EFFECT OF PHYSICAL MODIFICATION ON STARCH STRUCTURE AND PHYSICOCHEMICAL PROPERTIES

THAVARATNAM VASANTHAN

EFFECT OF PHYSICAL MODIFICATION ON STARCH STRUCTURE AND PHYSICOCHEMICAL PROPERTIES

BY

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ABSTRACT

Lipids from wheat, oat, lentil and potato starches were extracted by acid hydrolysis and by selective solvent extraction with chloroform-methanol 2:1 v/v [CM] at ambient temperature, followed by *n*-propanol-water 3:1 v/v [PW] at 90-100°C. The acid hydrolysed extracts which represented the total starch lipid [TSL] content ranged from 0.1% (w/w) (potato) to 1.13% (w/w) (oat). The combined action of CM and PW resulted in almost complete removal of starch lipids (>98.6%) from most of the starches, the exception being wheat, where the solvent extraction efficiency (%TSL) was 96.3%. The free lipids in the CM extracts (%TSL) ranged from 5.4% (wheat) to 22.1% (lentil), whereas the free and bound lipids (amylose-lipid complex) in the PW extracts ranged (%TSL) from 77.7% (lentil) to 90.8% (wheat). Neutral lipids (NL) formed the major lipid class in the CM extracts of all starches, while in PW extracts there were NL and phospholipids (PL) in potato, glycolipids (GL) in lentil, and PL in wheat and oat starch preparations. There was a great variation among the starches with respect to the major components of the lipid classes in both CM and PW extracts. Monoacyl lipids were most abundant in cereal starches (>78% TSL). The fatty acid composition of NL, GL and PL in CM and PW extracts was determined.

The physicochemical properties of PW defatted starches of wheat, oat, lentil and potato were determined by monitoring changes in relative crystallinity

(RC), Brabender viscosities, swelling factor (SF), amylose leaching (AML), susceptibility towards acid and α-amylase, thermal properties and retrogradation. The RC of potato and lentil starches increased by 21 and 7.8%, respectively, on defatting, while that of the other starches remained virtually unchanged. Defatting eliminated the pasting peak of cereal starches and increased the thermal stability and reduced the hot paste consistency of all starches. However, these changes were larger in potato and lentil. The SF of all starches decreased on defatting, with the decrease being more pronounced in potato and lentil. In comparison with their native counterparts, the extent of AML at different temperatures was higher in defatted wheat and oat starches. The extent of acid hydrolysis of native and defatted starches of wheat and oat were similar throughout a common time interval (2-20 days). However, during the first stage of hydrolysis, defatted granules of lentil and potato were hydrolysed (4 and 7 days, respectively) to a greater extent than were their native counterparts. Defatted granules of all starches were hydrolysed by porcine α-amylase to a greater extent than were native starches. The gelatinization temperature of defatted wheat and oat starch preparations were slightly lower than their native counterparts. Defatted granules of potato and lentil starch gelatinized over broader and higher temperature ranges than did native starches. The results suggested that amylose and amylopectin chains in native granules are more associated with each other in potato and lentil than

in the other starches. Defatting did not hasten or delay the onset time of the retrogradation endotherm in wheat, potato and lentil starches. However, the retrogradation endotherm of defatted oat starch appeared earlier than that of native starch. The differences in the onset (T_o), peak (T_p) and conclusion (T_c) temperatures of the retrogradation endotherm of native and defatted starch gels were only marginal and remained practically unchanged during the time course of retrogradation. The transition temperatures of retrograded gels were lower than those of gelatinized starches. The enthalpies of retrogradation (ΔH_R) of defatted starches were higher than those of their native counterparts. At the end of 20 days storage, this increase amounted to 4.7, 3.6, 1.7 and 2.2 J/g in defatted wheat, oat, lentil and potato starches respectively. The gel strength (after a storage period of 24h at 25°C) of all four native starches increased upon defatting. The results suggest that the interactions between reassociating starch chains are of a stronger order of magnitude (due to lipid removal) in defatted than in untreated starches.

A heat-moisture treatment study was carried out with native wheat, oat, lentil, yam and potato starches. The starch samples were heat treated at 100°C for 16 h at moisture contents between 10 and 30 %. The heat-treatment did not change granule size and shape. In oat starch, granules were less compactly packed after heat-treatment. The X-ray diffraction intensities increased in wheat, oat and lentil starches, but decreased in potato and yam.

The X-ray patterns of wheat and oat starches remained unchanged, while those of lentil, potato and yam starches became more cereal like. In all starches, the swelling factor and amylose leaching decreased, being more pronounced in potato. Heat-treatment induced complex formation between amylose and native lipids. Differential scanning calorimetry of the heat-treated samples showed a broadening of the gelatinization temperature range and a shifting of the endothermal transition towards higher temperatures. These changes were more pronounced in potato starch. The gelatinization enthalpy of wheat, oat and lentil starches remained unchanged, but those of potato and yam starches decreased on heat-treatment. Heat-treatment increased the 95°C Brabender consistency of wheat starch, but decreased those of oat, lentil, potato and yam starches. In all starches, thermal and shear stability increased after heat-treatment. Acid hydrolysis decreased on heat-treatment of wheat and lentil starches, but increased in oat, potato and yam starches. However, in potato and yam starches the foregoing trend was evident only during the first seven days of hydrolysis. Thereafter, acid hydrolysis was more pronounced in native than in heat-treated starches. The susceptibility towards hydrolysis by porcine pancreatic α-amylase decreased on heat-treatment of wheat and lentil starches, whereas increases were observed for oat, potato and yam starches. The results indicated that the extent of starch chain associations within the amorphous regions and the degree of crystalline order

are altered during heat-moisture treatment. The magnitude of these changes were found to be dependent upon the moisture content during heat-treatment and on the starch source. The effect of heat-moisture treatment (100°C, 30% moisture for 16 h) on the thermal and textural characteristics of retrograding starch gels from wheat, oat, lentil and potato were also investigated. The heat-treatment did not hasten or delay the onset time of the retrogradation endotherm in wheat, potato and lentil starches. However, in oat starch, heat-moisture treatment advanced the onset time from 15 days (native) to 6 days. The differences in T_o, T_p and T_c of the retrogradation endotherm of native and heat-moisture treated starch gels were only marginal, and remained practically unchanged during the time course of retrogradation. However, the enthalpy of retrogradation (ΔH_R) increased upon heat-moisture treatment in wheat, oat and lentil starches but decreased in potato. Furthermore, in native and heat-moisture treated starches, the ΔH_R increased with storage (at 25°C) time. The gel strength (after a storage period of 24h at 25°C) of all four starches increased upon heat-moisture treatment and more marked in potato starch. The results indicated that the changes in thermal characteristics and texture of a heat-moisture treated starch gel on storage is determined by the interplay of the changes in crystallinity, SF and AML on heat-moisture treatment.

Annealing was studied with native wheat, oat, lentil and potato starches. The starch samples were annealed at various starch/water ratios at 50°C for

time intervals ranging from 0.5 to 72 h. Annealing did not change granule size and shape. Oat starch granules were less compactly packed after annealing. X-ray diffraction patterns remained unchanged, and X-ray intensities changed only marginally in all starches. The SF, AML and the gelatinization temperature range (GTR) decreased on annealing. The extent of decrease in SF and AML followed the order: lentil > wheat > potato > oat, while the corresponding order for GTR was: wheat > lentil > oat > potato. The gelatinization transition temperatures (GTT) and enthalpy (ΔH) increased on annealing. However, the increases in GTT and ΔH did not begin concurrently during the time course of annealing. Increases in ΔH were slower and were evident only after 1, 2, 6 and 48 h respectively, in lentil, potato, oat and wheat starches. The extent and rate of increase in GTT and ΔH followed the order: potato > lentil > wheat > oat. The magnitude of changes in GTT and ΔH increased with increase in annealing moisture content. The susceptibility of oat starch to enzyme and acid hydrolysis increased on annealing. However, decreases occurred in the other starches (lentil > wheat > potato). Thermal and shear stability of starch granules increased on annealing (potato > lentil > wheat > oat). The results showed that the above changes in physicochemical properties were due to increased interaction between and among starch components during annealing. Annealing (50°C, 75% moisture for 72 h) did not hasten or delay the onset time of the retrogradation endotherm in wheat potato and lentil starches. However, in oat starch, annealing advanced the onset time from 15 days (native) to 6 days. The differences in T_o, T_p and T_c of the retrogradation endotherm of native and annealed starch gels were only marginal and remained practically unchanged during the time course of retrogradation. The ΔH_R increased in all four native starches not only upon annealing, but also with the time of storage. The increase in gel strength was marginal in wheat and oat starches after annealing, but was more pronounced in potato and lentil starches. The results indicated that the degree of chain reassociation in an annealed starch gel is influenced by the double helical content within the annealed granule prior to gelatinization.

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LIST OF ABBREVIATIONS

AML = Amylose leaching

BU = Brabender units

CM = Chloroform-methanol

cps = Counts per second

^{13}C-CP-MAS-NMR = Carbon (13) cross polarization magic angle spinning nuclear magnetic resonance

db = Dry basis

DSC = Differential scanning calorimetry

FFA = Free fatty acid

GL = Glycolipids

NL = Neutral lipids

PL = Phospholipids

PW = Propanol-water

SEL = Solvent extracted lipids

SF = Swelling factor

T_g = Glass transition temperature

T_m = Melting temperature

TSL = Total starch lipids

w/v = Weight/volume

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INTRODUCTION

Starch, an α-D-glucan biopolymer, a reserve carbohydrate of most plants, exists in a granular form. It is composed of two molecular entities, namely: a linear fraction, amylose, and its branched counterpart, amylopectin. From a human nutrition point of view, it is the major component of the diet in all populations. However, in our culture the main purpose of starch utilization in foods remain aesthetic rather than nutritional (Biliaderis, 1991). This biopolymer constitutes an excellent raw material for modifying food texture and consistency. Not only is the amount of starch important for the texture of a food product, but starch type is equally critical.

Presently, starch from six major plant sources such as maize, waxy maize, wheat, rice, potato and tapioca are commonly used in foods. The composition and properties of these starches vary with the plant source from which they are derived. However, native (unmodified) starches, due to their poor functional properties, are not preferred for incorporation into foods. Therefore, they are often modified by chemical or physical means to overcome one or more of the shortcomings and thus expand the usefulness of starch for a myriad of industrial applications.

Various chemical modification techniques (molecular scission, oxidation, cross linking and substitution) have been introduced in the past. The impact of these modifications on starch properties has been extensively studied, and hence their application has been highly exploited to produce starches with various unique functionality, for food and non food use.

Physical modification techniques such as pregelatinization (Snyder, 1984), extrusion cooking (Davidson *et al.*, 1984; Chinnaswamy and Hanna, 1988a,b), defatting (Morrison, 1981), heat-moisture treatment (Sair, 1967; Lorenz and Kulp, 1978; Donovan *et al.*, 1983; Stute, 1992) and annealing (Krueger *et al.*, 1987a,b; Knutson, 1990; Larsson and Eliasson, 1991; Stute, 1992) have been shown to modify starch properties. The structural changes within starch granules during extrusion cooking have been well documented (Mercier and Feillet, 1975; Mercier *et al.*, 1979; Mercier *et al.*, 1980). However, there is a dearth of information about the effect of defatting, heat-moisture treatment and annealing on granule structure, rheological properties, gel texture, retrogradation and susceptibility towards enzyme and acid hydrolysis. Furthermore, the literature is replete with conflicting information with regard to the effect of the above modification techniques on starch crystallinity and pasting properties.

The objective of this study was to elucidate the structural changes within the amorphous and crystalline regions of cereal, tuber and legume starch granules during defatting, heat-moisture treatment and annealing by monitoring changes in the physicochemical properties such as crystallinity, thermal characteristics, enzyme and acid resistance, swelling, amylose leaching and gel texture.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch - General

Starch, a polysaccharide, plays an important role as a renewable raw material. Current world production of starch is about 21 million tons per year. Starches have been used in food and non food (paper, textiles, mining pharmaceutical, etc.) industries for their various functional properties. However, the major applications are still in food and feed sectors.

Starch occurs in the form of tiny white granules in various sites of plants, for example in cereal grains (wheat, oat, maize, rice, barley, rye), in roots (sweet potatoes, cassava, arrow root), in tubers (potato), in stems (sago palm) and in legume seeds (peas and beans). The granules are semicrystalline, water insoluble and vary in shape and size (Table 2.1) depending upon the plant source. The granule is a polymer mixture with variable proportions of the essentially linear poly-(1-4)-α-D-glucan, amylose, and the branched molecule, amylopectin, where linear (1-4)-α-D-glucan chains are connected through (1-6)-α linkages. The general characteristics of these molecules are presented in Table 2.2.

Table 2.1 Shape and size of some starch granules[a]

Starch source	Shape	Diameter (μm)
Cereal		
Oat	Irregular compound	7-10
Wheat	Lenticular Polyhedric	2-38
Maize	Polyhedric	5-25
Rice	Polyhedric	3-8
Legume		
Lentil	Oval, round ellipsoidal	15-30
Smooth pea	Reniform	5-10
Root		
Manihot	Spherical, semi spherical	5-35
Tuber		
Potato	Ellipsoidal oval	15-100

[a] Adapted from Blanshard (1987), with permission.

Table 2.2 The general characteristics of amylose and amylopectin[a]

Property	Amylose	Amylopectin
Molecular structure	Essentially linear α-(1-4)	Branched α-(1-4) and α-(1-6)
Degree of polymerization[b]	~10^3	~10^4-10^5
Molecular weight	1.5×10^5-10^6	(50-500) × 10^6
Average chain length[c]	~10^3	20-25
Iodine complex (λmax) (colour)	600-640 nm (blue)	530-550 nm (purple)
Digestibility (%)		
β-amylase	~70	~55
β-amylase and debranching enzyme	~100	~100
Stability of dilute aqueous solutions	Unstable (retrogrades)	Stable
Gel texture	Stiff, thermally irreversible below 100°C.	Soft, thermally reversible below 100°C
Film properties	Strong, coherent	Brittle

[a] Adapted from Biliaderis (1991), with permission.
[b] Average number of glucose residues per molecule
[c] Average number of glucose residues per chain

The physical and chemical properties of starches from various plant origins depend on their differences between molecular structure and in the morphology of the starch granule itself. Structure refers to both, fine structure (shape, size, branching pattern and chain length) of the component molecules (amylose and amylopectin), and the supra molecular order (the manner in which the starch components are organized within the granule).

2.2 Starch structure

2.2.1 Fine structure of amylose

Amylose is an essentially linear glucan polymer, made up of α-1,4 glycosidic linkages. The amylose content of starches varies from 17 (rice) to 38% (lentil). Properties of some amyloses are summarized in Table 2.3.

Early work on the structure of amylose led to the conclusion that it was a linear polymer, since it was completely degraded to maltose by β-amylase (an enzyme that acts only on linear α-(1,4)-D-glucan linkages and produces maltose as the sole product). However, Peat *et al.* (1949) showed that highly pure crystalline preparations of β-amylase from sweet potato produced only about 70% hydrolysis of several samples of amylose. The above observation was supported by the findings of Greenwood and Thompson (1962) who reported that the β-amylolysis ranges from 72-95% for various purified amyloses from different sources. The crude amorphous β-amylase

Table 2.3 Physicochemical characteristics of amyloses from various botanical sources.

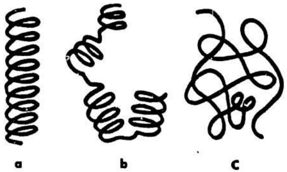
Starch source	IBC[a] (g/100g)	Intrinsic viscosity[b] ml/g	β-amylolysis limit (%)	DP_n mean[c]	Source
Cereal					
Wheat	19.9	-	32	570	Takeda *et al.* (1984)
Oat	19.5	246-299	78	-	Paton (1986)
Maize	20.1	166-183	81-84	930-990	Takeda *et al.* (1984)
Rice	20.5	180-216	73-84	920-1110	Takeda *et al.* (1986)
Legume					
Lentil	19.6	188	89	1400	Biliaderis *et al.* (1981a)
Smooth pea	18.8	194	85	1400	Biliaderis *et al.* (1981a)
Root					
Manihot	20.0	384	75	2660	Takeda *et al.* (1984)
Tuber					
Potato	19.0	430	78	3200	Biliaderis *et al.* (1981a)
	-	-	-	4920	Hizukuri and Takagi (1984)

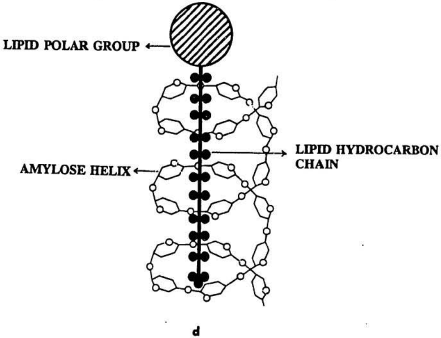
[a] Iodine binding capacity (amount (g) of iodine bound by 100 g (db) of amylose)
[b] At 22.5°C in 1 M KOH
[c] Average number of glucose residues per chain

preparations, used in the early studies of the structural analysis of amylose were later found to contain a small amount of debranching enzyme which allowed the barrier to β-amylase action to be by passed (Manners, 1985). The above experiments rationalized that incomplete β-amylolysis of amylose is due to an irregularity in its primary structure. The branching must be sufficiently long and infrequent to allow amylose to behave like a linear polymer (forming complexes and films). In most starches one-third to two-thirds of the amylose fraction has secondary chains attached through occasional α(1-6) branch points, and in some species amylose has a few phosphate groups probably at C-6 of glucose residues. The branch chains are usually moderately long, but a few may be as small as glucosyl or malto tetrasoyl (G1-G4). Banks and Greenwood (1975) and Takeda *et al.* (1984) showed that the amylose leached from starch granules, immersed in water just above their gelatinization temperature (GT) has a lower molecular weight and higher β-amylolysis limit (90-100%) than amylose leached at higher temperatures (β-amylolysis limit 70-80%) which is more extensively branched. Amylose conformation tends to be slightly helical due to the natural twist present in the chair conformation of the glucose units. However, the conformation and behaviour of amylose in aqueous solution has been the subject of much controversy, as can be seen from the models (Fig. 2.1) that have been proposed (Hollo *et al.*, 1961; Banks and Greenwood, 1971; Senior and Hamori, 1973). Hollo *et al.* (1961) proposed a tightly-wound helix (Fig. 2.1.a) for the structure of the amylose

Fig. 2.1. Models proposed for amylose conformation in aqueous solution (with permission).

a) Tightly wound helix (Hollo *et al.*, 1961).
b) Helical segment interspersed by regions of random coil (Hollo *et al.*, 1961).
c) Random coil (Banks and Greenwood, 1971).
d) Lipid-Amylose complex (Carlson *et al.*, 1979).


a
b
c


LIPID POLAR GROUP
LIPID HYDROCARBON CHAIN
AMYLOSE HELIX
d

chain. The above authors further suggested that the amylose chain is composed of helical segments (helical regions stabilized by intermolecular hydrogen bonds) interspaced by regions of random coil (Fig. 2.1.b). Banks and Greenwood (1971) postulated that amylose possesses no helical character but is present in the form of a random coil (Fig. 2.1.c) in neutral aqueous solution, and that the helical conformation is forced upon amylose by the addition of complexing agents such as iodine, lipids, etc. Senior and Hamori (1973), proposed that amylose conformation involves regions of loose and extended helices which alternate with shorter random coil sections. Based on this model, the formation of the amylose-iodine or amylose-lipid complex can be visualized as the entrapment of iodine atoms or a lipid side chain, by the contraction of the loose helical regions of amylose into tight helices referred as "V"amylose (Fig. 2.1.d). In aqueous phase, amylose can transform its conformation from one type to another. However, such conformational changes in the crystal phase are not easy because of the restriction in the flexibility of the chains.

