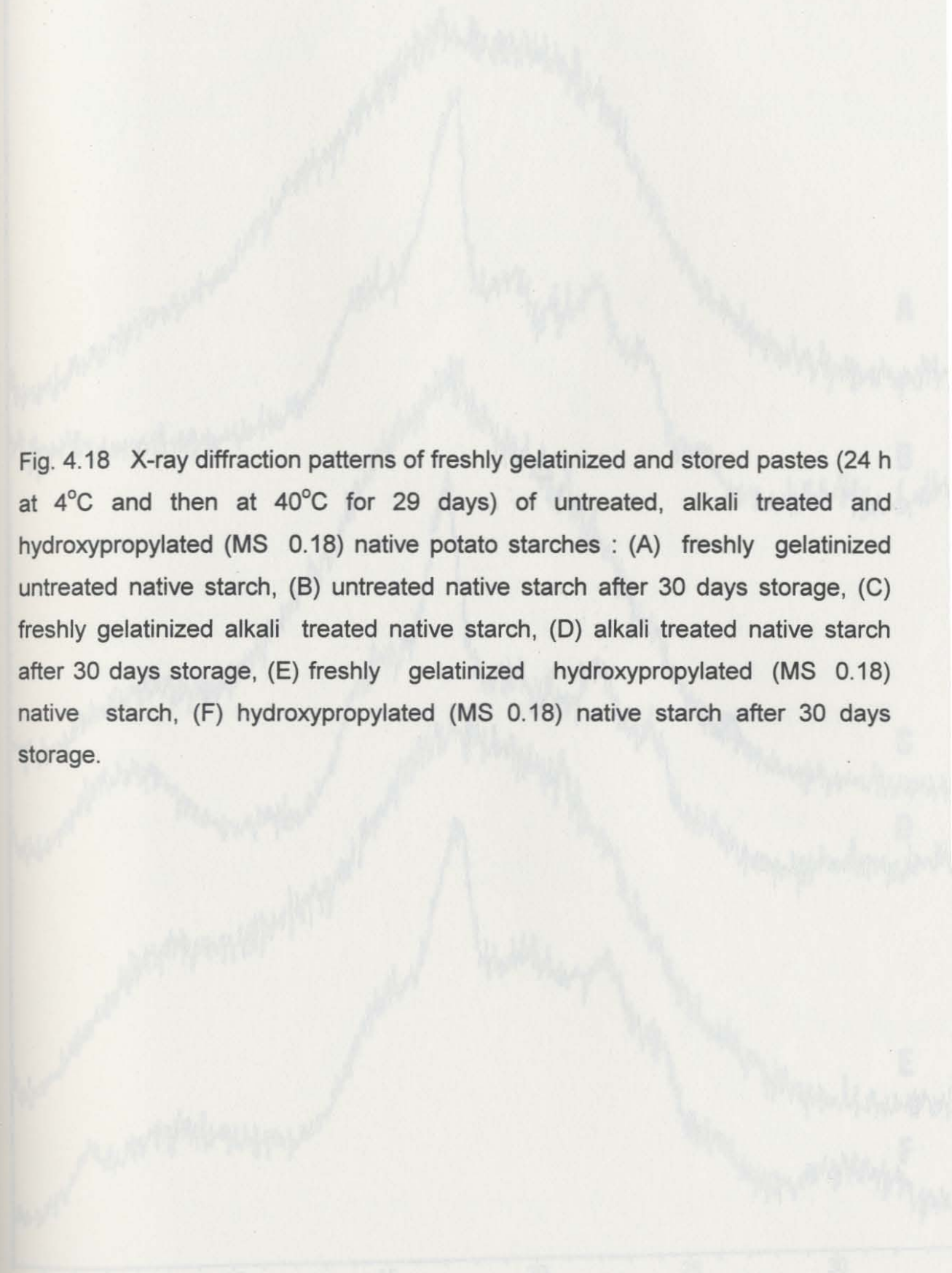


Fig. 4.18 X-ray diffraction patterns of freshly gelatinized and stored pastes (24 h at 4°C and then at 40°C for 29 days) of untreated, alkali treated and hydroxypropylated (MS 0.18) native potato starches : (A) freshly gelatinized untreated native starch, (B) untreated native starch after 30 days storage, (C) freshly gelatinized alkali treated native starch, (D) alkali treated native starch after 30 days storage, (E) freshly gelatinized hydroxypropylated (MS 0.18) native starch, (F) hydroxypropylated (MS 0.18) native starch after 30 days storage.