The solution behaviour of amylose is dominated by two properties: the ability to form inclusion complexes with iodine, surfactants, lipids and primary aliphatic alcohols and the ability to participate in strong intermolecular interactions leading to precipitation or gelation. Amylose inclusion complexes are also called "V" amyloses (water insoluble at room temperature) in which amylose exists in a helical form where the ligand resides within the central hydrophobic core (0.5 nm in diameter) of the helix (Mikus *et al.*, 1946;

Morrison, 1981). The diameter of the amylose helix in the inclusion complexes is controlled by the size of the complexing agent. Evidence has been presented indicating that linear alcohols, iodine and fatty acids form helices having 6 D-glucosyl residues per turn (Mikus *et al.*, 1946; Takeo *et al.*, 1973), branched chain alkyl compounds (e.g., tert-butyl alcohol) form helices of 7 D-glucosyl residues per turn (Takeo *et al.*, 1973) and other bulkier molecules (e.g., 1-napthol) yield helices of 8 D-glucosyl residues per turn (Yamashita *et al.*, 1973).

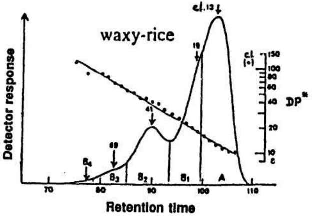
Amylose forms an intense blue complex with iodine reagent (I_2/KI solution). This behaviour has been used as the basis for quantitation of amylose (Gilbert and Spragg, 1965; Williams *et al.*, 1970; Chrastil, 1987). In I_2/KI solution the guest molecules are polyiodide ions, which, depending on solute concentrations, may be up to I_{13}^- but are mostly I_3^- or I_5^- (Teitelbaum *et al.*, 1978). At high concentrations some iodine appears to be bound outside the helix cavities. The colour and λ_{max}. of the complexes vary with chain length and analytical conditions. Inclusion complexes with lower alcohols such as thymol and butan-1-ol are insoluble and heat-labile and have been used to isolate amylose (Whistler, 1965). Inclusion complexes with fatty acids, monoglycerides and certain surfactants are also insoluble, and dissociate on heating in water at 90-120°C (Morrison, 1988; Raphaelides and Karkalas, 1988).

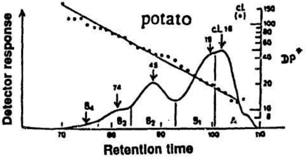
2.2.2 Fine structure of amylopectin

Amylopectin, like amylose, is a polymer of α-D-glucan. General characteristics of this molecule are summarized in Table 2.2. It is a highly branched starch molecule composed of many linear chains (α-(1-4)-D-glucan) and 4-5% branch points (α-(1-6)-D-glucosidic linkages). The presence of these 4-5% branching points give rise to an infinity of possible arrangements of the linear chains. Because of its general dominance of granule composition, structure and properties, amylopectin has been widely studied in terms of molecular size, branching and inner and outer chain lengths (French, 1972; Robin *et al.*, 1974; Hizukuri, 1985, 1986).

Investigation of amylopectin structure is a case in point where progress is dependent on methodology development; in this instance, both enzymatic and instrumental. Hydrolysis of amylopectin with a debranching enzyme and separation of the digest (consisting of linear chains) using exclusion gel filtration technique normally gives a bi or trimodal elution profile (Fig. 2.2), indicating the presence of chains with different chain length or molecular weight (Hizukuri, 1985). Measurements of the molecular weight or degree of polymerization (DP) of the resulting linear chains, give an excellent profile of branch lengths. Linear chains in amylopectin form red to purple polyiodide complexes ($\lambda_{max.}$= 530-585) in I_2/KI solution, but normally do not complex appreciably with alcohols or monoacyl lipids.

Fig. 2.2. Gel chromatography elution profile of enzymatically debranched amylopectin from waxy rice and potato (Hizukuri (1986), with permission).





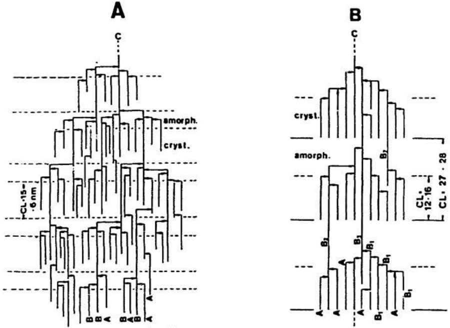
CL = Chain length

* DP = Degree of polymerization

Linear line represents the graph between retention time vs DP.

Fig. 2.3. Models proposed for amylopectin (with permission).

A) Cluster model (Robin *et al.*, 1974)
B) Cluster model (Hizukuri, 1986)



A - Outer most branches.
B - Inter branches.
C - Only chain in an amylopectin molecule that carry the reducing group.

amorph = AMORPHOUS REGION
cryst = CRYSTALLINE REGION
CL = chain length

Table 2.4 The average degree of polymerization of A- and B-chains of amylopectin from various botanical sources

	Average degree of polymerization[a]			
Starch source	Whole[b]	B-chain	A-chain	Reference
Cereal				
Oat	55	45	15	Paton (1986)
Maize	28	60	17	Hizukuri (1985)
Waxy maize	24	51	18	Hizukuri (1985)
Wheat	25	56	17	Hizukuri (1985)
Amylomaize	44	65	19	Hizukuri (1985)
Legume				
Lentil	23	50	16	Biliaderis *et al.* (1981a)
Root				
Manihot	26	52	17	Hizukuri (1985)
Tuber				
Potato	34	62	20	Hizukuri (1985)
Yam	28	55	19	Hizukuri (1985)

[a] Average number of glucose residues per chain.
[b] Average chain length of debranched starch.

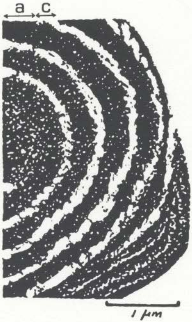
To explain the physicochemical properties of this macromolecule, several structural models have been proposed with different branching patterns. Laminated structure (Haworth *et al.*, 1937), herringbone structure (Staudinger and Husemann, 1937) and the randomly branched structure (Meyer and Bernfield, 1940) were some of them. In the last 20 years, however, the "cluster" model by French (1972), has received general acceptance. While their basic concept remains, modifications have been proposed (Robin *et al.* 1974; Hizukuri, 1986) (Fig. 2.3.A,B) since the model was introduced. All these models are depicted by three types of chains, referred to as A-, B- and C-chains (e.g., Fig. 2.3). A-chains are unbranched and linked to B-chains through an α-(1-6) bond at its reducing end; B-chains can either link to another B-chain or to a C-chain in the same manner; C-chains function like B-chains and carry the one functional reducing group in the molecule. The main features in the "cluster" model by Robin *et al.* (1974) (Fig. 2.3.A) are the presence of three chain populations, with chain lengths of 15-20 (for A-chains), 45 (for B-chains) and about 60 (for C-chains) and the ratio of A-chains to B-chains is about 1:1. This ratio is an important property of amylopectin which indicates the multiplicity in branching (Hizukuri, 1986; Manners, 1989). Hizukuri (1986), assigned a ratio of 0.8 and Manners (1989) indicated a range in ratios of 1.1 to 1.5 for potato amylopectin. The average chain length or degree of polymerization of A- and B- chains of the amylopectin molecule, obtained from different plant sources is presented in Table 2.4.

2.2.3 Super molecular order of starch granules.

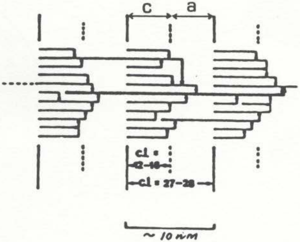
The presence of crystalline and amorphous regions within the starch granules is now widely accepted. Furthermore, there is indirect evidence to indicate that amylopectin is the main crystalline component of the starch granule. The involvement of amylopectin in the crystallites, originally proposed by Meyer and Bernfeld (1940), is supported not only by the fact that waxy maize starch containing no amylose is semi crystalline, but also by the fact that amylose may be leached out from the starch granules under appropriate conditions without seriously impairing granule crystallinity (Montogomery and Senti, 1958; Zobel, 1988b). Super molecular order, the organization of starch chains within the granule (in both amorphous and crystalline regions) through various degrees of intra and inter molecular associations, governs most of the physicochemical properties of starch. Features of starch granule organization can be detected by a number of experimental techniques which give information relevant to a nanometre through micrometre distance scale. Examination of internal granule features by scanning electron microscopy of fracture surfaces from amylase-treated starches, as well as transmission electron microscopy of chemically treated thin sections of starches have shown a radial periodicity or "growth rings" (French, 1984) (Fig. 2.4) and suggest "growth ring" spacings of the order of 100 nm. In the light of developments in understanding of the fine structure of amylopectin and its suggested radial

Fig. 2.4. Starch granule inner structure, showing the crystalline and amorphous shells (growth rings) (Gidley (1992), with permission).

Growth rings



Amylopectin molecule

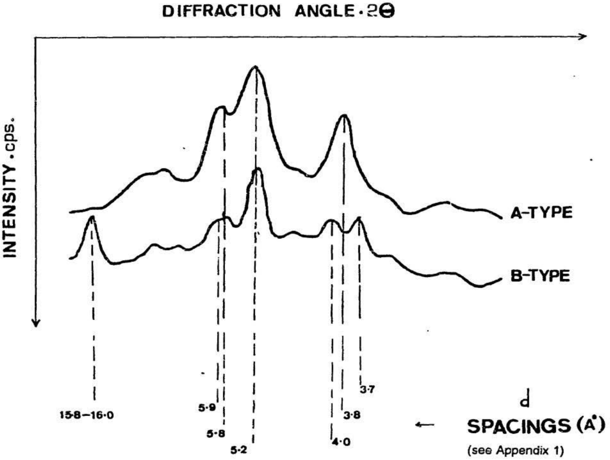


a = AMORPHOUS REGION

c = CRYSTALLINE REGION

cl = chain length

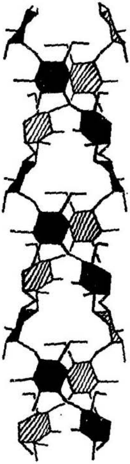
Fig. 2.5. X-ray diffractograms of A- and B-type starch with characteristic d-spacings (for each crystal type) (Zobel (1988b), with permission).


DIFFRACTION ANGLE · 2Θ
INTENSITY · cps.
A–TYPE
B–TYPE
15·8–16·0
5·9
5·8
5·2
4·0
3·8
3·7
d
← SPACINGS (Å)
(see Appendix 1)

orientation (having the reducing end towards the centre of the granule (French, 1972)), it seems likely that the molecular origin of this feature lies in the regularity of clusters of branch points in amylopectins, giving rise to regular alternation of carbohydrate density on a distance scale of 10 nm (Fig. 2.4).

Starch granules being partially crystalline, give distinct X-ray diffraction patterns that have been known since the pioneering work of Katz and Van Itallie (1930). Three crystalline polymorphs of native starches, designated as A-, B- and C-types, are generally recognized from their wide angle X-ray scattering diffractograms; that are still used for native and retrograded starch showing crystalline structures. The above nomenclature was based on the characteristic d-spacings, calculated from X-ray diffraction patterns (Fig. 2.5). The A-type pattern is exhibited by cereal starches and the B-type pattern is shown by tuber, fruit and high amylose (>40%) corn starches as well as by retrograded starch. C-type pattern, which is intermediate between A- and B-type is commonly observed in legume seed starches. Although this nomenclature is currently accepted and widely used, Katz and Van Itallie (1930) did not provide information about the super molecular arrangement of the starch linear chains in those crystal polymorphs. Hence, studies have persisted to determine the size, molecular conformation and chain packing that describe the basic repeating element (unit cell) of a starch crystallite (Kainuma and French, 1972; French, 1972; French and Murphy, 1977; Wu and Sarco, 1978a,b; Imberty *et al.*, 1987a,b; Imberty *et al.*, 1988; Imberty and Perez, 1988).

Fig. 2.6. Double helix model for starch chains (French and Murphy (1977), with permission).

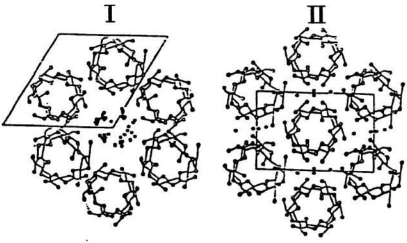


The idea of double stranded rather than single helices for native starch polymorphs was first presented by Kainuma and French (1972). Feasibility for helices being formed by both amylose and amylopectin was demonstrated using space filling molecular models (French, 1972). French (1972), proposed that in starch granules double helices are formed between the flexible outer branches (A-chains) of amylopectin. Two of these chains from the same molecule or neighbouring molecules fit together compactly with the hydrophobic zones of the opposed monomer units in close contact, and the hydroxyl groups are located outwards for strong interchain hydrogen bonding (helical order or short range order). French and Murphy (1977), produced the first detailed computer model (Fig. 2.6) for starch double helix with no intra chain hydrogen bonds. The stability of the helix is attained by inter chain hydrogen bonding between hydroxyl groups at positions **C2** and **C6** and from Van der Waal's forces. The helical core is highly hydrophobic and compact so that there is no room for water or any other molecule to reside in it. Previous research has shown that granule crystallinity is due to close packing of neighbouring double helices (crystalline or long range order) *via* direct or indirect (through a bridge of water molecules) hydrogen bonding (Wu and Sarco, 1978a,b; Imberty *et al.*, 1987a,b; Imberty *et al.*, 1988; Imberty and Perez, 1988)

Wu and Sarco (1978a,b), published B- and A- structures with double helices using data gathered from X-ray patterns taken of crystalline amylose

Fig. 2.7. Double helical packing arrangement in A- and B-type starch crystals (Wu and Sarco (1978a,b), with permission).

I) B-type: hexagonal packing.
II) A-type: orthogonal packing.

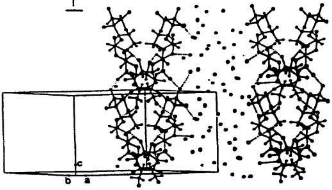


(●), Represents water molecules.

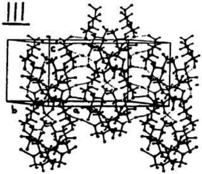
Fig. 2.8. Double helical packing arrangement in A- and B-type starch crystals [Imberty *et al.* (1987a,b and 1988); Imberty and Perez (1988), with permission].

I) B-type: three dimensional representation along the fibre axis (c).
II) B-type: projection of the structure onto the a,b plane.
III) A-type: three dimensional representation along the fibre axis (c).
IV) A-type: projection of the structure onto the a,b plane.

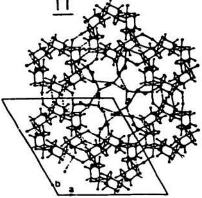
I



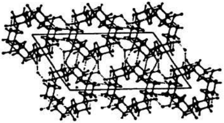
III



II



IV



Hydrogen bonds are indicated as broken lines.

(•), indicate water molecules.

films and computer modelling. The data indicated a best fit for unit cells with two right handed, parallel stranded double helices that are arrayed antiparallel to each other (12 glucose residues per unit cell). The unit cell packing for B and A type was hexagonal and orthogonal (Fig. 2.7.I & II), and each contained 36 and 8 molecules of water per unit cell respectively.

Imberty *et al.* (1987a,b), Imberty *et al.* (1988) and Imberty and Perez (1988), put forward new three dimensional structures for both native starch polymorphs. Structure development incorporated electron diffraction data from micron sized single crystals, X-ray powder diffraction data, and fibre pattern data from Wu and Sarco (1978a,b). Double helical packing (Fig. 2.8.I, II) and the lattice parameters proposed by Imberty *et al.* (1987a,b and 1988) and Imberty and Perez (1988) for B-type polymorph are the same as those of Wu and Sarco (1978a,b). However, Imberty *et al.* (1987a,b and 1988) and Imberty and Perez (1988), reported that the B-type unit cell has two left handed, parallel-stranded double helices that are arrayed in parallel: there are 12 glucose residues and 36 water molecules per unit cell. Half the water is tightly bound to the double helices and the other half is centered around a 6-fold screw axis parallel to the c or fibre axis (Fig. 2. 8.I, II). The repeating unit is α-(1,4) linked maltose with a symmetry generating a double helix with six residues per turn of each chain. The repeat distance is 21 Å. The calculated cell density is 1.41 compared to 1.45 determined experimentally.

Imberty *et al.* (1987a,b and 1988) and Imberty and Perez (1988), further reported that like B-, the A-type unit cell has the same helix conformation (left handed parallel stranded double helix) and 12 glucose residues. However, there are only four water molecules per unit cell. Crystallization occurs in a monoclinic lattice (Fig. 2.8.III, IV). Interstrand stabilization is provided by O_2 – O_6 hydrogen bonds and Van der Waal's forces. The helix inner channel and outside dimension is 3.5 Å (too small for water inclusion) and 10.3 Å. The individual helical repeat of 2c = 21.38 Å is generated by a two fold screw axis which means the asymmetric repeat unit for "A" is a maltotriose residue. Calculated cell density for A is 1.48 and close to the observed density 1.51.

The starch crystal polymorphism is mainly attributed to the differences in double helical packing with minor differences proposed in the helix geometry. However, in both A- and B-type of starch crystals, the double helical packing is stabilized by a network of hydrogen bonds (Fig. 2.8). The centre of the A-type unit cell is occupied by an amylosic double helix rather than a column of water, as in B-type (Fig. 2.8). Furthermore, it has been postulated that adjacent double helices in crystallites of A-type are mainly linked by direct hydrogen bonding (Imberty *et al.*, 1987a,b and 1988), while in B-type, adjacent double helices are mainly linked by hydrated water bridges and to a limited extent by direct hydrogen bonding (Imberty and Perez, 1988).

Gidley and Bulpin (1987), have shown (*in vitro*) that the length of the A-chain influences the development of a crystal type. The above authors synthesized starch crystals from amylose fragments with various degrees of polymerization (DP) and compared their X-ray patterns with standard A- and B-type X-ray patterns. They concluded that fragments with DP < 10 did not crystallize; chains with DP=10-12 yielded A-type; chains longer than DP=12 yielded B-type and chains longer than DP=50 did not form the single crystals, instead developed a tangled network.

2.2.4 Starch granule amorphous region

Although about 70% of the starch granule is believed to be amorphous, relatively little attention has been directed to elucidating its structure. Biliaderis *et al.* (1986a) have postulated that two different types of amorphous materials are present within the starch granules: a) A bulk amorphous region (mainly amylose), and (b) An intercrystalline amorphous phase (mainly intercrystalline regions of dense branching in the amylopectin). Many investigations have been carried out to elucidate the organization of amylose chains within the amorphous regions of the starch granule (Boyer *et al.*, 1976; Jane *et al.*, 1986, 1992; Blanshard, 1986; Zobel, 1988b). Boyer *et al.* (1976), compared the amylose content in starch at different stages of maturities, and suggested that amylose is more concentrated at the granule periphery. Jane *et al.* (1986), reported that amylose in starch interacts with amylopectin, thereby preserving starch granule integrity. Blanshard (1986), proposed that amylose is present in bundles at amorphous regions in wheat starch, but partly co-crystallized with amylopectin in potato starch. Zobel (1988b), presented several arguments to support the concept that amylose is more closely associated with amylopectin in potato than in cereal starches. The above author postulated that lipids in starches may be responsible for effecting an amylose separation within the granules. This would imply that the starch components 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 and corn). Although this may be partly true, it is also plausible that the extent to which the starch components are associated with each other within the native granule may also depend on their respective chain length (CL). Long amylose CL may facilitate easier association with the chains of amylopectin. It is therefore likely that in potato starch the degree of association between starch components may be higher than in other starches, due to its low lipid content and long amylose chain length. Recently, Jane *et al.* (1992), showed by gel permeation chromatography on native and cross-linked corn and potato starches, that cross-linking between amylose molecules does not increase the size of amylose. However, the susceptibility of the amylose to sequential hydrolysis by isoamylase and β-amylase decreased. A comparison of the blue values of amylopectin (isolated from cross-linked starches and debranched with isoamylase) peaks revealed that amylose was cross-linked to amylopectin. The results from this study showed that amylose is interspersed among amylopectin molecules in corn and potato starch granules

2.3 Minor components of starch

The minor components of starch are generally believed to be contaminants which enter starch during the extraction process. According to their location, they may be considered under three categories: a) Particulate material or fragments of non-starch material that separate with starch (e.g. insoluble proteins and cell wall material). These materials can lead to unwanted flavors or colors in starch and also affect starch properties such as water binding capacity and viscosity; b) Surface components - materials associated with the surface of granules and that may be removed by extraction procedures which do not cause disruption of the granule internal structure (e.g. soluble proteins, other carbohydrates, inorganic material and lipids). Washing with water, organic solvents (chloroform and methanol) and mild alkali solution (0.05 *N* NaOH) will remove most of these contaminants and c) Internal components - materials buried within the granule matrix and inaccessible to extraction without granule disruption (e.g. proteins and lipids). Starches from different sources vary in their content of internal, non starch materials. Cereal starches in general contain lipids that appear to be present free or associated with the amylose fraction of starch. Tuber starches contain little lipid; on the other hand potato starch contains phosphate groups esterified to glucose residues of amylose and amylopectin and undoubtedly, play an important role in determining the physical properties of potato starch. Although cereal starches contain phosphate groups, these are present in the phospholipid components of the starch lipids. All starches contain small amounts of nitrogenous material as shown by Kjeldahl nitrogen analysis, usually taken to indicate the presence of protein. The presence of lipids and proteins (Morrison, 1981) in high amounts, affect starch properties such as hydration, swelling, viscosity and resistance to α-amylolysis.

2.3.1 Surface and internal 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 triglycerides (TG), followed by free fatty acids, glycolipids (GL), and phospholipids (PL), and they include those that may have been present on the granule surface *in situ* within the plant tissue and the non starch lipids, which are absorbed into the surface layer of the starch granules during the isolation procedures (Morrison, 1981; Galliard and Bowler, 1987). The non starch lipids occur as spherosomes and 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 and Bowler, 1987). The internal lipids of cereal starches are predominantly monoacyl lipids, with the major components being lysophospholipids (LPL) and FFA (Hargin and 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 (Acker, 1977) in which the hydrocarbon chain of a lipid resides within the central hydrophobic core of the amylose helix (Mikus *et al.*, 1946; Morrison, 1981) or linked *via* ionic or hydrogen bonding to hydroxyl groups of the starch components. A "V" X-ray diffraction pattern (characteristic for the amylose inclusion complex) is seen when lipid containing starches are subjected to extrusion cooking (Mercier *et al.*, 1980), and after addition of monoacyl lipids to starch under appropriate conditions (Hoover and Hadziyev, 1981; Biliaderis *et al.*, 1986b). Native (untreated) cereal starches do not exhibit the "V" pattern (Galliard and Bowler, 1987). This means that either the complexes do not exist in native starch and are formed only on heating (Galliard and Bowler, 1987), or more probably, that they do exist but only in partially helical or crystalline conformation (Galliard and Bowler, 1987). Controversy still exists with regard to the lipid binding ability of the short linear (15-20 glucose units) portions of the outer branches of amylopectin (Lagendijk and Pennings, 1970; Krog, 1971; Goering *et al.*, 1975; Evans, 1986; Biliaderis and Vaughan, 1987; Hahn and Hood, 1987; Gidley and Bociek, 1988).

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 internal) has been generally found to be in the range 0.7-1.2% in cereals (Morrison and Milligan, 1982; Takahashi and Seib, 1988), 0.01-0.87% in legumes (Hoover and Sosulski, 1991) and 0.08-0.19% in tubers and roots (Emiola and Delarosa, 1981; Goshima *et al.*, 1985).

Starch damage has been shown to occur during isolation of cereal starches (Evers *et al.*, 1984). The type of damage may range from cracks, cuts and other abrasions on the surface to cleavage of amylose and amylopectin. Therefore, there is always a possibility that cross-contamination of surface (free and bound) with internal (free and bound) lipids and vice versa, could occur respectively during cold and hot solvent extractions. It is our opinion that classification of starch lipids as surface and internal is valid only if the starch granules were highly purified (60-80 mg N/100g of dry starch) and were undamaged. However, since the latter criterion is very difficult to achieve in practice, an alternative classification based on solvent (cold and hot) extractability would be more meaningful.

2.4 Starch properties

2.4.1 Gelatinization

Starch granules heated in the presence of sufficient water exhibit an order-disorder phase transition called gelatinization, which results in near solubilization of starch. The above phase transition is associated with the diffusion of water into the granule, hydration and swelling of the starch granules, uptake of heat, loss of crystallinity and amylose leaching (Lelievre, 1973; Donovan 1979; Hoover and Hadziyev 1981). Most technological uses of starch in food involve such disruption of granular order and consequent rheological and textural changes.

The semicrystalline nature of the starch granule led many researchers (Donovan, 1979; Biliaderis *et al.*, 1980; Hoover and Hadziyev, 1981) to use the Flory-Huggins (Flory, 1953) thermodynamic equation to interpret the relationship between water content and crystallite melting (melting temperature and enthalpy) observed during starch gelatinization.

$1/T_m - 1/T_m^\circ = (R/\Delta H_u)(V_u/V_1)(v_1 - X_1 v_1^2)$ → Flory Huggins equation

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° = true melting point of the undiluted polymer

v_1 = volume fraction of the diluent

X_1 = the polymer solvent interaction parameter

In the application of this equation, it was assumed that the starch-water system is homogeneous and gelatinization occurs under equilibrium conditions. Evans and Haismann (1982) reported that the starch water system is not homogeneous, since it consists of individual granules suspended in a variable amount of liquid phase. Furthermore, these authors have postulated that, once starch granules reach their maximum swelling capacity, further changes in the amount of water added will not affect granule composition. Therefore, volume fractions for the Flory-Huggins equation should be based on granule composition, rather than on the composition of the entire system. The detection of a glass transition (Tg) point just prior to the gelatinization endotherm led Maurice *et al.* (1985); Biliaderis *et al.* (1986b) and Slade and Levine (1988), to postulate that the process of starch gelatinization is inherently non equilibrium in character, in that it occurs when starch granules are subjected to heat in the presence of plasticizing water in which crystalline melting is indirectly controlled by the kinetically constrained continuous amorphous environment, which was in a glassy state prior to gelatinization. Based on these observations, the above authors came to the conclusion that the applicability of the Flory-Huggins equation to starch water systems is inappropriate. However, many researchers (Biliaderis *et al.*, 1986b; Paton, 1987; Russel, 1987) continued to use the Flory-Huggins equation, since it provides a reliable means of comparing the behavior of starches from different sources under identical experimental conditions.

The glass transition (Tg) of a macromolecule defines the temperature region in which the chain motion (Brownian motion) commences. In pure amorphous macromolecules the transition entails a change, over a temperature range of about 5-20°C, from an immobile solid to a rubbery liquid. In starch granules, the presence of microcrystals (which inter-connect the amorphous regions) provide stability to amorphous region chains and thereby increase the temperatures and range over which the softening occurs (Wunderlich, 1990). However, a range of values is possible since the glass to rubber transformation depends on kinetic factors and is not an equilibrium process. The glass transition (Tg) of starch is a function of the previous thermal history of the sample. For instance, heating of moist starch granules at temperatures in the region between the glass and melting temperatures caused annealing and crystallization within the granule (Slade and Levine, 1987; Knutson, 1990). Glass softening is much more subtle than melting and consequently more difficult to detect. Differential scanning calorimetry (DSC) traces normally signal the presence of a glass transition by a shift in the heat capacity in the absence of an enthalpic change (a small depression in the baseline just before the onset of the gelatinization endotherm). Plasticization of the amorphous polymer depresses the temperature at which the glass to rubber transformation occurs. The plasticizer increases the segmental motion of the starch chains. Water (low molar mass and high free volume) is a very effective plasticizing agent and small quantities can cause T_g to decrease markedly (Slade and Levine, 1988; Wunderlich, 1990).

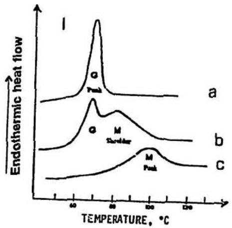
Because gelatinization is of great importance to many food processing operations, several analytical techniques have been employed to probe the molecular mechanism of this phenomenon. These include light microscopy, electron microscopy, light transmission, viscometry, swelling and solubility measurements, X-ray diffraction, nuclear magnetic resonance and differential scanning calorimetry (DSC). Of these methods, DSC in particular, has attracted most interest in studies of phase transitions of aqueous starch systems (Stevens and Elton, 1971; Lelievre, 1973; Donovan, 1979; Biliaderis *et al.*, 1980; Slade and Levine, 1987).

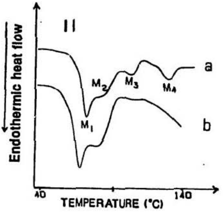
The effect of the moisture content on DSC traces has been extensively investigated to understand the molecular basis of starch gelatinization by many researchers (Donovan, 1979; Biliaderis *et al.*, 1980). These studies showed that at relatively high moisture levels (volume fraction of water, $v_w > 0.85$), a single endotherm is apparent at about 60°C. The precise position of the peak depends on the starch variety being investigated (Donovan, 1979; Biliaderis *et al.*, 1980). Donovan (1979) observed (Fig. 2.9.I) a single endotherm at $v_w >$ 0.7, (Fig. 2.9.I.a), and at $v_w = 0.65$ (medium moisture), a shoulder was apparent on the leading edge of the thermogram (Fig. 2.9.I.b). This shoulder developed into a single peak and melted at increasing temperatures with further decrease in water content. At $v_w = 0.6$ (low moisture), the first peak was virtually absent (Fig. 2.9.I.c). Furthermore, a gradual increase in melting temperature and a decrease in gelatinization (G) enthalpy occurred when the concentration of the starch slurry was gradually increased. Donovan (1979) suggested that the initial DSC peak in biphasic endotherms results from the stripping of polymer chains from the surfaces of the crystallites due to stress developed by hydration and swelling of amorphous regions, while the second peak represents melting (M) at low diluent (H_2O) volume fractions. According to this model, in excess water the resultant stress due to hydration and swelling is high enough that only the stripping mechanism operates. Since Donovan (1979) considered different mechanisms to be responsible for each section of the biphasic endotherm, the peaks were labelled G and M respectively. Biliaderis *et al.* (1980), obtained similar endotherms (Fig. 2.9.II) with native and defatted wheat starch at 50% moisture level. As these endotherms are due to the melting of some kind of molecular order within the

Fig. 2.9. DSC thermograms of starch-water system (with permission).

I) Native potato starch at various moisture levels: a) high, b) medium and c) low (Donovan, 1979).

II) a) Native and b) Defatted wheat starches (Biliaderis *et al.*, 1980).





M_1 & M_2 are endotherms represent gelatinization and crystallite melting respectively.
M_3 & M_4 are endotherms represent amylose lipid complex melting.

starch granule, the above authors labelled them as M1, M2, M3 and M4 (Fig. 2.9.II.a). Endotherms M1 and M2 are comparable to the G and M endotherms of Donovan (1979). The M3 and M4 endotherms were attributed to the melting of amylose lipid complex (since they disappeared on defatting (Fig. 2.9.II.b). From the standpoint of granular structure, the above data (Donovan, 1979; Biliaderis *et al.*, 1980) reflected a crystallite population with varying degrees of internal order (Zobel, 1988a).

Evans and Haisman (1982) proposed another explanation for the biphasic endotherms at low moisture levels. These authors postulated that since water migrates from one location to another within the sample as the various granules gelatinize, the peaks must correspond to melting transitions of crystalline material with different stabilities, occuring at different diluent levels. Therefore, if the water content of the suspension is sufficiently high, each granule absorbs moisture without restriction and a single endothermic peak is observed. This explanation is consistent with reports that X-ray crystallinity changes occur during the first and second DSC peaks (Liu *et al.*, 1991) and that the loss of birefringence begins with the passage through the first endotherm but is not complete until the second peak has been traversed (Burt and Russell, 1983). Biliaderis *et al.* (1985) and Maurice *et al.* (1985) have suggested that the lower temperature peak is a consequence of chain mobilization in the amorphous regions of the granule. Russell (1987) interpreted that the double endotherms observed in limited water systems to be due to disruption of double helices associated with short range ordering involving amylose and amylopectin followed by melting of crystallites. Biliaderis (1990) suggested that recrystallization can contribute to the second endothermic peak.

Cooke and Gidley (1992) determined the loss of both molecular (^{13}C-CP-MAS-NMR spectroscopy) and crystalline (X-ray diffraction) order for a range of starches as a function of gelatinization extent (DSC), in order to probe any sequential loss of structural order and to investigate the structural origin(s) of the endothermic event. The above authors showed that crystalline and molecular order are lost concurrently during gelatinization, which indicated that thermal stability of double helices within granules is not affected significantly by the presence or absence of a crystalline environment. Furthermore, a close relationship was found to exist between the loss of enthalpy and loss of helical order. This indicated that DSC endothermic enthalpy values primarily reflect the loss of double helical rather than loss of crystalline order. From the standpoint of starch granule ultrastructure, these results suggested that the forces holding the starch granule together are largely at the double helical level and that the observed crystallinity may function as a means of achieving close packing rather than as a primary provider for structural stability.