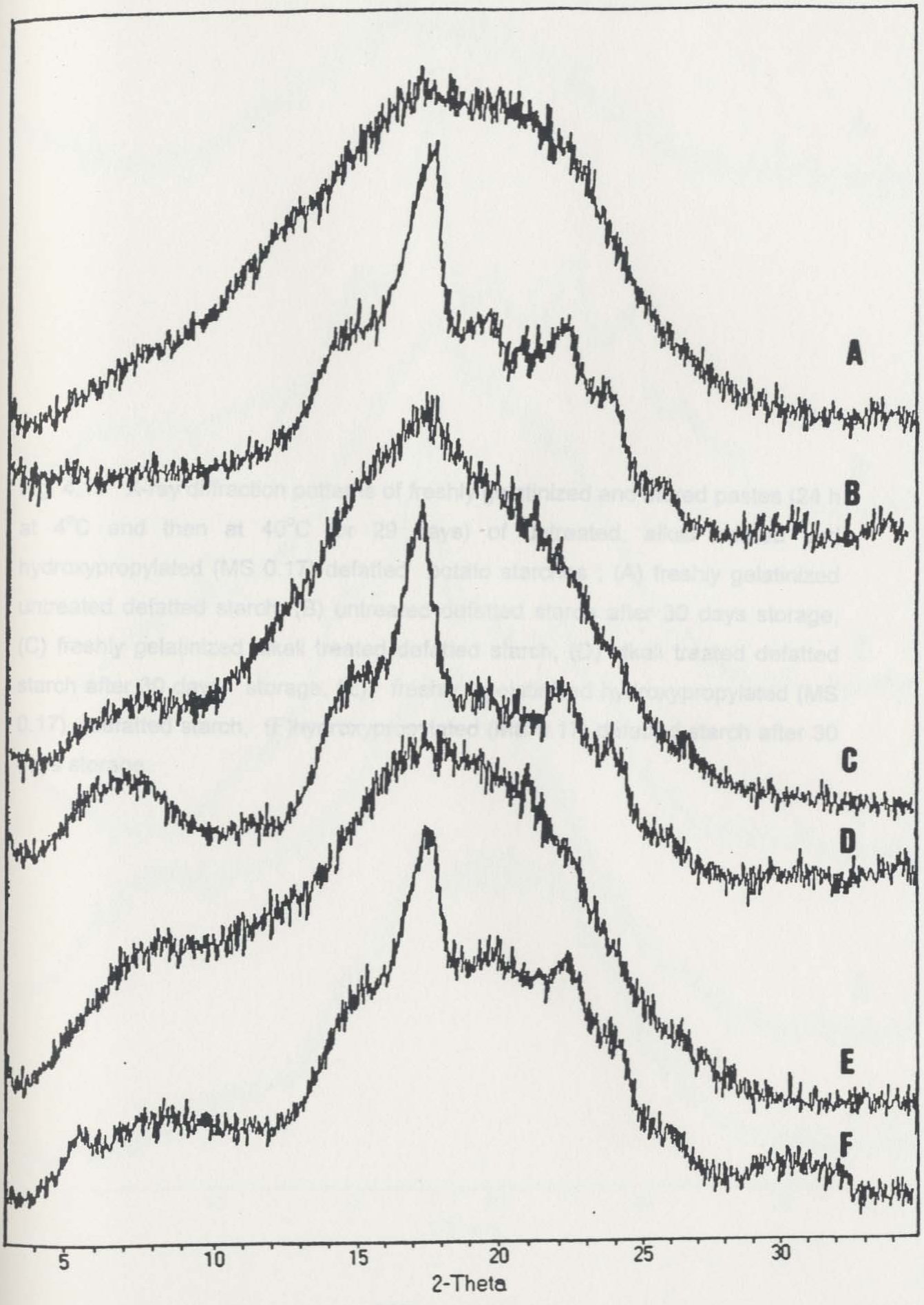
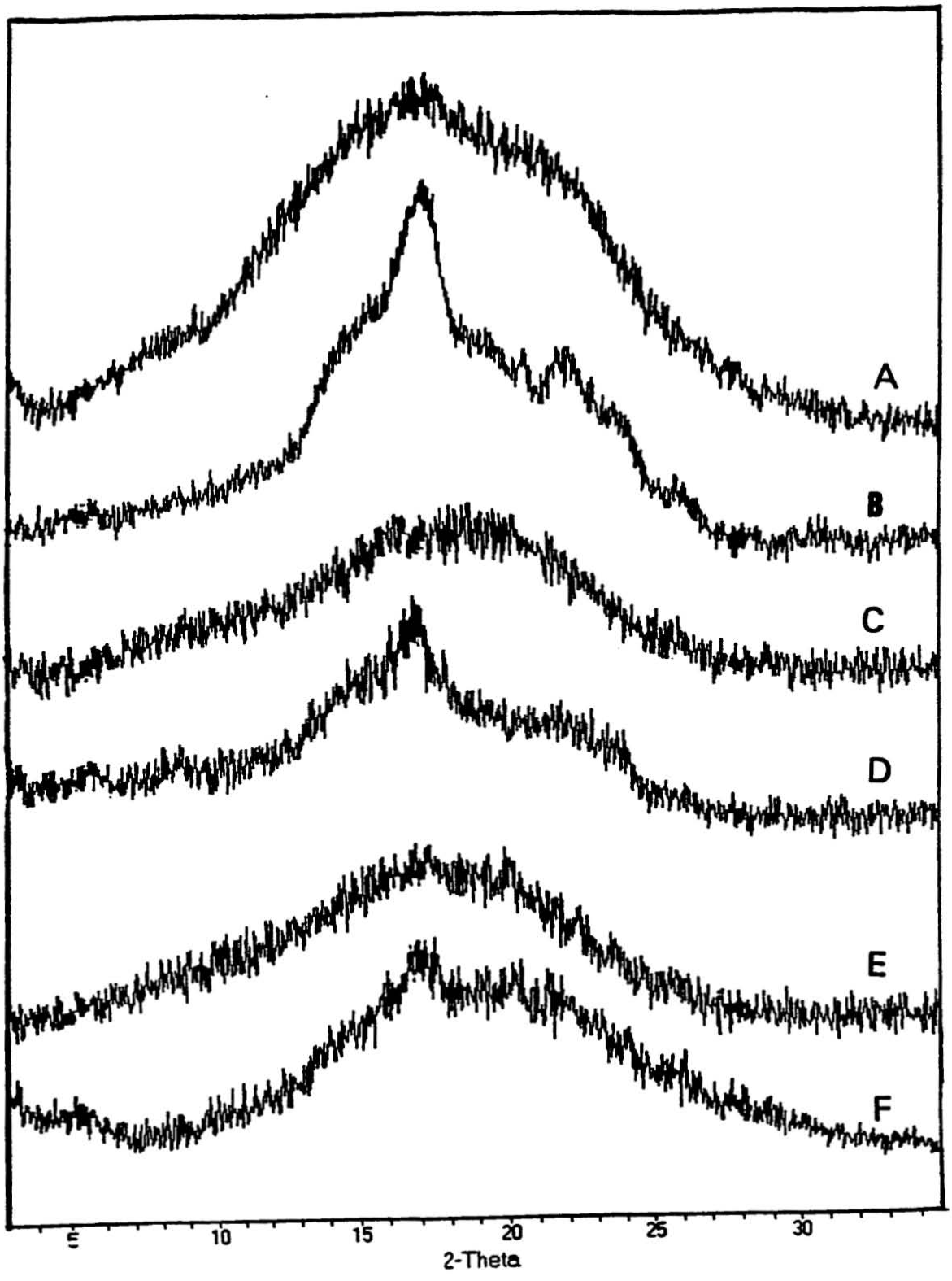

A
B
C
D
E
F
5
10
15
20
25
30
2-Theta

Fig. 4.19 X-ray diffraction patterns of freshly gelatinized and stored pastes (24 h at 4°C and then at 40°C for 29 days) of untreated, alkali treated and hydroxypropylated (MS 0.17) defatted potato starches : (A) freshly gelatinized untreated defatted starch, (B) untreated defatted starch after 30 days storage, (C) freshly gelatinized alkali treated defatted starch, (D) alkali treated defatted starch after 30 days storage, (E) freshly gelatinized hydroxypropylated (MS 0.17) defatted starch, (F)hydroxypropylated (MS 0.17) defatted starch after 30 days storage.

Fig. 4.20 X-ray diffraction patterns of freshly gelatinized and stored pastes (24 h at 4°C and then at 40°C for 29 days) of untreated, alkali treated and hydroxypropylated (MS 0.20) heat-moisture treated potato starches : (A) freshly gelatinized untreated heat-moisture treated starch, (B) untreated heat-moisture treated starch after 30 days storage, (C) freshly gelatinized alkali treated heat-moisture treated starch, (D) alkali treated heat-moisture treated starch after 30 days storage, (E) freshly gelatinized hydroxypropylated (MS 0.20) heat-moisture treated starch, (F) hydroxypropylated (MS 0.20) heat-moisture treated starch after 30 days storage.


A
B
C
D
E
F
10
15
20
25
30
2-Theta

4.19a) and heat-moisture treated starches (Fig. 4.20a). The 'B' type X-ray pattern which is typical of retrograded starch (d spacings at 16, 5.2, 3.9 and 3.7) was evident (in all three starches) at the end of the storage period (Figs. 4.18b, 4.19b, 4.20b). The intensity of the spacing centred at 5.2 Å followed the order : defatted > native > heat-moisture treated (Table 4.24). As described earlier, the distance separating the outer branches of amylopectin chains in gelatinized starches followed the order : heat-moisture treated > native > defatted. Thus, due to closer proximity of the outer branches in gelatinized defatted starch, double helical formation and packing of double helices during storage would be much easier and more ordered in defatted than in other starches. This would then account for the stronger X-ray diffraction pattern of retrograded defatted starch (Fig. 4.19b). It has been shown that the 'B' pattern originates from hexagonal packing of double helices during retrogradation of both amylose (Gidley, 1989) and amylopectin (Zobel, 1988b). As shown in Fig. 4.13, granule remnants are present in gelatinized pastes of defatted (Fig. 4.13c) and heat-moisture treated starches (Fig. 4.13e). The size of these remnants being greater in the latter. The presence of granule remnants could hinder aggregation and packing of double helices of both amylose and amylopectin. Thus, the recorded intensities are probably influenced by the interplay of two factors : 1) degree of proximity of the outer branches of amylopectin chains to each other in gelatinized pastes; and 2) size of the granule remnants in the gelatinized paste.