2.4.2 Retrogradation

Starch granules gelatinize as a result of heating in the presence of water, i.e. the granules swell, crystalline regions melt, starch chains become hydrated and some amylose leaches out of the granules. After being cooled, ageing of the gelatinized starch suspension is accompanied by increasing turbidity, firmness, degree of crystallinity and phase separation between polymer and solvent (syneresis) due to reassociation of solubilised starch molecules. "Retrogradation" was first used to refer to the return of crystallinity in the starch of staling bread (Katz, 1928) and is also used to refer to the analogous process in starch. The several processes accompanying ageing are important for texture and stability of starch-containing foods.

Starch gels are metastable, and therefore, undergo structure transformation (further chain aggregation and crystallization) during storage. Amylose (essentially, a linear and smaller molecule) in the gelatinized starch suspension tends to associate quickly to form a gel network (starch gels may be regarded as complex composites in which swollen gelatinized granules are embedded in an amylose rich matrix) (Lampitt *et al.*, 1948). Therefore, most of the initial changes in the physicochemical properties (e.g., texture, crystallinity, water holding capacity, etc) of a gel are attributed to amylose association. Formation of hydrogen bonds between hydroxyl groups of starch chains are responsible for the parallel association of solubilized starch molecules (molecular ordering, Miles *et al.*, 1985). The above author reported that the gelation of amylose may be regarded as a partial crystallization process and it arises through phase separation, which produces polymer rich and polymer deficient regions. Neither the initial gelation nor the development of the crystallinity of amylose can be reversed by heating to 100°C (Miles *et al.*, 1985).

In contrast to amylose, amylopectin retrogrades very slowly. Molecules of amylopectin, because of their very high molecular weight and branching, can associate only with difficulty (Lampitt *et al.*, 1948). Therefore, the changes observed in the physico-chemical properties of the gel, on long term storage (more than 4-5 days) is mainly attributed to amylopectin association. Unlike amylose, amylopectin crystallization is thermo reversible at temperatures below 100°C (Ring *et al.*, 1987). DSC measurements have shown that recrystallized amylopectin melts at temperatures around 60°C (Eliasson, 1985).

Miles *et al.* (1985), suggested a three step mechanism (Fig 2.10), (based on the nucleation theory of Avarami (1939, 1940)) to explain crystal development during amylose gelation. The mechanism involves the following sequential steps: 1) nucleation - formation of critical nuclei by initiation of ordered chain segments, i.e. double helix formation between the ends of amylose molecules, favouring chain elongation (Fig. 2.10.a); 2) propagation - growth of crystals from nuclei by intermolecular aggregation of ordered segments, i.e. packing of double helical regions by chain folding (Fig. 2.10.b); 3) maturation - crystal perfection by annealing of metastable microcrystallites.

Katz and Van Itallie (1930), showed that retrograded starch gels always display a "B" X-ray pattern regardless of the initial crystalline pattern of the native granule. Hellman *et al.* (1954) and Dragsdorf and Varriano-Marston, (1980), observed an increase in the intensity of the "B" pattern during ageing of starch gels. Furthermore, the "B" pattern was also obtained from retrograded gels from amylose and amylopectin (Miles *et al.*, 1985; Ring *et al.*, 1987). Thus, starch retrogradation was attributed to changes in both amylose and amylopectin. However, the rate of retrogradation of isolated amylose was not similar to that of starch (Miles *et al.*, 1985). Radley (1953) observed that the rate and extent of retrogradation of any particular amylose-amylopectin mixture is directly proportional to the amount of amylose present.

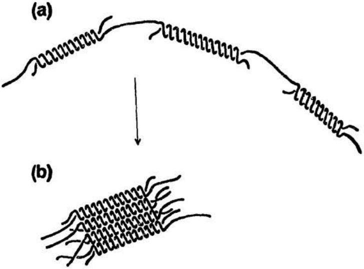
The Avarami model of polymer crystallization has been widely used to kinetically model retrogradation (McIver *et al.*, 1968; Colwell *et al.*, 1969; Russell, 1987; Levine and Slade, 1988). Based on crystalline phase changes vs time, the Avarami equation is expressed as follows:

$\Theta = 1 - e^{(-K t^n)}$

where Θ is the percent crystallized starch at time t, K is a rate constant, and n is the Avarami exponent. The Avarami exponent (n) provides qualitative information on the nature of nucleation and growth process. For instance, an exponent of 1 is said to represent rod-like crystalline growth from instantaneous nuclei, and an exponent of 2 can either represent rod-like

Fig 2.10. Mechanism of amylose gelation (Miles *et al.* (1985), with permission).

a) Chain elongation by double helical formation.
b) Double helical packing by chain folding.


(a)
(b)

crystalline growth from sporadic nuclei or disk like growth from instantaneous nuclei (Sharples, 1966). The rate constant (K) is a combined function of nucleation and growth rate, providing quantitative information on the course of crystallization (Sharples, 1966).

2.4.2.1 Factors influencing starch retrogradation

2.4.2.1.1 Concentration

Starch concentration influences the extent of retrogradation. Hellman *et al.* (1954), showed that the most intense B-pattern is obtained for wheat starch gels with a water content of 47-50% (w/w). For a starch gel with 63% (w/w) water stored 8 days at 24°C, the intensity of the B-pattern is only one-fifth the intensity observed for a starch gel with 50% water treated in the same way. Zeleznak and Hoseney (1986) prepared starch gels containing 15-80% starch, aged them for 7 days, then heated them in the DSC. They showed a minimum enthalpy at 15% and 80% and maximum enthalpy values in gels of 50-60% starch (these corresponded respectively, to amylopectin contents of 37.5 and 45%). Slade and Levine (1987), reported similar results in a study of wheat starch gels (10 to 77% starch). These authors postulated that the decrease in enthalpy from 50% to 77% starch was due to decreasing plasticization by water, while the decrease from 50% to 10% starch (increase in water from 50%-90%) was due to a dilution effect.

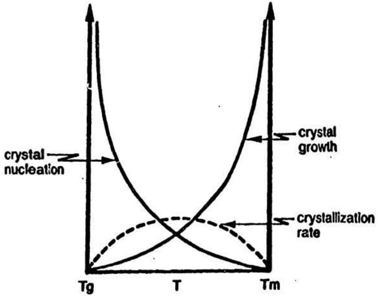
2.4.2.1.2 Moisture content

At very high moisture contents (>95%) the amylose in a gelatinized starch suspension rapidly phase separates, recrystallizes and precipitates. However, at low moisture contents (<60%) this suspension will form an elastic gel, upon cooling. Studies of the gelatinization and aging of starch (Miles *et al.*, 1985; Russell, 1987) have usually been conducted at high moisture contents (>20%) since this condition is often encountered in food processing. At moisture contents above 20%, the amylose and amylopectin within a starch gel recrystallizes to the B form (Miles *et al* 1985). This occurs because at moisture levels above 20%, the glass transition temperature (T_g) drops below room temperature, allowing the starch with sufficient mobility to rearrange into the crystalline form (Slade and Levine, 1988).

2.4.2.1.3 Temperature

The starch retrogradation process is very sensitive to temperature. This is evident from using DSC and X-ray diffraction as well as rheological measurements (Morris, 1990). Crystallization has been shown to occur (Fig. 2.11) only when $Tg < T < Tm$ (Morris, 1990). The higher the degree of supercooling, the more crystal seeds will be formed, but the growth of crystals is favoured at higher temperature (Fig. 2.11) (Morris, 1990). However, in the case of a starch gel, supercooling cannot be brought too far; at some

Fig. 2.11. The effect of temperature on starch retrogradation (Morris (1990), with permission).



Tg = Glass transition temperature

Tm = Crystal melting temperature

temperature, T_g of the starch as well as the freezing point of the water will be passed, and during these conditions no crystallization will occur at all. The best conditions for crystallization of amylopectin therefore, seem to be just above 0°C (Eliasson and Larsson, 1993). The retrogradation process might then be avoided or delayed by storage at the correct temperature. To bring about as much starch retrogradation as possible, the correct treatment would be to store the starch gel first at a low temperature (e.g. 4°C) to promote nucleation and then at a higher temperature (e.g., 25–40°C) to promote crystal growth (Morris, 1990). Such experiments have been performed, and after 24h at 4°C starch gels were stored for another 24 h at 4, 12, 25 and 40°C. The highest values of ΔH were obtained after storage at 12 and 25°C (Zeleznak and Hoseney, 1987).

2.4.2.1.4 Starch source

The extent of retrogradation is influenced by the starch source. Russell (1987) showed that the enthalpy of recrystallization decreased in the order: waxy maize > potato > wheat > amylomaize. Orford *et al.* (1987) investigated retrogradation of wheat, maize, potato and pea starches. Gels from all sources showed substantial increases in shear modulus over 7 days. Pea starch showed the highest increase, followed by potato maize and wheat. Crystallinity (X-ray and DSC) paralleled the long term development of shear modulus, indicating that this was due to a crystallization process. All gels exhibited a B-type X-ray pattern. However, the above authors made no attempt to explain the differences in recrystallization between various starch sources.

The influence of starch source on retrogradation can be related to variations in the fine structure of the component molecules. Whistler and Johnson (1948) suggested that there was an optimum chain length for amylose retrogradation. Gidley *et al.* (1986) found the minimum chain length required for retrogradation (0.5-3% starch solution) was eight or nine glucose units. Sterling (1978) reported that β-amylase treated amylopectin (external branches reduced to 2-3 glucose units) retrograded less readily than native amylopectin.

2.4.2.1.5 Addition of other components

Retrogradation can be delayed by adding suitable substances. The most common additives with this function are the monoacyl lipids (monoglycerides) and free fatty acids. Although the exact mechanism by which the lipids retard retrogradation is far from clear, the role of lipids is generally attributed to the formation of helical inclusion complexes between starch molecules (mainly amylose) and lipid hydrocarbon chains. Whittam *et al.* (1986) reported a reduction in amylose gel rigidity, when lipids were added in the form of fatty acid (C_{10}-C_{18}) or monoglyceride; and the effect being dependent both on lipid concentration and lipid acyl chain length. In the presence of monoacyl lipids

(Mikus *et al.*, 1946) amylose undergoes rapid conformational ordering in solution (coil → helix), which promotes aggregation of helices into partially crystalline structures (V-crystals).

Many authors (Eliasson, 1983; Russell, 1983; Eliasson and Ljunger, 1988) have shown that recrystallization of amylopectin or waxy maize starch (100% amylopectin) is decreased (decreased enthalpy) in the presence of emulsifiers. However, the mechanism by which lipids hinder amylopectin recrystallization has been the subject of much controversy (Gray and Schoch, 1962; Lagendijk and Pennings, 1970; Kugimiya *et al.*, 1980; Evans, 1986).

2.5 Physical modification of starches

2.5.1 Defatting

Many researchers (Medcalf *et al.*, 1968; Goering *et al.*, 1975; Lorenz, 1976; Melvin, 1979; Maningat and Juliano, 1980; Lorenz and Kulp, 1983; Goshima *et al.*, 1985; Kawano *et al.*, 1989) have used different lipid extractants, many of which are now known to differ in their ability to extract firmly bound lipids (Morrison, 1981; Morrison and Coventry, 1985). Therefore, it is difficult to interpret coherently the effect of defatting on starch properties.

Leach *et al.* (1959), postulated that the bonding forces within the starch granule influence the extent of swelling. Thus, highly associated starch granules should be relatively resistant to swelling and amylose leaching. Tester and Morrison (1990a) reported that the swelling behaviour of cereal starches was primarily a property of their amylopectin content; amylose acts both as a diluent and as an inhibitor of swelling, especially in the presence of lipids (natural components of non-waxy cereal starch granules). Tester and Morrison (1990b) showed by studies on waxy rice starches (no native lipids) that crystallites within the amylopectin molecule determine the onset of swelling and gelatinization. There are conflicting reports in the literature (Lorenz and Kulp, 1983; Goshima *et al.*, 1985; Tester and Morrison, 1990a) with regard to the effect of defatting on swelling and amylose leaching. Lorenz and Kulp (1983), reported that defatting with 80% methanol increased the swelling power and solubility of wheat starch, whereas in potato starch the swelling power remained unchanged while the solubility decreased. Goshima *et al.* (1985), reported that the swelling power and solubility of potato starch increased on defatting with 99% methanol. Tester and Morrison (1990a), reported that partial extraction of lipids from wheat starch with anhydrous methanol at 100°C increased the swelling factor by 30%.

The literature is replete with conflicting information with regard to the effect of defatting on pasting (thickening of starch/water slurry upon heating) properties (Lorenz, 1976; Melvin, 1979; Takahashi and Seib, 1988). Lorenz (1976), reported that lipid removal (0.54-0.61%) from wheat starch with 80% methanol did not affect the peak viscosity at 92°C. Melvin (1979), reported that lipid removal (0.4-0.5%) from corn and wheat starches by slurrying at 70°C with water-saturated n-butanol (5 h), or by Soxhlet extraction using 85% aqueous methanol (72 h) reduced the pasting temperature but increased the pasting peak and paste consistencies. Takahashi and Seib (1988), however, showed that lipid removal from wheat (1.0%) and corn (0.82%) starches with boiling 75% ethanol eliminated the pasting peak, reduced consistency and set-back and decreased the pasting temperature. Biliaderis and Tonogai (1991) observed increased gel firmness and viscosity when lipids were extracted from rice (0.82%) and wheat (0.64%) starches using 85% methanol (4 h). They monitored viscosity and gel texture changes using the Bohlin VOR rheometer. The above researchers attributed the discrepancy between their results and those of Takahashi and Seib (1988) to differences in starch concentration (20-30%) versus (6.5-7.5%). Lorenz and Kulp (1983) showed that lipid removal from potato starch with 80% methanol (48 h) did not significantly alter the amylograph consistencies. Goshima *et al.* (1985), however, reported that lipid removal (0.064%) from potato starch with 99% methanol (15 h) in a Soxhlet extractor reduced the pasting temperature, increased paste consistency at 67.8°C and caused no change in thermal stability during the holding period.

There is very little information in the literature with respect to the effect of defatting on starch crystallinity. Lorenz and Kulp (1983), observed that wheat and potato starches showed no changes in their X-ray pattern on defatting with 80% methanol for 48 h. Furthermore, defatting was shown to cause a decrease in relative crystallinity (RC), which amounted to 1.7% and 6.8% in potato and wheat starch respectively.

2.5.2 Heat-moisture treatment

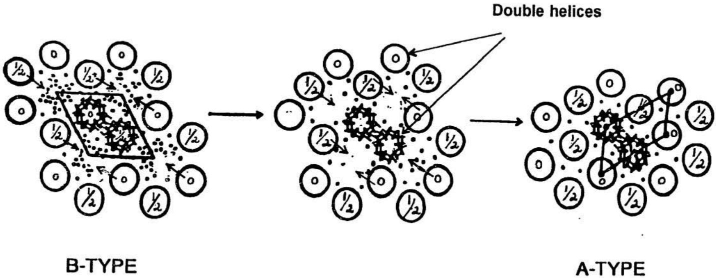
Heating (100-120°C) of starches at limited moisture (18-27%) contents (heat-moisture treatment) for 16 h dramatically changes the properties of starches without actual gelatinization occurring (Sair and Fetzer, 1944; Leach *et al.*, 1959; Sair, 1964,1967; Fukui and Nikuni, 1969; Kulp and Lorenz, 1981; Lorenz and Kulp, 1982; Donovan *et al.*, 1983; Stute, 1992).

Sair (1967) and Stute (1992) showed that the X-ray pattern of B-type starches (e.g., potato) are transformed into a mixture of "B" and "A" patterns upon heat-moisture treatment. However, the X-ray diffraction patterns of cereal starches remain unaffected, by heat-moisture treatment (Buleon *et al.*, 1982). Lorenz and Kulp (1982) showed that the degree of crystallinity of cereal starches decreases on heat-moisture treatment. However, similar studies have not been reported for tuber and legume starches.

The double helical packing in the unit cells of the A-type crystal is more compact than B-type. Thus, the "B" to "A" transition could occur by a simple shifting of helices following removal of water. On the other hand, "A" to "B" transformation cannot take place without the destruction of "A" and recrystallization (Zobel, 1988a). A stepwise description (Fig. 2.12) of the "B" to "A" transformation has been proposed from computer modelling studies (Imberty *et al.*, 1991). Fukui and Nikuni (1969), showed by X-ray diffraction studies, that maize starch develops a "V"-crystalline structure (due to formation of amylose-lipid inclusion complexes) on heat-moisture treatment. This suggests, that starch lipids and amylose occur in close proximity within the granule.

Heat-moisture treated (100°C, 16h, 18-27% moisture) potato and wheat starches have been shown to exhibit a broadening of the gelatinization temperature range and a shifting of the endothermal transition towards higher temperatures, compared to the untreated starches (Lorenz and Kulp, 1982; Donovan *et al.*, 1983). The enthalpy of this transition decreased with increasing moisture contents. The change was greater for potato than for wheat starch. The endotherm of the treated (27% moisture) samples (wheat and potato) was biphasic, as well as having an elevated Tp (mid-point temperature of gelatinization) and increased range. The above authors suggested, that this may have been due to the development of more stable crystallites resulting from changes within the crystallite structure. An obvious question is whether these crystallites are derived from modifications of the pre-existing crystallites, or whether they are formed *de novo* from the previously amorphous regions (Blanshard, 1987). Donovan *et al.* (1983), postulated from the above studies

Fig. 2.12. Model of the polymorphic transition from the B to the A starch, in the solid state (Imberty *et al.* (1991), with permission).



C axis is perpendicular to the diagram

The parallel double-helices which form the duplex are labelled 0 and ½ (this indicates their relative translation along the "c" axis). The water molecules are shown as dots (•).

that heat-moisture treatment induces starch chain reorientation in crystallites which is accompanied by movement or chain reorientation in the amorphous or glassy regions, and alterations occur in the amorphous domains which have a profound effect on the ability of these domains to destabilize crystallites upon absorbtion of water by the granule and thus upon the gelatinization temperatures. Blanshard (1987) suggested that, at 27% moisture and 100°C, the glass transition temperature of the system may have been exceeded and therefore, the granules would be in the rubbery state.

Heat-moisture treatment of cereal and tuber starches have been shown to reduce swelling power (Sair, 1964, 1967; Kulp and Lorenz, 1981; Lorenz and Kulp, 1983). However, the extent of amylose leaching increases in cereal starches, but decreases in tuber starches (Kulp and Lorenz, 1981; Lorenz and Kulp, 1982). Lorenz and Kulp (1982) have postulated that the difference in the solubility behaviour of the cereal and tuber starches may be due to differences in the physical state of the amylose components in the native granules. In cereal starch granules amylose is present in a helical form, complexed with lipids; while in tuber starches the lipids are absent and the amylose occurs in the amorphous state and is converted by heat-moisture treatment into a less soluble helical form.

It is evident from the above review, that most of the studies have been concentrated on understanding changes within the crystalline region during heat moisture treatment. However, changes within the amorphous regions still remain an area of uncertainty which merits further investigation. A proper understanding of the structural changes within the amorphous and crystalline domains of the starch granule would emerge, if studies are conducted on starches differing not only in amylose content but also in the degree of separation of the starch components within the native granule.

2.5.3 Annealing

Annealing of starch is a process whereby the material is held at a temperature somewhat lower than its gelatinization temperature which permits molecular reorganization to occur within the granule and a more organized structure of lower free energy to form (Blanshard, 1987). Typical conditions for such treatments are temperatures of 50-55°C, moisture contents of 50-75% and treatment times of 48-72 h. Such a process may have industrial significance since the steeping of wheat and corn grains to soften the grain prior to wet milling in some instances is performed at 50°C for some 20 h (warm conditioning). The impact of annealing on starch structure and properties have been investigated by various researchers (Gough and Pybus, 1971; Marchant and Blanshard, 1978; Hoseney *et al.* 1986; Yost and Hoseney, 1986; Krueger *et al.* 1987a,b; Knutson, 1990; Larsson and Eliasson, 1991; Stute, 1992).

Birefringence studies of Gough and Pybus (1971), on annealed wheat starch showed that the treatment narrowed the gelatinization temperature range and raised the peak temperature of gelatinization. Marchant and Blanshard (1978), postulated that during annealing a realignment of starch chains occur within the crystalline and amorphous regions of the starch granule. The relationship between annealing and the glass transition temperature in starch granules was studied by Hoseney *et al.* (1986), Yost and Hoseney (1986) and Larsson and Eliasson (1991). The above authors discussed that the physical aim of annealing is to approach the glass transition temperature which thereby enhances molecular mobility without, at the same time, triggering total gelatinization.

DSC has been used to investigate the effect of annealing on the thermal characteristics of starches. Krueger *et al.* (1987a), annealed corn starch, both laboratory extracted and commercially obtained, at 50°C and 75% moisture for various time intervals up to 48 h. DSC analysis showed that untreated commercial corn starch had a relatively narrower gelatinization temperature range and a higher peak temperature and enthalpy than that of laboratory extracted starch. Furthermore, upon annealing, the magnitude of increase in gelatinization temperatures and enthalpy were higher in laboratory extracted than commercial starches. This demonstrated that partial annealing of starch granules occurs during commercial extraction (wet milling) of corn starch. Krueger *et al.* (1987b), studied the effect of annealing (50°C, 75% moisture for 48 h) on normal and mutant varieties (waxy and high amylose) of corn starches. The above authors have reported that annealing had no effect on the gelatinization enthalpy of waxy maize starch but increased the enthalpy of amylose containing corn starches. This indicated a possible interaction between amylose and linear chains of amylopectin. The extent of the change varied with the composition of the starch.

Knutson (1990), investigated the effect of multistep annealing process (which allows higher annealing temperatures) on maize starches [normal, waxy and high amylose (50-70% amylose)] by DSC. Normal and waxy maize starches showed an increase in To and Tp, and a decrease in the range with relatively little change in ΔH upon annealing at 75% moisture for 72 h at various temperatures between 50 and 70°C. However, in amylomaize (high amylose content), To, Tp and ΔH increased dramatically when annealed at temperatures beyond 55°C. The above author interpreted the results as evidence that interaction between amylose and amylopectin (increased order within the granule) occurs in amylomaize granules during annealing, and further suggested that since waxy starch contains no amorphous amylose regions, annealing effects at higher temperatures occur in intercrystalline amylopectin regions or in crystallites.

The effect of temperature, between 25 and 50°C and time, up to 48 h (when annealing starches at an intermediate starch/water ratio (1:1 w/w) on the gelatinization behaviour of starches from maize, waxy-maize, wheat and potato have been investigated by DSC (Larsson and Eliasson, 1991). The above authors reported that gelatinization temperatures of maize and waxy-maize starches remained unaffected by annealing temperatures up to

40°C. However, T_o and T_p of potato and wheat linearly increased with temperatures between 25 and 50°C. Changes in T_c was marginal in all starches. The closer the annealing temperature was to T_o of the untreated starch, the more pronounced was the increase of T_o. Potato starch when annealed at 52°C at a moisture content of 75% for 92h (Stute, 1992) showed an increase in T_o, T_p and T_c and a decrease in the gelatinization temperature range. However, ΔH, the extent of crystallinity and the X-ray pattern remained virtually unaffected. On the strength of these observations, the above author postulated that annealing treatment causes structural changes within the amorphous region of the granule.

It is evident from the above review that a great deal is known about the effect of annealing on gelatinization parameters (T_o, T_p, T_c and ΔH) of cereal starches. However, changes in X-ray intensities, swelling factor, amylose leaching, Brabender viscosities and susceptibility towards acidic and enzyme hydrolysis on annealing have not received much attention. Furthermore, there have been no reports on the response of other cereal starches such as oat (exhibits properties that are not similar to those of other cereal starches (Paton, 1977)) and legume starches to annealing. As a result, it is still not clear how annealing modifies the structural organization of the starch chains within the amorphous and crystalline domains of the granule.

CHAPTER 3

MATERIALS AND METHODS

3.1 Starch sources

Wheat and potato starches were obtained from Sigma Chemical Co., St. Louis, MO. Yam (*Dioscorea dumetorum*) and seeds of lentil (*Lens culinaris medicus var eston*) were obtained from a local supplier. AC Hill oat grains (*Avena nuda, var chinensis*) which is a spring type, day length sensitive cultivar were obtained from the Central Experimental Farm (Agriculture Canada) in Ottawa.

3.2 Starch isolation and purification

Lentil seeds or oat grains were steeped overnight in water at room temperature. A mixture 1 part soaked grains or seeds to 3 parts distilled water was blended for three minutes at low speed and followed by another three minutes at high speed, in a Waring blender. The resultant slurry was passed through a double layer cheese cloth and centrifuged at 5000×g for 15 min. The supernatant was discarded and the sediment suspended in excess 0.02% NaOH to remove the residual proteins. After standing for 1 h the supernatant was removed. This procedure was repeated three times. The final sediment was suspended in distilled water, and subjected to filtration through a 70 micron polypropylene screen, neutralized to pH 7.0, filtered on a Buchner funnel and thoroughly washed on the filter with distilled water. The filter cake was dried overnight at room temperature (25°C).

The extraction of starch from yam was carried out in the same manner but the yam was peeled to remove the skin, thoroughly washed and cut into small pieces before the blending process.

Commercial wheat and potato starches were purified by washing with excess alkali (0.02% NaOH), neutralized to pH 7, filtered on a Buchner funnel and thoroughly washed on the filter with distilled water. The filter cake was dried overnight at room temperature (25°C).

3.3 Estimation of starch damage

The extent of starch damage was determined enzymatically (AACC, 1983). The method determines the percentage of starch granules which are susceptible to hydrolysis by α-amylase. The enzyme used was a fungal α-amylase from *Aspergillus oryzae* (Sigma Chemicals Co., St. Louis, MO). It had pH and temperature optimums of 4.8 and 53°C respectively. The starch (1.0 g, db) was digested with α-amylase (12,500 Sigma units) in a 30°C bath for exactly 15 min, followed by addition of 3.68 N H_2SO_4 (3.0 ml) and Na_2WO_4. 2 H_2O (2.0 ml). The mixture was allowed to stand for 2 min, and then filtered. Sample aliquots (1 ml) of the carbohydrate solution were mixed with 2.0 ml of chilled 3,5-dinitrosalicylic acid and diluted to 4.0 ml with distilled water. The tubes were heated in a boiling water bath for 5 min. The reaction mixture was diluted by adding 8 ml of distilled water and the absorbance measured at 540 and 590 nm (for absorbance above 1.5) against the blank from which the enzyme was omitted (Bruner, 1964). A calibration curve was established (at 540 and 590 nm) with maltose (0.2-2.0 mg in 2ml H_2O). The % damaged starch was calculated as:

% damaged starch = $(M \times 1.64)/(W \times 1.05) \times 100$

where M = mg maltose equivalents in the digests; 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 starches. The latter is an emperical factor which assumes that under the conditions of the experiment, the maximum hydrolysis is 61%.

3.4 Chemical composition of native starches

All chemicals used in this study were ACS grade and the results of all experiments were calculated on starch dry weight basis (db).

3.4.1 Moisture content

Moisture content was determined by drying a preweighed (4-5g) amount of material in a forced air oven (Fisher Scientific, Isotemp 615G, USA) at 105°C until it reached a constant weight. The moisture content was calculated as a percentage of weight loss of the sample due to drying (AOAC, 1990).

3.4.2 Ash content

Samples weighing 2.0-3.0 g were transferred into clean porcelain crucibles, charred using a flame and then placed in a temperature-controlled furnace (Lab Heat, Blue M, Illinois) which was preheated to 550°C. Samples were held at this temperature until grey ash remained and then transferred to a desiccator, cooled and weighed immediately. Ash was calculated as percentage weight of the remaining matter (AOAC, 1990).

3.4.3 Total protein content

Samples (100-200 mg) were weighed on nitrogen-free papers and placed in the digestion tubes of a Buchi 430 (Buchi Laboratoriums-Technik AG, Flawil/Schweiz) digester. The samples were digested with two Kjeltabs catalyst pellets (Profamo, Quebec) and 20 ml of concentrated H_2SO_4 in the Kjeldahl digester (Buchi 430) until a clear solution was obtained. Digested samples were diluted with distilled water (50 ml); alkali (150 ml of 25% NaOH) was added and the released ammonia was steam distilled (Buchi 321) into 4% H_3BO_3 (50 ml) containing twelve drops of an end point indicator (N-point indicator, EM Science, New Jersey) until 200 ml distillate was collected. The content of ammonia in the distillate was determined by titrating it against 0.1*N* H_2SO_4 (AOAC, 1990). The content of crude protein in samples was calculated by multiplying the percentage of nitrogen by a factor of 6.25.

3.4.4 Amylose content

The apparent amylose content of native and dimodan (35% mono- and 65% diglycerides) complexed starches were determined by the method of Chrastil (1987), after complete dispersion of samples in KOH solution followed by neutralization with HCl at room temperature. The total amylose content of the starches was determined by the same procedure, but the samples were defatted with hot n-propanol-water (3:1 v/v) for 7 h, prior to determination.

3.4.4.1 Preparation of starch dispersions

Starch samples (20 mg db) were dispersed in 10 ml of 0.5 *N* KOH in 20 ml screw cap glass tubes. The contents were transferred into volumetric flasks and diluted to 100 ml. Aliquots (10 ml) were neutralized with 5 ml of 0.1 *N* HCl prior to dilution to 50 ml.

3.4.4.2 Chrastil's method of amylose determination

Sample aliquots (0.1 ml) of the neutralized solution were mixed with 5.0 ml of 0.5% trichloroacetic acid (TCA) and 0.05 ml of 0.01 *N* I_2-KI solution (1.27 g of I_2 per litre + 3 g of KI per litre). The absorbance of the blue colour was read at 620 nm (after 30 min at 25°C) in a spectrophotometer (LKB Biochrom, Novaspec). The absorbance of the reaction blanks with water was zero, and with pure amylopectin (corn amylopectin from Sigma Chem. Co., purified by precipitation with acetone) was less than 0.03. The amylose content was approximated by the following formula (obtained through a calibration graph) and represented as mg of amylose per 100 mg dry starch:

Absorbance × 45.8 = mg of amylose / litre in cuvette.

3.4.5 Lipid content

3.4.5.1 Lipid extraction

Wheat starch [5.0 g dry basis (db)] was used for comparing the lipid extraction efficiency of various solvent systems. Extraction at ambient temperatures (25-27°C) was with 100 ml each of chloroform-methanol 2:1 v/v (CM), and water saturated *n*-butanol (WSB) under vigorous agitation in a wrist action shaker (up to 12 h). At elevated temperatures (90-100°C) lipids were obtained by Soxhlet extraction with 100 ml each of n-propanol-water 3:1 v/v (PW), and WSB (up to 12 h). However, in the determination of lipid composition, all starches were subjected to the following extraction procedures: (1) starches (5 g db) were extracted under vigorous agitation in a wrist action shaker with 50 ml of CM at 25-27°C for 1 h; (2) the residues from CM extraction were solvent extracted with 100 ml of PW at 90-100°C for 7 h in a Soxhlet apparatus; (3) to determine the total native starch lipids, starches (2.0g, db) were hydrolysed with 25 ml of 24% HCl at 70-80°C for 30 min, and the hydrolysate then extracted three times with n-hexane (Goshima *et al.*, 1985).

3.4.5.2 Lipid purification

The crude lipids from the above extracts were purified by extraction with chloroform/methanol/water (1:2:0.8 v/v/v) and by forming a biphasic system (chloroform/methanol/water (1:1:0.9 v/v/v)) by addition of chloroform and water, at room temperature (Bligh and Dyer, 1959); the chloroform layer was then diluted with benzene and brought to dryness on a rotary evaporator.

3.4.5.3 Lipid fractionation

The purified lipids were fractionated using silicic acid chromatography (Kates, 1972). The silicic acid (325 mesh, Sigma Chemical Co., St. Louis, MO) was washed several times with deionised water, filtered through a Buchner funnel and rinsed with anhydrous methanol. The washed silicic acid was dried at 120°C for 24 h and stored in a desiccator until used. Each column was prepared by suspending a 10.0 g portion of the silicic acid in 50 ml chloroform and pouring into a 30 cm long by 2 cm diameter glass column. A 5 ml aliquot of the total purified lipid extract was applied to the top of the silicic acid column. Neutral lipids (NL), glycolipids (GL) and phospholipids (PL) were eluted with 50 ml chloroform, 50 ml acetone and 75 ml methanol respectively. All fractions were concentrated using a rotary evaporator, transferred to vials (4 ml) and dried under nitrogen to a constant weight. The lipid fractions were resolubilized in chloroform and stored under nitrogen at -15°C.

3.4.5.4 Thin layer chromatography

Lipid class components (NL, GL and PL) were examined by thin layer chromatography (TLC) on pre-coated silica gel plates (Fisher Scientific Co.) in one of the following solvent systems: (1) petroleum ether/diethyl ether/acetic acid (70:30:1v/v/v) (2) chloroform/methanol/acetone/diethylamine/H_2O (120:35:37:6:4 v/v/v), and (3) chloroform/acetone/methanol/acetic acid/water (10:4:2:2:1 v/v/v/v/v). Neutral lipids were separated by development in solvent system 1. The GL were resolved in solvent system 2 while the PL were resolved in solvent system 3. Lipids were detected on TLC plates by charring with 50% H_2SO_4 or by spraying with specific spray reagents (Kates, 1972) and comparison of their R_f values with those of pure samples.

3.4.5.5. Densitometry

A Pharmacia Ultrascan XL enhanced laser (helium-neon) densitometer (Model LKB 2222-20, Uppsala, Sweden) and a Pharmacia XL software (code 2400) system was used to determine the percentage distribution of the lipid constituents within each lipid class. For the densitometer scanning, lipid components were visualized by charring the TLC plate by spraying lightly with sulfuric acid-dichromate reagent (1.2 g of $K_2Cr_2O_7$ in 200 ml of 50% (by weight) reagent grade H_2SO_4) as a fine mist, and heating in a forced-draft oven at 180°C for 20-40 min.

3.4.5.6 Fatty acid composition

Fatty acid methyl esters (FAME) of the three lipid classes (NL, GL, PL) from CM and PW extracts were prepared by transmethylation with 6% H_2SO_4 in 99.9 mole% of methanol at 65-70°C for 15 h (Keough and Kariel, 1987). The methyl esters were analyzed using a Perkin-Elmer 8310 gas chromatograph equipped with a 30 m × 0.25 mm column (SP 2330, Supelco, Oakville, Ontario). Oven temperature was 180°C and the injection port and flame ionization detector temperatures were 230° and 250°C, respectively. The flow rate of the helium carrier gas was 25 ml/min. Identification of FAME was based on the comparison of their retention times with those of FAME standard mixture (Supelco Inc., Oakville, Ontario). Quantification was accomplished with the data handling and control unit of the instrument.