Table 4.24 X-ray diffraction intensities (at 5.2Å) of stored[1] potato starch gels

Starch source & treatment	Moisture (%)	Intensity (cps[2]) at 5.2Å
Native	9.6	1535
Native (alkali treated)[3]	9.5	1496
Native (MS[4] 0.18)	9.6	990
Heat-moisture treated[5]	9.5	1225
Heat-moisture treated (alkali treated)[3]	9.6	232
Heat-moisture treated (MS[4] 0.20)	9.5	194
Defatted[6]	9.4	1895
Defatted(alkali treated)[3]	9.5	1383
Defatted (MS[4] 0.17)	9.5	715

[1]One day at 4°C and then at 40°C for 29 days.

[2]Counts per second.

[3]Parent starch treated with NaOH and Na_2SO_4 at 40°C.

[4]Molar substitution.

[5]Heat-moisture treatment (16 h / 100°C /30% moisture).

[6]Defatted with 75% propanol for 7 h.

4.7.3.2 X-ray diffraction of fresh and stored gels of alkali treated native, defatted and heat-moisture treated starches

No significant peak could be distinguished in freshly gelatinized pastes of native (Fig. 4.18c), defatted (Fig. 4.19c) and heat-moisture treated (Fig. 4.20c) starches. In all alkali treated starches, the X-ray diffraction pattern and the intensity of the 5.2 Å peak after 30 days of storage was weaker than in their untreated counterparts (Table 4.24). The extent of this intensity reduction followed the order : heat-moisture treated > defatted > native (Table 4.24). This decrease can be attributed to the size of granule remnants (heat-moisture treated > defatted > native) in the alkali treated pastes, which would hinder the formation, aggregation and packing of double helices in a crystalline array.

4.7.3.3 X-ray diffraction of fresh and stored gels of hydroxypropyl native, defatted and heat-moisture treated starches

No significant peaks could be distinguished in freshly gelatinized pastes of hydroxypropylated native (Fig. 4.18e), defatted (Fig. 4.19e) and heat-moisture treated (Fig. 4.20e) starches. The intensities at 5.2 Å decreased in stored hydroxypropylated starch gels (Table 4.24). The extent of this decrease in native (MS 0.18), defatted (MS 0.17) and heat-moisture treated (MS 0.20) hydroxypropylated starches were 35.5, 62.2 and 84.2%, respectively. This decrease is due to a decrease in chain aggregation resulting from the interplay of two factors : 1) steric effects imposed by hydroxypropyl groups on adjacent

starch chains; and 2) aggregation of large granule remnants of hydroxypropylated defatted (MS 0.17) [Fig. 4.17d] and heat-moisture treated (MS 0.20) [Fig. 4.17g] starches.

4.7.4 Differential scanning calorimetry (DSC)

4.7.4.1 DSC parameters of native, defatted and heat-moisture treated starches after gelatinization and storage

In all starches, the retrogradation endotherm occurred only after 2 days of storage (Table 4.25). Retrogradation enthalpy (ΔH_R) at the end of the storage period (7 days) was more pronounced in native (6.7J/g) than in defatted (4.5 J/g) or heat-moisture treated (4.8 J/g) starches. The transition temperatures T_o, T_p and T_c of the retrogradation endotherm of native starch were higher than those of defatted and heat-moisture treated starches. However, differences between T_o, T_p and T_c of defatted and heat-moisture treated starches were only marginal (Table 4.25). In all starches, T_o, T_p and T_c remained practically unchanged over the day 7 of storage. The storage of gels beyond 7 days produced endotherms which were too broad for accurate determination of transition temperatures and ΔH_R .

Recrystallization of starch molecules occurs during gel storage. Reheating of an aged starch gel in a DSC produces an endothermic transition which is not present in the DSC scan of freshly gelatinized samples. Such a transition is

Table 4.25 DSC parameters of native, defatted and heat-moisture treated potato starches after gelatinization and storage

Starch source and treatment	Storage time[1] (days)	Transition Temperatures (°C) To[2]	Tp[2]	Tc[2]	Enthalpy of retrogradation (J/g)
Native	1	-	-	-	0.0±0.0[d]
	2	63.3±0.3[a]	72.2±0.8[a]	81.6±0.6[a]	3.8±0.0[c]
	5	62.9±0.2[a]	71.7±0.1[a]	81.5±0.4[a]	4.9±0.2[b]
	7	63.1±0.4[a]	73.4±0.4[a]	82.1±0.3[a]	6.7±0.0[a]
Heat-moisture treated[3]	1	-	-	-	0.0±0.0[h]
	2	60.9±0.6[e]	67.7±0.7[e]	76.8±0.1[e]	3.3±0.0[g]
	5	61.1±0.5[e]	67.8±0.2[e]	76.8±0.5[e]	4.2±0.2[f]
	7	61.3±0.0[e]	67.4±0.1[e]	77.0±0.1[e]	4.8±0.0[e]
Defatted[4]	1	-	-	-	0.0±0.0[i]
	2	59.5±0.1[j]	67.7±0.4[i]	75.7±0.3[j]	2.9±0.3[k]
	5	60.5±0.6[i]	67.0±0.3[i]	76.7±0.1[i]	3.2±0.1[j]
	7	60.3±0.5[i]	67.4±0.2[i]	76.1±0.2[i]	4.5±0.1[i]

[1]. Storage beyond 7 days gave endotherms that were too broad for accurate determination of DSC parameters.