3.5 Preparation of defatted starches

To study the effect of defatting on starch structure and physicochemical properties, the starches were extracted in a Soxhlet apparatus with PW at 90-100°C for 7 h. The solvent was removed by vacuum filtration and the starch was dried for 72 h at room temperature.

3.6 Preparation of starch/dimodan complexes

Dimodan PM (a mixture of 90% monoester, 1.5% FFA and 1% free glycerol) (Grindsted Products Inc., Rexdale, Ontario) (1 part by weight) was slowly added to water (9 parts by weight) and the mixture was preheated to 70°C, with agitation, until a translucent dispersion was obtained. This dispersion was cooled to ambient temperature (25-27°C) and added to a starch/water suspension (10% w/w) preheated to 40°C in the starch to dimodan weight ratio of 5:1. The combined volume was heated at 40°C with agitation for 6 h. The suspension was then cooled to ambient temperature, and the insoluble fraction was recovered by filtration through a Buchner funnel. Any non-complexing dimodan, was removed from the insoluble fraction by extraction at ambient temperature with chloroform/methanol (2:1, v/v) and agitation for 10 min. The solid fraction remaining was dried under vacuum at 40°C.

3.7 Preparation of heat-moisture treated starches

The method of heat-moisture treatment was that of Sair (1964), with minor modifications. Native starch samples (15 g db) were weighed into glass containers. Starch moisture content was brought to 10, 20 and 30% (w/w) by adding the appropriate amounts of distilled water into the containers. Starch samples were mixed thoroughly during the addition of water. The containers were sealed (a double layer of aluminum foil was placed in-between the lid and the container, to obtain a tight seal), kept for 24h at ambient temperature, and then placed in a forced air oven (Fisher Scientific, Isotemp 615G, USA) for 16h at 100°C. Afterwards, the containers were opened, and the starch samples air dried to a uniform moisture content (≈10.2%). The effect of treatment time (1-

24 h) on gelatinization transition temperatures and enthalpy, was studied on starches heat-treated (100 °C) at 30% moisture.

3.8 Preparation of annealed starches

The method of annealing was essentially that of Krueger *et al.* (1987a). Native starches (25 g db) were weighed into glass containers and the moisture content of the samples was brought to 10, 30, 40, 50, 66.6, and 75% by adding appropriate amounts of distilled water. Starch samples were mixed thoroughly during the addition of water. The sealed samples were heated at 50°C for 72 h in an air oven. The influence of annealing time was investigated by heating starch samples (starch:water ratio, 1:3 w/v) at 50°C for time intervals ranging from 0.5 to 72 h. Annealed starches were separated by filtration, washed several times with distilled water and air dried to uniform moisture content (~ 10%).

3.9 Physico-chemical properties

3.9.1 X-ray diffraction

X-ray diffractograms were obtained with a Rigaku RU 200 R X-ray diffractometer connected to a data acquisition and processing station. The starch powder (≈ 10% moisture, wet basis) was scanned through the 2Θ range of 3-35°. Traces were obtained using a Cu-Kα radiation detector with a nickel filter and a scintillation counter operating under the following conditions: 40 KV, 50 mA, 1°/1° divergence slit/scattering slit, 0.3 mm receiving slit, 1 s time constant and scanning rate of 3°/min. Relative crystallinity was measured by the method of Nara *et al.* (1978), (Appendix 1). Quartz was used as the 100% reference crystal.

3.9.2 Differential scanning calorimetry

Starch gelatinization and fusion of retrograded amylopectin were studied on a Perkin-Elmer DSC-2 differential scanning calorimeter (Perkin Elmer Ltd., England) equipped with a data acquisition and processing station. The thermograms were recorded with water as reference. Indium was used for calibration. The thermal transitions (appendix 2) of starch were defined in terms of temperature at To (onset), Tp (peak) and Tc (conclusion). The symbol ΔH refers to the enthalpy associated with the transition. This enthalpy corresponds to the area enclosed by drawing a straight line between To and Tc (appendix 2) and is expressed in terms of joules per unit weight of dry starch (J/g^{-1}). All DSC experiments were replicated at least thrice.

3.9.2.1 Starch gelatinization

Water (8 μl) was added with a microsyringe to starch (2.5 mg) in aluminum DSC pans (Perkin-Elmer, kit no. 219-0062), which were then closed with aluminum lids and hermetically sealed (using a screw press), reweighed and allowed to stand overnight at room temperature. The scanning temperature range and the heating rate were, respectively, 20-120°C and 10°C min^{-1}.

3.9.2.2 Starch retrogradation

Fusion of retrograded amylopectin at various time intervals of storage (0.5 h-20 days) was determined by weighing (3-4 mg db) of the stored (at 25°C) gels (40% w/v) into DSC aluminum pans. Which were then closed with aluminum lids, sealed and scanned from 20-100°C at 5°C min$^{-1.}$

3.9.3 Brabender viscoamylography

Pasting characteristics of starch slurries at a concentration of 6% (w/v) and pH 5.5 were determined using the Brabender viscoamylograph, Model VA-V (C.W.Brabender Instruments, Inc., South Hackensack, NJ), equipped with a 700-cm.g sensitivity cartridge, operating at a bowl speed of 75 rpm. The starch slurry was heated from 30 to 96°C at the rate of 1.5°C/min, maintained at 96°C for 30 min, and then cooled (30 min) to 51°C at the same rate. The viscosity was measured in Brabender units (BU). The pasting temperature was when viscosity reached 10 BU during the heating period. The thermal stability was evaluated from the viscosity breakdown during the holding period (at 96°C for 30 min).

3.9.4 Swelling factor

The swelling factor (SF) of starches when heated at 50-95°C in excess water was measured according to the method of Tester and Morrison (1990a). Starch samples (50-200 mg dry basis, depending on the anticipated SF) were weighed into 10 ml screw cap tubes, 5.0 ml of water added, and the sealed tubes were incubated with constant shaking in a water bath at the required temperature for 30 min. The tubes were then cooled rapidly to 20°C, 0.5 ml of blue dextran (Pharmacia, M_r 2×10^6, 5 mg/ml) was added and the contents were mixed gently by inverting the closed tubes several times. After centrifuging at 1,500×g for 5 min the absorbance of the supernatant (A_s) was measured at 620 nm. The absorbance of the reference (A_r) tube that contained no starch was also measured.

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

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

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

where A_r and A_s are absorbance of the reference and sample respectively.

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

$$V_o = W/1,400$$

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

$$V_l = 5.0 - FW$$

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

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

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

3.9.5 Extent of amylose leaching

Starch samples (10-15 mg) in distilled water (10 ml) were heated (50-95°C) in volume calibrated sealed tubes for 30 min. The tubes were cooled to ambient temperature and centrifuged at 3500 rpm for 10 min. Aliquots (0.1 ml) of the supernatant, were assayed for solubilized amylose, by the method of Chrastil (1987). Percentage amylose leaching was calculated and represented as mg of amylose leached per 100 mg dry starch.

3.9.6 Scanning electron microscopy (SEM)

Granule morphology of native, defatted and heat treated starches, and the mode of action of porcine pancreatic α-amylase and acid (2.2N HCl) on the above starches were studied by scanning electron microscopy. Starch samples were mounted on circular aluminum stubs with double sticky tape, and then coated with 20 nm of gold and examined and photographed in a Hitachi (S570) scanning electron microscope at an accelerating potential of 20 KV.

Enzyme and acid hydrolysed granules were prepared for SEM by rapidly freezing in liquid nitrogen and freeze drying at -80°C. The dried samples were prepared for viewing as described above.

3.9.7 Acid hydrolysis

The starches were hydrolysed with 2.2 N HCl at 35°C (1.0 g starch/40 ml acid) for 20 days. The starch slurries were shaken by hand daily to resuspend the deposited granules. At specified time intervals, aliquots (1.0 ml) of the reaction mixtures were neutralized and centrifuged (3500 rev/min, for 10 min.) and the supernatant liquid was assayed for total carbohydrate (Bruner, 1964). Controls without acid but subjected to the above experimental conditions were run concurrently. The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial dry starch.

3.9.8 Enzymatic Hydrolysis

Enzymatic digestion studies on starches were carried out using crystalline porcine pancreatic α-amylase in 0.5 M NaCl containing 3 mM $CaCl_2$ (Sigma Chemical Co., St. Louis, MO), in which the concentration of α-amylase was 23.9 mg ml^{-1}, and the specific activity was 1240 units per milligram of protein. One unit was defined as the α-amylase activity which liberated 1 mg maltose in 3 min at 20°C and pH 6.9.

The procedure used was essentially that of Knutson *et al.* (1982). However, a higher concentration of enzyme was used in this study. Starch granules (100 mg) were 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 0.1M phosphate buffer (pH=6.9) containing 0.006 M NaCl were added to the slurry. The mixture was gently stirred before adding 4 μl α-amylase suspension. The reaction mixtures were shaken by hand daily to resuspend the deposited granules. Then 1.0 ml aliquots were removed at specified time intervals, pipetted into 0.2 ml of 95% ethanol, and centrifuged. 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.

3.9.9 Retrogradation

Changes in gel texture and thermal characteristics, during storage were monitored by a "Lloyd" texture testing machine and differential scanning calorimetry respectively, at various time intervals (1-20 days).

3.9.9.1 Starch gel preparation

Gels (40% w/v) were prepared as described by Krusi and Neukom (1984). Native, defatted, heat-moisture treated and annealed starch samples (4 g db) were carefully weighed into circular aluminum moulds with removable tops and bases and mixed with distilled water (10 ml) containing 0.02% $Na_2S_2O_3$ (as preservative). The moulds were tightly closed and the contents were thoroughly mixed for 30 min in a wrist action shaker before being heated in a water bath at 95°C for 30 min. The resulting gels were allowed to cool within the moulds for 30 min at 4°C, prior to storage at 25°C.

3.9.9.2 Thermal characteristics of the gel

Fusion of retrograded amylopectin was studied on a differential scanning calorimeter, as described under section 3.7.2.

3.9.9.3 Gel texture measurement

The resistance to penetration of the gel, after 1 day of storage (at 25°C), was determined with a model 6000 R Lloyd texture testing machine (Omnitronix Instruments Ltd., Mississauga, Ontario) equipped with a data acquisition and processing station. The 5 and 50 N load cells were used. The gels within the aluminum moulds were placed on the compression table. The cross head of the machine, fitted with an appropriate load cell and a cylindrical probe (5 mm diameter) was driven down so as to just touch the gel surface. The probe was then driven at a constant speed (0.5 mm/min.) into the gel for a distance of 6 mm. The load at 1 mm compression was termed firmness. The resulting readings were in units of load grams.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 PHYSICAL CHARACTERISTICS OF STARCHES

The physical characteristics of the starches, used in this study, are summarized in Table 4.1. The nitrogen content of isolated starches represents the contributions from endosperm storage proteins, lipids that contain choline, ethanolamine and serine, and proteins located inside the starch granules (Morrison, 1981). The nitrogen content of the purified starches were in the range 0.01-0.04% (db), indicating the absence of endosperm protein and by implication, most of the non starch lipids (Morrison, 1981). Starch damage has been shown to occur during isolation of cereal starches (Evers *et al.*, 1984). The observed values for starch damage were within the maximum acceptable limit (1.5%). The difference between the total and apparent amylose content provides information about the amount of amylose complexed by native lipids. The amount of complexed amylose followed the descending order: wheat > oat > lentil > potato. The granule surface of all starches appeared to be smooth with no evidence of cracks or damages. Oat starch granules were found to exist in clusters (Fig. 4.1) and they ranged from polygonal to irregular in shape.

Table 4.1. Physical characteristics of some cereal, legume and tuber starches[a]

Starch source	Moisture (%)	Nitrogen (%)	Starch damage (%)	Amylose content (%)		Lipids (mg/100g dry starch)		
				Apparent	Total	Acid hydrolysed[b]	Solvent extracted	
							CM[c]	PW[d]
Wheat	10.7±0.1	0.04±0.01	0.8±0.06	21.1±0.4	27.3±0.2	704.0±5.8	36.0±1.1	639.9±8.8
Oat	10.2±0.4	0.05±0.02	1.3±0.05	16.7±0.2	19.4±0.3	1120.0±7.5	70.0±0.9	1050.0±5.9
Lentil	10.9±0.3	0.02±0.01	0.6±0.10	36.7±0.5	38.9±0.6	136.0±3.2	30.0±0.5	105.8±2.5
Potato	14.5±0.2	0.03±0.01	1.2±0.08	21.9±0.4	23.0±0.4	107.0±2.1	17.3±0.5	89.3±1.7
Yam	10.5±0.3	0.07±0.02	1.1±0.10	26.5±0.3	27.1±0.2	132.0±1.9	28.8±0.9	103.1±3.2

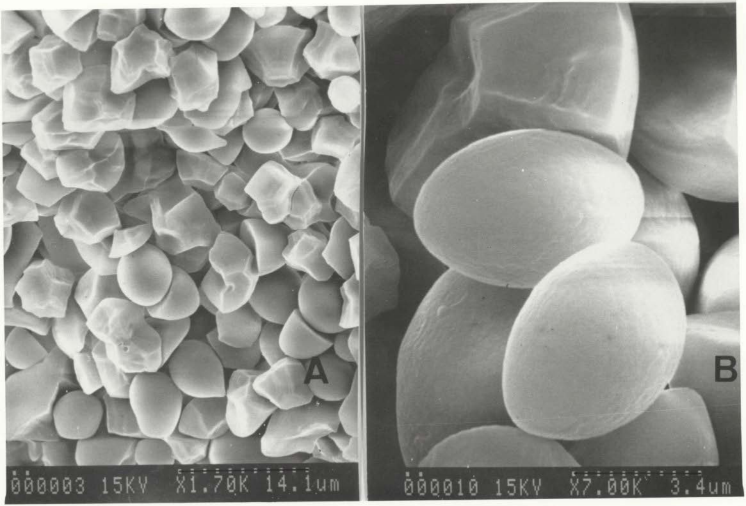
[a] Values are averages of three determinations.

[b] Lipids obtained by acid hydrolysis (24% HCl) of native starches at 70-80°C for 30 min.

[c] Lipids extracted by chloroform-methanol (2:1, v/v) at 25-27°C.

[d] Lipids extracted by propanol-water (3:1, v/v) at 90-100°C from the residue left after CM extraction.

Fig. 4.1. Scanning electron photomicrographs of native oat starch granules: (A) 1700×; (B) 7000×.


A
000003 15KV X1.70K 14.1um
B
000010 15KV X7.00K 3.4um

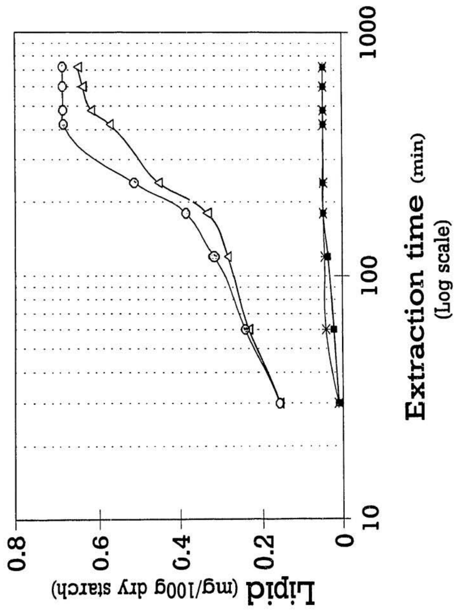
4.2 A COMPARATIVE STUDY OF THE COMPOSITION OF LIPIDS ASSOCIATED WITH STARCH GRANULES FROM VARIOUS BOTANICAL SOURCES

4.2.1 The lipid extracting ability of various solvent systems

The lipid extracting ability of solvent systems at ambient and elevated temperatures (Fig. 4.2) was determined on wheat starch due to its high lysophospholipid (LPL) content (Hargin and Morrison, 1980). LPL occurs in the starch granule mainly in the form of amylose-lipid complexes (Acker, 1977) and is thus not easily extractable by lipid solvents (Wren and Merryfield, 1970; Morrison and Coventry, 1985). Neither chloroform-methanol (CM) nor water saturated butanol (WSB) was capable of extracting significant amounts of lipids at 25°C (Fig. 4.2). At this temperature, the extraction ability of CM was slightly superior to that of WSB. However, higher yields were obtained (Fig. 4.2) when the residues from CM and WSB extractions (at 25-27°C) were subjected to lipid removal (in a Soxhlet apparatus) at 90-100°C by propanol-water (PW) and WSB, respectively. After 7 h, PW had extracted 16.2% more lipid than WSB (Fig. 4.2). Although there was hardly any increase in extracted lipid after 7 h with PW, the yield of lipid continued to increase with WSB, and reached a plateau after 12 h. However, this yield was still 4.3% less than that obtained with PW during this same time period (Fig. 4.2). Thus due to their superior extraction abilities, CM and PW were used at ambient and elevated temperatures, respectively, to characterize the starch lipids.

Fig. 4.2. Solvent extraction of lipids from wheat starch.

—✳—✳— CM extraction at 25°C;
—■— ■— WSB extraction at 25°C;
—●—●— PW extraction at 100°C;
—Δ—Δ— WSB extraction at 100°C.


Lipid (mg/100g dry starch)
0.8
0.6
0.4
0.2
0
10
100
1000
Extraction time (min)
(Log scale)

4.2.2 Total starch lipids (TSL)

The total starch lipids obtained by acid hydrolysis are presented in Table 4.1. They ranged from 0.1% (potato) to 1.1% (oat).

4.2.3 Solvent extracted starch lipids (SEL)

SEL refers to the starch lipids obtained by the combined action of CM and PW. The sequential use of these two solvents resulted in complete removal of starch lipids from most of the starches, the exception being wheat, where the total solvent extraction efficiency was 96.3% (%TSL) (Table 4.1). Comparisons between results of this study and some of those cited are difficult because of differences in extraction procedures and solvents.