[2]T_o, T_p & T_c indicate respectively, the temperature of onset, mid point and end of retrogradation endotherm.

[3]Heat-moisture treatment (16 h / 100°C /30% moisture).

[4]Defatted with 75% propanol for 7 h.

[a-i] Means within a column with different superscripts are significantly different ($p \leq 0.05$).

generally attributed to the melting of crystallized amylopectin. This was also proven by the thermo-reversible crystallization of amylose free (obtain by leaching) swollen starch granules (Miles *et al.*, 1985b). The thermo-reversibility is attributed to the involment of outer branches of amylopectin which gives rise to low melting crystals (Morris, 1990). The enthalpy of retrogradation is generally considered to correspond to order-disorder transitions of crystallites (i.e. double helices present in extended ordered arrays) and regions of lesser crystalline order. Numerous researchers (Ring *et al.*, 1987; Russel, 1987; Kalichevsky *et al.*, 1990; Ward *et al.*, 1994) have observed retrogradation endotherm at similar temperatures well below the temperature range for gelatinization. Recrystallization of the amylopectin branch chains in a less ordered manner than what existed for the native starch is an explanation for the observed amylopectin retrogradation endotherm at a temperature range below that for gelatinization (Ward *et al.*, 1994).

Amylose crystals formed in starch gels contain longer helical sequences which melt at higher temperatures. Thus, the melting of amylose crystallites were not observed within the heating temperature range studied (20-100°C) in DSC. The absence of retrogradation endotherm on day 1 suggests that amylose crystallization takes place mainly during the first 24 h of storage, after which amylopectin crystallization occurs at a slow rate, which was implied by the gradual increase in ΔH_R.

Hoover & Vasanthan (1994a) have shown that during heat-moisture treatment crystallinity is disrupted within granules of potato starch. Thus, after gelatinization the degree of separation between the outer branches of adjacent amylopectin chain clusters would be more pronounced in heat-moisture treated than in native starch. Consequently, double helical formation (during storage) between adjacent amylopectin chains of gelatinized heat-moisture treated starch would be much slower and reduced in number than in native starch. This would explain the slower increase in ΔH_R (during storage) and its decreased magnitude after heat-moisture treatment (Table 4.25). Vasanthan & Hoover (1992a) have also shown that defatting increases granule crystallinity in potato starch. Thus, the number of ordered double helices that disrupt on gelatinization would be much lower in defatted than in native starch. Consequently, the number of new double helices that form during retrogradation would be much decreased after defatting. This would explain the slower increase in ΔH_R (during storage) and its decreased magnitude after defatting (Table 4.25).

4.7.4.2 DSC parameters of alkali treated native, defatted and heat-moisture treated starches after gelatinization and storage

In all starches, retrogradation endotherm appeared on the day 2 of storage (Table 4.26). The ΔH_R of alkali treated starch gels increased with increase in storage time, whereas the transition temperatures remained unchanged during the time course of retrogradation. However, the magnitude

Table 4.26 DSC parameters of alkali treated[1] native, defatted and heat-moisture treated potato starches

Starch source and treatment	Storage time[2] (days)	Transition temperature (°C) To[3]	Tp[3]	Tc[3]	Enthalpy of retrogradation (J/g)
Native	1	-	-	-	0.0±0.0^{d}
	2	62.1±0.4^{a}	70.6±0.5^{a}	80.3±0.2^{a}	3.7±0.1^{c}
	5	62.5±0.5^{a}	70.4±0.3^{a}	80.2±0.7^{a}	4.6±0.2^{b}
	7	62.5±0.4^{a}	71.0±0.3^{a}	81.1±0.2^{b}	6.3±0.0^{a}
Heat-moisture treated[4]	1	-	-	-	0.0±0.0^{h}
	2	61.4±0.1^{f}	70.6±0.5^{f}	81.1±0.8^{f}	4.3±0.1^{g}
	5	62.6±0.4^{e}	71.6±0.8^{ef}	81.5±0.5^{f}	6.1±0.1^{f}
	7	62.1±0.3^{e}	71.7±0.4^{e}	82.9±0.2^{e}	7.3±0.1^{e}
Defatted[5]	1	-	-	-	0.0±0.0^{i}
	2	63.3±0.2^{i}	73.0±0.0^{i}	80.6±0.2^{i}	2.0±0.1^{k}
	5	63.3±0.2^{i}	72.4±0.3^{j}	80.8±0.6^{i}	3.2±0.1^{j}
	7	62.1±0.2^{j}	72.2±0.9^{ij}	80.6±0.1^{i}	4.5±0.2^{i}

[1]Parent starch treated with NaOH and Na_2SO_4 at 40°C

[2]Storage beyond 7 days gave endotherms that were too broad for accurate determination of DSC parameters.

[3]T_o, T_p & T_c indicate respectively, the temperature of onset, mid point and end of retrogradation endotherm.

[4]Heat-moisture treatment (16 h / 100°C /30% moisture).

[5]Defatted with 75% propanol for 7 h.

[a-l] Means within a column under the same treatment with different superscripts are significantly different ($p \leq 0.05$).

and the rate of increase in ΔH_R (during storage) decreased slightly after alkali treatment. The difference in magnitude of ΔH_R between untreated and alkali treated starches was more pronounced in defatted than in native and heat-moisture treated starches (Table 4.26). The results indicate that the number of double helices that form on storage is reduced after alkaline treatment. This can be attributed to repulsion between negatively charged oxygens on adjacent interacting starch chains.

4.7.4.3 DSC parameters of hydroxypropylated native (MS 0.11), defatted (MS 0.10) and heat-moisture treated (MS 0.11) starches after gelatinization and storage

In hydroxypropylated starches (Table 4.27), the retrogradation endotherm occurred only near the end (7 days) of the storage period. Hydroxypropylation decreased ΔH_R in all starches (Table 4.27). At the same MS (0.11), the extent of decrease in ΔH_R (Day 7) was more pronounced in native than in heat-moisture treated starch (Table 4.27). This decrease in ΔH_R suggests that hydroxypropyl groups within the bulk amorphous and intercrystalline amorphous regions hinder double helical formation by preventing proper alignment of the outer branches of the amylopectin chains during storage. The reduction in ΔH_R on hydroxypropylation was more pronounced in native than in defatted starch (Table 4.27) due to two reasons : 1) the lower MS level of defatted starch (MS

Table 4.27 DSC parameters of native, defatted and heat-moisture treated hydroxypropyl potato starches

Starch source and treatment	Molar substitution[1]	Storage time (days)[2]	Transition temperature (°C) To[3]	Tp[3]	Tc[3]	Enthalpy of retrogradation (J/g)
Native	0.11	7	63.7±0.4[a]	73.3±0.9[a]	82.7±0.5[a]	2.7±0.0[b]
Heat-moisture treated[4]	0.11	7	62.5±0.6[a]	72.8±0.4[a]	83.3±0.5[a]	3.4±0.2[a]
Defatted[5]	0.10	7	62.8±0.3[a]	72.4±0.2[a]	83.4±0.2[a]	2.1±0.3[c]

[1]In all starches, a detectable endotherm was not observed at high molar substitution levels (> 0.11).

[2] Starches were stored at 4°C for 24h and then at 40°C. Endotherm was not observed before 7 days of storage.

[3]T_o, T_p & T_c indicate respectively, the temperature of onset, mid point and end of retrogradation endotherm.

[4]Heat-moisture treatment (16 h / 100°C /30% moisture).

[5]Defatted with 75% propanol for 7 h.

[a-c] Means within a column under the same treatment with different superscripts are significantly different ($p \leq 0.05$).

0.10); and 2) the presence of higher amounts of crystallites in the gelatinized defatted starch paste (defatting increases crystallinity) which partially negates the influence of hydroxypropyl groups on chain alignment.

Furthermore, the crystallites formed during storage of hydroxypropylated defatted and hydroxypropylated heat-moisture treated gels melted at a broader temperature range (hydroxypropylated defatted, Tc-To = 20.6°C; hydroxypropylated heat-moisture treated, Tc-To = 20.8°C [Table 4.27]) than did their untreated counterparts (defatted, Tc-To = 15.8°C, heat-moisture treated, Tc-To = 15.7°C [Table 4.25]). This broadening is possibly due to the formation of loosely packed gels (as shown by the scanning electron micrographs Fig. 4.17d,h) which are composed of crystallites with different stabilities. However, broadening of crystallite melting temperature range was not observed in retrograded native starch gels after hydroxypropylation.

CHAPTER 5

SUMMARY AND CONCLUSIONS

This study has shown that defatting causes clustering of the outer 'A' chains of amylopectin, resulting in the formation of crystallites which are perfectly arrayed to diffract X-rays, whereas, crystallite disruption and/or reorientation of the double helices (within the crystalline array) occurs on heat-moisture treatment. Both defatting and heat-moisture treatment changed the X-ray pattern from 'B' to 'A+B'. However, the amount of 'A' unit cells was higher on heat-moisture treatment. Interactions between starch chains (AM-AM and AM-AP) occurred on defatting and heat-moisture treatment (heat-moisture treatment > defatting). Double helical content within the amorphous and crystalline domains decreased on heat-moisture treatment, but increased slightly on defatting. These structural changes decreased the SF, Brabender viscosity (at 95°C) and AML, and increased the thermal stability and gelatinization transition temperatures of both defatted and heat-moisture treated starches. However, ΔH decreased on heat-moisture treatment, but increased on defatting.

The reagents (NaOH and Na_2SO_4) used during hydroxypropylation altered crystallite orientation, disrupted double helices (within the amorphous regions), increased SF, and decreased Brabender viscosity (at 95°C). The extent of the above changes followed the order : native > defatted > heat-moisture treated. However, starch chain interactions did not occur under these reaction conditions.

The degree of accessibility of hydroxypropyl groups into the granule interior (heat-moisture treated > native > defatted) was found to be influenced by granule crystallinity (defatted > native > heat-moisture treated). X-ray diffraction patterns showed that hydroxypropylation occurs mainly in the amorphous regions.