4.2.3.1 Chloroform-methanol (CM) extracted starch lipids

The lipid content in these extracts followed the order: lentil > potato > cereals (Table 4.1). The values (% TSL) were 22.1% (lentil), 16.2% (potato), 6.2% (oat) and 5.4% (wheat). Since the degree of penetration of CM into the granule interior and its capacity to extract bound lipids is negligible at ambient temperatures, the extracted lipids probably represent to a large extent the free surface lipids. However, since the granules were slightly damaged the probability of contamination with free internal lipids cannot be ruled out.

Table 4.2. Composition of neutral lipids associated with some cereal, legume and tuber starch granules

Starch source	Extraction method	Total neutral lipids[a] (mg/100g dry starch)	Neutral lipid composition[b] (mg/100g dry starch)					
			FFA[c]	MG	DG	TG	FS	SE
Wheat	CM[e]	19.9±0.4	8.5	0.3	1.7	5.8	3.2	0.4
	PW[f]	34.9±1.6	19.1	1.6	5.2	1.7	3.4	3.9
Oat	CM	55.9±1.3	22.2	8.7	5.6	8.9	7.8	2.7
	PW	434.0±3.8	273.4	tr[d]	25.6	134.5	tr	tr
Lentil	CM	20.0±0.5	tr	tr	2.8	tr	2.1	15.1
	PW	48.4±1.0	12.1	tr	5.3	8.5	10.7	11.8
Potato	CM	9.7±0.5	1.4	tr	tr	1.6	tr	6.7
	PW	39.5±1.2	3.2	tr	tr	7.1	9.2	tr

[a] Values are averagesof three determinations.

[b] Based on densitometric absorbance (average SD = 5% of the value).

[c] FFA, free fatty acid; MG, monoacylglycerol; DG, diacylglycerol; TG, triacylglycerol; FS, free sterol; SE, sterol ester.

[d] tr = trace (< 0.5% of total neutral lipids).

[e] Chloroform-methanol (2:1, v/v) at 25-27°C.

[f] n-Propanol-water (3:1, v/v) at 90-100°C from the residue left after CM extraction.

Table 4.3. Composition of glycolipids associated with some cereal, legume and tuber starch granules

Starch source	Extraction method	Total glycolipids[a] (mg/100g dry starch)	Glycolipid composition[b] (mg/100g dry starch)					
			MGMG[c]	MGDG	DGDG	DGMG	CEI	CEII
Wheat	CM[e]	9.9±0.5	tr[d]	tr	6.8	tr	tr	1.9
	PW[f]	19.2±1.2	3.8	1.7	5.6	7.8	tr	tr
Oat	CM	tr	tr	tr	tr	tr	tr	tr
	PW	200.3±4.8	5.6	10.6	166.6	tr	4.2	12.8
Lentil	CM	tr	tr	tr	tr	tr	tr	tr
	PW	30.4±1.5	tr	2.6	tr	15.8	tr	11.6
Potato	CM	tr	tr	tr	tr	tr	tr	tr
	PW	10.1±0.6	tr	tr	tr	7.2	tr	tr

[a] Values are averages of three determinations.

[b] Based on densitometric absorbance (average SD = 5% of the value).

[c] MGMG, monogalactosylmonoglyceride; MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; CE I, cerebroside I; CE II, cerebroside II.

[d] tr = trace (< 0.5% of total neutral lipids).

[e] Chloroform-methanol (2:1, v/v) at 25-27°C.

[f] n-Propanol-water (3:1, v/v) at 90-100°C from the residue left after CM extraction.

Table 4.4. Composition of phospholipids associated with some cereal, legume and tuber starch granules

Starch source	Extraction method	Total phospholipids[a] (mg/100g dry starch)	Phospholipid composition[b] (mg/100g dry starch)						
			LPC[c]	LPE	PC	PE	PS	PG	PA
Wheat	CM[e]	8.2±0.8	tr	tr	5.1	3.1	tr	tr	tr
	PW[f]	586.0±7.1	482.0	75.2	19.9	tr	tr	tr	9.0
Oat	CM	17.0±0.5	15.8	tr	1.2	tr	tr	tr	tr
	PW	413.0±6.3	291.2	54.9	21.5	36.7	4.1	4.5	tr
Lentil	CM	10.3±0.4	2.8	tr	4.6	tr	tr	tr	2.6
	PW	27.6±1.3	7.3	2.3	11.3	tr	tr	tr	6.5
Potato	CM	7.6±0.5	7.6	tr	tr	tr	tr	tr	tr
	PW	39.8±0.9	31.8	1.7	2.4	3.9	tr	tr	tr

[a] Values are averages of three determinations.

[b] Based on densitometric absorbance (average SD = 6% of the value).

[c] LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; PA, phosphatidic acid.

[d] tr = trace (< 0.5% of total neutral lipids).

[e] Chloroform-methanol (2:1, v/v) at 25-27°C.

[f] n-Propanol-water (3:1, v/v) at 90-100°C from the residue left after CM extraction.

Table 4.5. Fatty acid distribution of the major lipid classes in CM and PW extracts of some cereal, legume and tuber starches

Starch source	Lipid class and Extraction method	Fatty acid composition (area %)[a]						
		16:0	18:0	18:1	18:2	18:3	20:0	other[b]
Wheat	Neutral lipids							
	CM	43.1	5.7	6.9	40.3	2.6	1.1	0.3
	PW	45.6	6.7	7.1	37.8	1.3	0.9	0.6
	Glycolipids							
	CM	42.2	3.9	9.8	42.6	tr[c]	0.9	0.5
	PW	39.6	6.1	10.1	41.5	0.5	2.1	0.1
	Phospholipids							
	CM	46.7	3.5	10.4	38.7	tr	0.4	0.2
	PW	39.7	3.2	7.3	47.9	1.3	0.5	0.1
Oat	Neutral lipids							
	CM	35.3	3.6	11.7	40.9	8.2	tr	0.3
	PW	38.7	4.3	8.9	41.5	4.8	1.6	0.2
	Glycolipids							
	CM	tr	tr	tr	tr	tr	tr	tr
	PW	46.6	0.5	10.1	40.2	tr	21.1	0.5
	Phospholipids							
	CM	72.4	tr	2.9	13.8	10.2	tr	0.7
	PW	74.2	0.5	5.4	17.1	1.1	0.8	0.9

Continued

Table 4.5. continued

Lentil	Neutral lipids							
	CM	23.6	4.8	18.9	36.8	14.1	1.2	0.6
	PW	36.5	8.2	29.1	22.3	2.5	1.4	tr
	Glycolipids							
	CM	30.6	6.7	13.6	35.2	13.8	tr	0.1
	PW	42.9	5.8	26.6	17.7	3.6	2.9	0.5
	Phospholipids							
	CM	34.8	8.1	11.2	30.7	12.8	1.8	0.6
	PW	30.1	5.2	13.3	34.7	15.3	1.2	0.2
Potato	Neutral lipids							
	CM	36.1	7.3	28.8	14.4	12.5	tr	0.9
	PW	38.2	9.2	30.1	15.8	6.3	tr	0.4
	Glycolipids							
	CM	tr	tr	tr	tr	tr	tr	tr
	PW	39.1	6.2	25.8	18.2	9.2	0.8	0.7
	Phospholipids							
	CM	44.9	9.7	17.5	16.9	8.4	1.9	0.7
	PW	40.4	6.4	22.2	18.5	11.8	0.4	0.3

[a] Values are averages of three determinations.

[b] Includes 14:0 and 22:0.

[c] Trace = less than 0.1%.

The lipid classes generally followed the order neutral lipid (NL) > phospholipid (PL) > glycolipid (GL) (Table 4.2 - 4.4). The concentration (% SEL) of NL ranged from a low of 2.9% in wheat to a high of 14.7% in lentil (Table 4.2). The major NL (Table 4.2) fractions (% total NL) were free fatty acid (FFA) in wheat (42.7%) and oat (39.7%); and sterol esters (SE) in lentil (75.5%) and potato (69.0%) GL were not detected in oat, potato and lentil. However, its concentration (% SEL) in wheat was 1.5% (Table 4.3). The major GL (Table 4.3) fraction (% total GL) in wheat was digalactosyldiglyceride (DGDG) (68.6%). The PL concentration (% SEL) ranged from a low of 1.2% in wheat to a high of 7.4% in lentil (Table 4.4). The major PL (Table 4.4) fractions (% total PL) were phosphatidylcholine (PC) in wheat (62.1%) and lentil (46.0%); and lysophosphatidylcholine (LPC) in oat (92.9%) and potato (100%). The major fatty acid (Table 4.5) in NL fractions was palmitic (16:0) in wheat and potato; and linoleic (18:2) in oat and lentil, while in GL fractions it was 18:2 in lentil. However, in PL fractions all starches had 16:0 as their major fatty acid.

4.2.3.2 Propanol-water (PW) extracted starch lipids

Lipids obtained by extraction of the CM residues with hot PW are presented in Table 4.2 - 4.4. These lipids probably represent most of the free and bound lipids in the granule interior and some bound lipids that may have been present on the granule surface. They ranged (% TSL) from 77.9% (lentil) to 90.8% (wheat).

The lipid classes followed the order: PL > NL > GL in wheat; NL > PL > GL in oat; NL > GL > PL in potato; and NL = PL > GL in potato (Table 4.2-4.4). The neutral lipid content was found to vary widely even among starches belonging to the same species (Table 4.2). In cereal starches these values (%SEL) were respectively 5.1 and 38.8% in wheat and oat. The corresponding values for lentil and potato were respectively 35.6% and 37.0%. The major NL (Table 4.2) fractions (% total NL) were FFA in wheat (54.7%), oat (63.0%) and lentil (25.0%); and free sterol (FS) in potato (23.3%). The GL (Table 4.3) contents also differed widely among cereal starches. The values (%SEL) were 2.7% (wheat) and 17.8% (oat). The corresponding values for lentil and potato were respectively 22.1% and 9.3%. The major GL (Table 4.3) fractions (% total GL) were digalactosylmonoglyceride (DGMG) in wheat (41.2%), lentil (52.6%) and potato (72.0%); and DGDG in oat (83.3%). Cereal starches also exhibited wide variations in their PL levels (Table 4.4). The values (%SEL) were 86.4% (wheat) and 36.8% (oat). These variations were similar to those seen in NL and GL fractions. The corresponding PL values for lentil and potato were 20.2% and 37.3%. The major PL (Table 4.4) fractions (% total PL) were lysophosphatidylcholine (LPC) in wheat (82.2%), oat (70.5%) and potato (79.8%); and phosphatidylcholine (PC) in lentil (41.2%). The major fatty acids in NL, GL and PL fractions of all starches were identical to those observed in CM extracts (Table 4.5). However, there were slight variations in their area percentages.

The monoacyl lipid content in both CM and PW extracts of wheat and oat amounted to, respectively 88.2% and 60.0%. The value for wheat is in general agreement with that reported by Hargin and Morrison (1980). The corresponding values for lentil and potato starches were respectively 29.5% and 49.6%. Since it is the monoacyl lipids that interact most strongly with the amylose helix, their removal by defatting would therefore result in greater functionality changes in starches.

4.3 EFFECT OF DEFATTING ON STARCH STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES

4.3.1 Lipid (dimodan) complexing study

The total amylose content and the apparent amylose content of native, and dimodan-complexed starches are presented in Table 4.6. A comparison of the apparent and total amylose gives an indication of the proportion of amylose complexed with lipid. In the case of native starches, the proportion of amylose complexed with naturally occurring lipids amounted to 22.7, 14.0, 11.3 and 5.6% in wheat, oat, potato and lentil respectively.

The amount of amylose complexed by native lipids and dimodan was 31.5% (wheat) and 21.1% (oat). The above values did not change significantly on the addition of dimodan to lipid-free wheat (33.3%) and oat (19.6%) starches. However, additional amylose complexation [(native complexed - defatted complexed)/total] was evident in defatted lentil (12.3%) and potato (13.5%) starches (Table 4.6).

4.3.2 X-Ray diffraction

The X-ray diffraction patterns and the relative crystallinity (RC) of native and defatted starches are shown in Fig 4.3. Native potato starch exhibited the typical "B" pattern of tuber starches, with peaks at 16.7, 5.9, 5.2, 4.0 and 3.7 Å. Defatting resulted in an increase in RC (21%) and the disappearance of the peaks at 16.7 and 5.9 Å (Fig 4.3 E,F). The X-ray pattern changed to a

Table 4.6. Amylose content of native and dimodan complexed starches

Starch source	Treatment	Amylose content[a] (% of total starch)	
		Apparent[b]	Total[c]
Wheat	Native	21.1±0.4	27.3±0.2
	Native complexed[d]	18.7±0.6	
	Defatted[e] complexed	18.2±0.2	
Oat	Native	16.7±0.2	19.4±0.3
	Native complexed	15.3±0.5	
	Defatted complexed	15.6±0.1	
Lentil	Native	36.7±0.5	38.9±0.6
	Native complexed	25.3±0.3	
	Defatted complexed	20.5±0.6	
Potato	Native	21.9±0.4	23.0±0.4
	Native complexed	10.7±0.2	
	Defatted complexed	7.6±0.5	

[a] Values are means of three replicates ± SD.

[b] Apparent amylose content was determined by iodine binding without removal of total native starch lipids and/or dimodan.

[c] Total amylose was determined by iodine binding after removal of total starch lipids with hot n-propanol/water (3:1, v/v).

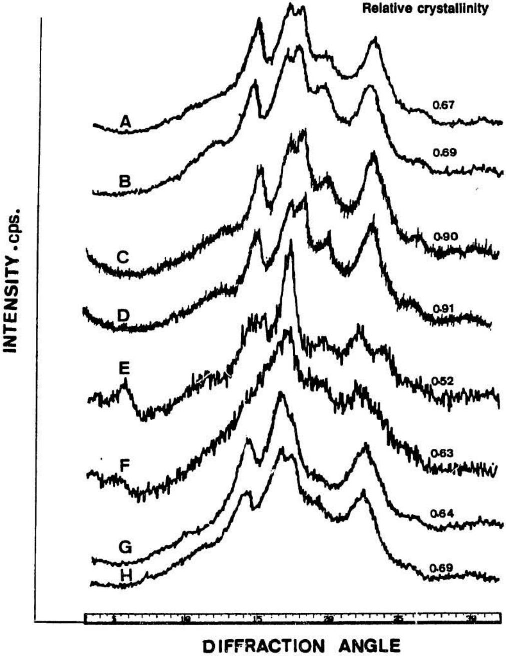
[d] Complexed with 5% dimodan starch dry weight basis. Non-interacting dimodan was removed with chloroform/methanol (2:1, v/v) at 25 C.

[e] Soxhlet extraction with hot n-propanol/water (3:1, v/v) for 7 h.

Fig 4.3. X-ray diffraction patterns and relative crystallinities of native and PW-defatted starches.

(A) native wheat; (B) defatted wheat;
(C) native oat; (D) defatted oat;
(E) native potato; (F) defatted potato;
(G) native lentil; (H) defatted lentil.

See Appendix 1 for relative crystallinity calculation.
cps = counts per second.


Relative crystallinity
A
0.67
B
0.69
C
0.90
D
0.91
E
0.52
F
0.63
G
0.64
H
0.69
INTENSITY . cps.
5
10
15
20
25
30
DIFFRACTION ANGLE

combination of one half "A" and one half "B" pattern, indicating that a rearrangement in the double helical packing of the native crystals may have occurred during defatting, resulting in the formation of a close-packed arrangement of double helices (Gidley, 1987). A similar transformation has been observed in heat-moisture treated potato starch (Donovan *et al.*, 1983).

The X-ray patterns of native and defatted wheat and oat starches were similar (A-type) showing spacings at 5.9, 5.2 and 3.8 Å. Both these starches showed no significant changes in their RC on defatting (Fig. 4.3). The "C"-type X-ray pattern was seen in both native and defatted lentil starches (Fig. 4.3). However, the RC increased by 7.8% on defatting (Fig. 4.3).

Long range molecular ordering (crystallinity) in starch granules has been attributed to regular packing of double helices formed from adjacent clusters of the short DP chains of amylopectin (French, 1984). It is therefore likely, that starches containing amylose chains entrapped between adjacent chain clusters of amylopectin would exhibit a lower degree of long range molecular order (by preventing close association of adjacent amylopectin chains) than those in which amylose and amylopectin chains are well separated within the granule. The unchanged X-ray pattern and the very low increase in intensities seen in defatted starches of wheat, oat and lentil indicates the degree of separation of amylose and amylopectin in the native granules to be in decreasing order: wheat - oat > lentil > potato.

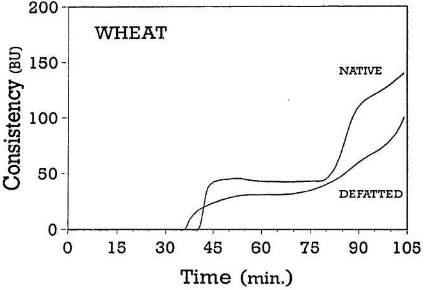
The additional amylose complexing (Table 4.6) and the increases in RC (Fig. 4.3) observed in defatted lentil and potato starches seem to suggest that the moisture and thermal energy that exist during refluxing increases the mobility of entrapped amylose chains, resulting in their release into the amorphous region of the granule. The discrepancy between this study (+7.8%, ▲RC) and that of Lorenz and Kulp (1983) (-1.7%, ▲RC) with regard to changes in potato starch crystallinity on defatting, is possibly due to differences in the composition of the extracting solvent systems. The authors' results showed that both propanol-water (PW) and 80% methanol extracted the same quantity of lipid from potato starch. However, the maximum temperature experienced by potato starch granules during refluxing with PW and 80% methanol for 7 h was 82 and 67°C, respectively. This would then explain the low degree of structural change observed by Lorenz and Kulp (1983), who defatted potato starch by refluxing with 80% methanol for 48 h. It is likely that at 67°C the thermal energy may have been insufficient to cause the release of entrapped amylose chains into the amorphous regions.