The SF (at different temperatures) of hydroxypropylated (at different MS levels) native, defatted and heat-moisture treated starches was influenced by the interplay between the extent of hydrogen bond disruption within the amorphous regions (due to hydroxypropylation) and the increased interaction that occurs between starch chains during defatting and heat-moisture treatment. Hydroxypropylation decreased amylose leaching. The DSC results showed that hydroxypropylation decreased T_o, T_p and T_c (due to hydrogen bond disruption) and ΔH (due to double helical disruption). The magnitude of this decrease was influenced by the MS level. The pasting temperatures of all three starches decreased with increased MS. Increase in the level of MS progressively increased Brabender viscosity (95°C) for defatted starch. However, for native and heat-moisture treated starches, an increase in the level of MS beyond 0.18 and 0.20, respectively, resulted in a decrease in Brabender viscosity (95°C). The changes in pasting temperature and viscosity with increase in MS levels were attributed to the interplay between changes in SF and hydrogen bond disruption.

The enzyme digestibility study showed that the susceptibility of potato starch granules towards hydrolysis by porcine pancreatic α-amylase increased

progressively with the time of defatting (due to the release of entrapped amylose chains into the amorphous domains of granules). However, defatting beyond 9 h, decreased hydrolysis (due to interactions between the outer branches of amylopectin chains and between the neighboring segments of the released amylose chains) to a level that was lower than that observed for native starch. Heat-moisture treatment was also found to increase granule susceptibility towards hydrolysis by α-amylase. A steep increase in hydrolysis occurred during the first 8 h of heat-moisture treatment (due to crystallite disruption), followed by a gradual decrease (due to AM-AM and AM-AP interactions). However, at all time intervals, the level of hydrolysis in heat-moisture treated starch was higher than that of native starch.

The alkaline conditions used during hydroxypropylation were found to increase the susceptibility of native, defatted and heat-moisture treated starches towards hydrolysis by α-amylase (native > defatted > heat-moisture treated). The extent of this increase was attributed to the interplay of 3 factors : a) disruption of double helices, b) ionization of the hydroxyl groups on adjacent starch chains, and c) an increase in granule solubility.

The susceptibility of native, defatted and heat-moisture treated starches towards hydrolysis by α-amylase increased with MS levels (due to increased granular swelling). However, hydrolysis decreased (due to steric effects imposed by the bulky hydroxypropyl groups) at MS levels of 0.29 (native), 0.28 (heat-

moisture treated) and 0.26 (defatted). The results showed that the structural changes that occur on defatting and heat-moisture treatment do not influence the mechanism by which hydroxypropyl groups influence the reactivity of the granule towards α-amylase.

The retrogradation properties of native defatted and heat-moisture treated potato starches before and after hydroxypropylation were monitored using different techniques. The results showed that changes in turbidity during storage of native, defatted and heat-moisture treated starch pastes were influenced by the interplay of : 1) interaction between leached starch chains, 2) interaction between granule remnants and the leached exudate. However, in alkali treated native, defatted and heat-moisture treated starch pastes, turbidity changes on storage were influenced by aggregation of granule remnants. The extent followed the order : native > defatted >heat-moisture treatment. Hydroxypropylation decreased the rate and extent of increase in turbidity during storage of all three starches. The change in turbidity during storage of hydroxypropylated starch pastes was influenced by the interplay between : 1) steric effects imposed by hydroxypropyl groups on chain aggregation, 2) aggregation between small granule remnants, and 3) settling of large granule remnants beneath the path of the spectrophotometer beam. Reactivity of gelatinized pastes of native, defatted and heat-moisture treated starches towards porcine pancreatic α-amylase decreased on storage. A similar trend was also observed after alkaline treatment. However, hydrolysis remained unchanged

throughout the storage period in hydroxypropylated starches. Stored pastes of native, defatted and heat-moisture treated starches gave a 'B' type X-ray pattern. A similar pattern was also observed after alkaline treatment, and hydroxypropylation. However, the X-ray intensity of the peak at 5.2Å decreased after alkaline treatment and hydroxypropylation. The retrogradation endotherm (monitored by DSC) occurred after 2 days storage in native, defatted and heat-moisture treated starches. A similar trend was also observed after alkaline treatment. However, the retrogradation endotherm appeared only after 7 days in hydroxypropylated starches. The enthalpy of retrogradation in all starches decreased on alkaline treatment and hydroxypropylation. These results showed that, retrogradation properties of native, defatted and heat-moisture treated starches are indirectly influenced by the structural arrangement of starch chains within the amorphous and crystalline regions of the ungelatinized granule, which in turn, influence the extent of granule breakdown during gelatinization, and the interactions that occur between starch chains during gel storage.

5.1 IMPORTANCE OF THIS STUDY TO STARCH CHEMISTS, AND DIRECTIONS FOR FUTURE RESEARCH

This research has provided additional information on the mechanism of starch chain rearrangements within the amorphous and crystalline domains of the potato starch granule under conditions of defatting and heat-moisture treatment. Conditions similar to heat-moisture treatment might be expected to occur during food processing when such conditions are met. Thus, this study would provide a deeper insight into the structure and physicochemical changes that occur during hydrothermal treatment of foods. This study has also shown that granule crystallinity changes on heat-moisture treatment. Since crystallinity influences starch functionality, heat-moisture treatment may be one way of modifying the poor functional properties of legume and certain tuber starches (cassava, yam). Research should thus be carried out to improve the functional properties of these starches by heat-moisture treatment (under different time / temperature / moisture conditions regimes).

The trend in the food industry is moving towards natural ingredients. For years, scientists have been researching for starches that have all of the properties of a modified starch but without the chemical treatment.

Presently, starches are modified by acetylation and hydroxypropylation to reduce retrogradation rates (high retrogradation rates lead to poor freeze thaw stability). This research has indicated that heat-moisture treatment may provide starches with superior freeze thaw stability (since retrogradation rates would be

drastically reduced after heat-moisture treatment, due to decrease in amylose leaching and granular swelling). Thus, starch chemists need to tailor the retrogradation properties of heat-moisture treated starches (by different time / temperature / moisture conditions) to a level that is presently met by chemical modification.

The mode of interaction of sugars and lipids with starch components is still in dispute (Evans & Haisman, 1982; Germani *et al.,* 1983; Eliasson, 1992; Bello-perez & Paredes-lopez, 1995). Furthermore, the mechanism by which sugars influence starch properties is still not clearly understood. This is because many of these studies have been conducted on native starches. However, if the above studies are conducted on native, defatted, heat-moisture treated and alkali treated starches, a deeper insight into the mechanism can be obtained.

This work has covered those areas in which the most exciting developments are likely to occur. It will provide the impetus for food scientists and food processors to look for physical means by which starch properties can be tailored to requirements whilst, avoiding any toxicological problems.

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Publications in Refereed Journals

1. Perera, C., Hoover, R. & Martin, A.M. (1997). The effect of hydroxypropylation on the structure and physicochemical properties of native, defatted and heat-moisture treated potato starches. *Food Res. Int.*, **30** : 235-247.

2. Perera, C. & Hoover, R. (1998). The reactivity of porcine pancreatic α-amylase towards native defatted and heat-moisture treated potato starches before and after hydroxypropylation. *Starch,* (in press).

3. Perera, C. & Hoover, R. (1998). Influence of hydroxypropylation on retrogradation properties of native, defatted and heat-moisture treated potato starches. *Food Chem.*, (in press).

4. Chavan, U., Shahidi, F., Hoover, R. & Perera, C. (1998). Isolation and physicochemical properties of beach pea starch. *Food Chem.*, (in press).

5. Hoover, R., Sinnott, A.W. & Perera, C. (1998). Physicochemical characterization of starches from amaranthus cruentus grains. *J. Food Biochem.*, (submitted for publication).

Papers in preparation

1. Perera, C. & Hoover, R. (1998). Influence of defatting, heat-moisture treatment and hydroxypropylaiton on the susceptibility of retrograded potato starch gels towards α-amylase. (In preparation).

Presentations

1. Perera, C., Hoover, R. & Martin, A.M. (1996). The influence of defatting and heat moisture treatment on the reactivity of potato starch towards hydroxypropylation. 79th Annual conference of Canadian Society for Chemistry. June 23-26. Newfoundland, Canada.

2. Perera, C., Hoover, R. & Martin, A.M. (1996). Effect of hydroxypropylation on the thermal characteristics of native, defatted and heat-moisture treated potato starches. 39th Annual conference of Canadian Institute of Food Science and Technology. August 17-22. Guelph, Canada.

3. Perera, C. & Hoover, R. (1997). Structural and physicochemical characteristics of native, defatted and heat-moisture treated potato starch granules before and after hydroxypropylation. Annual Meeting of Institute of food technologists. June 14-18, Orlando, USA.

4. Perera, C., Hoover, R. (1997). The reactivity of alpha-amylase towards native, defatted and heat-moisture treated hydroxypropyl potato starches. Annual conference of Canadian Institute of Food Science and Technology. September 20-24. Montreal, Canada.

5. Hoover, R. & Perera, C. (1998). Retrogradation studies on physically and chemically modified potato starches. Production and uses of starches. Association of Applied Biologists, April 6-8. Edinburgh, UK.

6. Perera, C. & Hoover, R. (1998). Role of starch modification on retrogradation of potato starch gels. CIC-APIC Atlantic Student Chemistry Conference. May 13-15, Newfoundland, Canada.

7. Perera, C. & Hoover, R. (1998). A comparative study of the rate and the extent of retrogradation of native, defatted and heat-moisture treated potato starches before and after hydroxypropylation. Annual Meeting of Institute of food technologists. June 20-24, Atlanta, USA.

Scholarships and Awards

1984-1988	Scholarship for Undergraduate studies, Sri Lanka
1991-1993	Scholarship for Postgraduate studies, UK
1995 -1998	Graduate Fellowship, Dept. Biochemistry, Memorial University of Newfoundland
1997	Graduate student paper award (Carbohydrate division), Annual Meeting of Institute of Food Technologists, Orlando, USA.
1998	Fellow of the Graduate School, Memorial University of Newfoundland
1998-1999	Postdoctoral Fellowship, Iowa State University, USA