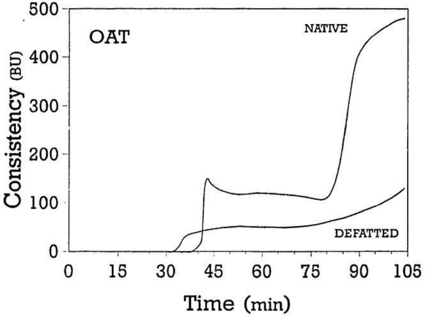
4.3.3 Pasting curves

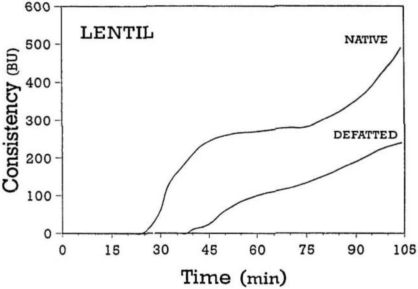
Removal of lipids decreased the pasting temperatures of wheat (Fig. 4.4.A) and oat (Fig. 4.4.B) by 3 and 5°C, respectively and increased those of lentil (Fig. 4.4.C) and potato (Fig. 4.4.D) by 21 and 26°C, respectively. Defatting also eliminated the pasting peak of wheat and oat starches (Figs.

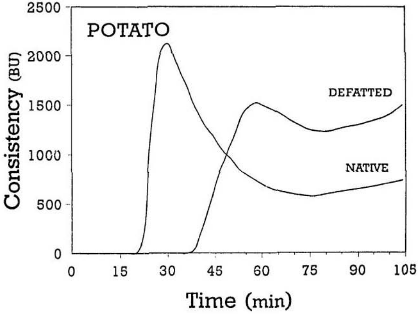
Fig. 4.4. Brabender amylograms of native and PW-defatted starches

BU = Brabender Units.


WHEAT
NATIVE
DEFATTED
Consistency (BU)
200
150
100
50
0
0
15
30
45
60
75
90
105
Time (min.)


OAT
NATIVE
DEFATTED
Consistency (BU)
500
400
300
200
100
0
0
15
30
45
60
75
90
105
Time (min)

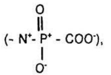

LENTIL
NATIVE
DEFATTED
Consistency (BU)
600
500
400
300
200
100
0
0
15
30
45
60
75
90
105
Time (min)


POTATO
DEFATTED
NATIVE
Consistency (BU)
2500
2000
1500
1000
500
0
0
15
30
45
60
75
90
105
Time (min)

4.4.A,B), increased the thermal stability and reduced the hot paste consistencies of all starches (Figs. 4.4.A-D). The magnitude of the changes in thermal stabilities and hot paste consistencies decreased in the order: potato > lentil > oat > wheat. The viscosity profile of defatted lentil and potato starches during the holding cycle at 96°C (Figs. 4.4.C,D) resembled that of cross-linked starches. The results suggest, that in potato and lentil starches, the interaction between amylopectin chain clusters during defatting may have been the main causative factor responsible for the large increase in pasting temperature, increased thermal stability and reduced hot paste consistency.

Native oat and wheat starches showed wide differences in peak viscosity and set back. These values were higher in oat. However, both starches showed identical pasting temperatures (Figs. 4.4.A and 4.4.B). Similar observations have been made by MacArthur and D'Appolonia (1979) on starch from three varieties of oat (Dal, Froker, Cayuse). The difference in peak viscosity can be attributed to higher quantities of amylose bound lipids in wheat starch. It is likely that in wheat and oat, the observed changes in pasting curves on defatting may reflect mainly the amount of bound lipids removed. This seems plausible, since the changes in pasting curves were greater in defatted oat than in wheat, probably due to the presence of solvent unextractable bound lipids still remaining within the granules of the latter (Table 4.1).

The set-back (viscosity at 50°C) is normally associated with the extent of retrogradation. The set-back of native and defatted starch gels was greater in oat than in wheat. This was rather surprising since oat starch is known to retrograde slowly due to co-leaching of both starch components during the pasting process (Doublier, 1987). Recently, Hansen *et al.* (1991), showed by means of oscillatory rheometry measurements that corn starch gelation is hindered in the presence of amylopectin. Furthermore, Miles *et al.* (1985), postulated that amylose gelation requires network formation, and network formation requires polymer chain entanglement. Therefore, the low rigidity of oat starch gels could be attributed to interaction between the exudates (branched component and amylose chains) in the continuous phase. Most likely this interaction restricts the ability of amylose chains to form a strong network by latteral association of double helical junction zones. Thus set-back values probably reflect the extent of water immobilization around the charged centres of starch components ($O^{\delta -}$-$H^{\delta +}$) and those of free and helically complexed lipid molecules



$$(-\,N^{+}-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^{-}}{|}}{P^{+}}}-\,COO^{-}),$$

rather than starch paste retrogradation. The association of water molecules with these charged centres would decrease the effective water concentration in the continuous phase, resulting in a rise in viscosity during the cooling cycle. The decreased set-back on lipid removal might thus be explained. Among native starches, the extent of water immobilization is higher in oat starch (high set back), due to the presence of more charged centres (provided by the leached branched component) in the continuous phase.

The amylogram of defatted wheat starch is in general agreement with those of Takahashi and Seib (1988), but differ from those of Lorenz (1976) and Melvin (1979). The amylograms of defatted potato starch differed from those reported by Goshima *et al.* (1985) and Lorenz and Kulp (1983). The author's results seem to indicate that discrepancies in the literature with respect to changes in rheological properties on lipid removal are due to differences in the maximum temperatures experienced by starch granules during lipid removal, and in the nature and composition of the extracting solvent system.

4.3.4 Swelling factor and amylose leaching

Swelling factor (SF) and amylose leaching (AML) at different temperatures are presented in Table 4.7 and 4.8 respectively. Both SF and AML of native and defatted starches increased with rise in temperature. The SF of wheat, potato and lentil starches decreased on defatting. However, in oat starch, defatting increased the SF at 50°C, but decreased at temperatures above 80°C (Table 4.7). Defatting also decreased the extent of AML in potato and lentil starches and increased those of wheat and oat (Table 4.8). The

Table 4.7 Swelling factor of native and defatted starches

Starch source	Treatment	Swelling factor[a]			
		Temperature (°C)			
		50°	70°	80°	95°
Wheat	Native	6.0±0.6	14.4±0.2	21.5±0.6	27.6±0.3
	Defatted[b]	4.9±0.5	12.7±0.1	16.2±0.4	25.0±0.2
Oat	Native	1.9±0.3	11.3±0.6	19.8±0.5	25.7±0.1
	Defatted	3.2±0.7	11.4±0.2	17.1±0.4	22.7±0.4
Lentil	Native	6.9±0.5	14.7±0.7	19.5±0.6	24.4±1.0
	Defatted	1.6±0.1	5.4±0.1	7.3±0.2	16.1±0.9
Potato	Native	10.6±1.1	31.5±1.5	50.5±1.3	93.1±1.8
	Defatted	4.7±0.4	12.3±0.6	18.3±0.8	29.9±1.3

[a] Values are means of three replicates ± SD.

[b] Soxhlet extraction with hot n-propanol/water (3:1, v/v) for 7 h.

% Reduction = $(SF_{native} - SF_{defatted})/SF_{native}$.

Table 4.8 Amylose leaching of *native* and *defatted* starches

Starch source	Treatment	Amylose leaching (% db)[a]			
		Temperature (°C)			
		50°	70°	80°	95°
Wheat	Native	0	4.3±0.1	7.1±0.2	10.9±0.4
	Defatted[b]	0.4±0.1	6.6±0.4	8.6±0.1	12.1±0.2
Oat	Native	0	4.9±0.2	7.8±0.1	12.3±0.3
	Defatted	1.8±0.3	10.6±0.5	13.3±0.5	14.4±0.1
Lentil	Native	0.4±0.1	18.7±0.3	24.6±0.8	29.4±0.8
	Defatted	0	14.6±0.5	17.8±1.2	20.6±1.1
Potato	Native	0.9±0.1	12.8±0.2	15.7±0.5	18.3±0.7
	Defatted	0.2±0.1	4.4±0.1	6.3±0.2	10.8±0.3

[a] Values are means of three replicates ± SD.

[b] Soxhlet extraction with hot n-propanol/water (3:1, v/v) for 7 h.

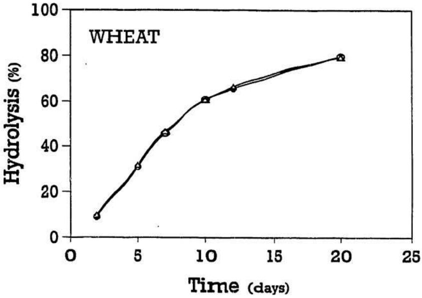
% Reduction = $(AML_{native} - AML_{defatted})/AML_{native}$.

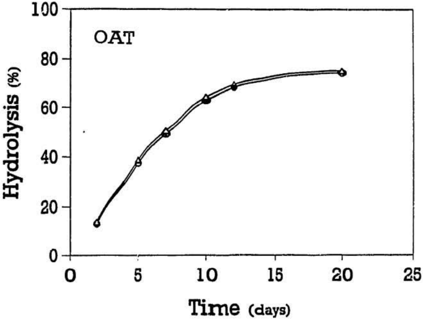
results seem to suggest that decrease in SF on defatting is indicative of increased granular stability arising from interaction between amylopectin chain clusters, the magnitude of this interaction being strongest in potato starch and weakest in cereal starches. The increase in granular stability is also responsible for the decreased AML seen in defatted potato and lentil starches. However, in cereal starches, the increased AML probably represents to a larger extent the removal of bound lipids. The discrepancies between these findings and those of Lorenz and Kulp (1983), Goshima *et al.* (1985) and Tester and Morrison (1990a) may have been due to the same differences as outlined in the previous section and to a lesser extent to differences in the methodology employed for measuring the extent of swelling. Lorenz and Kulp (1983) and Goshima *et al.* (1985) used the method adopted by Leach *et al.* (1959) for measurement of swelling factor, which does not distinguish between intragranular water and intergranular or interstitial water. However, the method (Tester and Morrison, 1990a) used in this study measures only the intragranular water and hence the true swelling factor at a given temperature is measured.

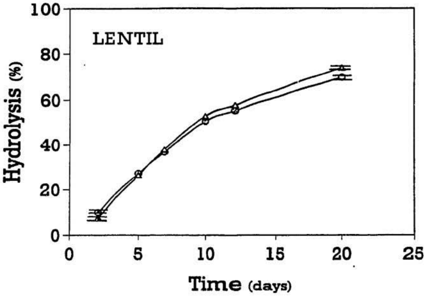
4.3.5 Acid hydrolysis

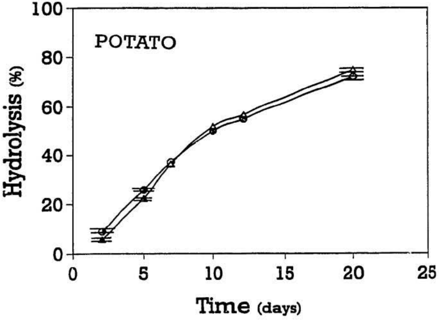
The solubilization patterns of native and defatted starches are shown in Fig. 4.5.A-D (values are presented in Appendix 3). All starches exhibited a two-stage hydrolysis pattern. A relatively faster rate of hydrolysis was observed

Fig. 4.5. Heterogeneous hydrolysis of native (—Δ—Δ—) and PW-defatted (—●—●—) starches in 2.2 N HCl at 35°C.


WHEAT
Hydrolysis (%)
0
20
40
60
80
100
0
5
10
15
20
25
Time (days)


OAT
Hydrolysis (%)
0
20
40
60
80
100
0
5
10
15
20
25
Time (days)


LENTIL
Hydrolysis (%)
0
20
40
60
80
100
0
5
10
15
20
25
Time (days)


POTATO
Hydrolysis (%)
0
20
40
60
80
100
0
5
10
15
20
25
Time (days)

during the first nine days of hydrolysis followed by a slower rate between days 9 and 12. Similar hydrolysis patterns have been reported for native corn, waxy corn, wheat, potato, cassava, rice, lima bean, mung bean, lentil, and wrinkled pea starches (Robin *et al.*, 1974; Biliaderis *et al.*, 1981b; Nara *et al.*, 1983; Komiya *et al.*, 1987; Hoover and Sosulski, 1991). The faster hydrolysis rate during the initial eight days has been shown to correspond to the attack on the amorphous regions of the starch granule. During the second stage the crystalline region is slowly degraded (Robin *et al.*, 1974; French, 1984). Lipid removal from wheat and oat did not cause any significant changes in the extent of hydrolysis (Fig. 4.5.A,B). This seems to indicate that native lipids complexed with amylose do not render the amorphous regions of the starch granule resistant to degradation by cold aqueous acid. Lentil and potato starches, however, showed increased rates of hydrolysis on defatting (Figs. 4.5.C and 4.5.D). This increase was higher in potato. The higher rate of hydrolysis was evident for the first five days in defatted lentil starch (Fig. 4.5.C) and the first seven days in defatted potato starch (Fig. 4.5.D). Thereafter, the rate of hydrolysis was lower than those of their native counterparts. The higher extent of degradation seen in defatted starches of lentil and potato during the first five and seven days of hydrolysis, respectively, is indicative of hydrolysis of released amylose chains that were originally a part of the amylopectin structure (in the native granule). Once the released amylose chains are hydrolyzed the effect of increased crystalline order becomes evident.

4.3.6 In-vitro digestibility of native and defatted starches by porcine pancreatic α-amylase

The extent of α-amylase hydrolysis (24 h) of native and defatted starches is presented in Table 4.9. Defatting increased the extent of hydrolysis in all starches. The percentage increase (with respect to native starch hydrolysis) followed the decreasing order: potato > lentil > oat > wheat. This seems to suggest that defatting increases the accessibility of amylose chains to α-amylase. In wheat and oat starches the increase in hydrolysis could be attributed to a change in amylose conformation (V-helix – random coil) upon lipid removal, with the result a larger surface area becomes available for enzyme action. Previous studies have shown that amylose/lipid complexes show reduced susceptibility to α-amylase digestion (Larsson and Meizis, 1979; Holm *et al.*, 1983; Seneviratne and Biliaderis, 1991). However, in potato and lentil starches, a change in amylose conformation is less likely (since these starches have been shown to contain very small quantities of complexed lipids (Table 4.1). Therefore, it seems plausible that in potato and lentil starches the increase reflects the action of α-amylase on the released amylose chains.

Table 4.9 In-vitro hydrolysis[a] of native and defatted starches by porcine pancreatic α-amylase

Starch source	Treatment	Hydrolysis[b] (%)	% Increase
Wheat	Native Defatted[c]	41.9±1.8 72.4±1.1	72.7
Oat	Native Defatted	24.6±1.5 57.2±1.2	132.5
Lentil	Native Defatted	19.2±0.9 53.3±1.7	177.6
Potato	Native Defatted	4.9±0.6 20.1±1.0	310.2

[a] Hydrolysis for 24 h in phosphate buffer (pH=6.9).

[b] Values are means of three replicates ± SD.

[c] Soxhlet extraction with hot n-propanol/water (3:1, v/v) for 7 h.

4.3.7 Gelatinization

The gelatinization parameters of native and defatted starches are presented in Table 4.10. Defatting slightly decreased the gelatinization temperatures (T_o, T_p and T_c) of wheat and oat. This may be due to lipid removal which facilitates easy hydration of starch granules during gelatinization. However, defatted starch granules from potato and lentil gelatinized over a broader and higher temperature range than did the corresponding untreated control starches (Table 4.10). The final gelatinization temperature of lentil starch increased on defatting from 67.5 to 75.1°C, while that of potato increased from 64.5 to 72.0°C. The range over which the starch granules gelatinized increased from 12.5 to 14.8°C in lentil and from 10.5 to 12.8°C in potato starch. These increases are an indication of increased order within the defatted starch granules of lentil and potato. Takahashi and Seib (1988), showed that extraction of wheat and corn starches with 75% ethanol did not cause any significant change in their gelatinization temperatures. Similar observations were reported by Lorenz and Kulp (1983) and Goshima *et al.* (1985) who used respectively, 80 and 99% methanol to defat potato starch. Biliaderis and Tonogai (1991), reported that the gelatinization temperature of rice starch decreased on extraction with 85% methanol. Differences in extracting solvent systems are mainly responsible for these discrepancies. The gelatinization temperatures of dimodan complexed native

Table 4.10 Gelatinization temperatures and enthalpy of native and defatted starches

Starch source	Treatment	Transition points[a] (°C)			Enthalpy[a] (J/g db)
		T_o	T_p	T_c	
Wheat	Native	56.2±0.4	61.1±0.2	66.6±0.4	9.6±0.4
	Defatted	55.1±0.3	60.0±0.4	65.2±0.4	10.1±0.5
	Native complexed[b]	64.0±0.5	66.0±0.3	69.1±0.3	9.2±0.4
	Defatted[c] complexed	64.7±0.2	66.5±0.2	69.8±0.2	9.7±0.2
Oat	Native	60.2±0.2	64.1±0.1	70.0±0.4	10.1±0.4
	Defatted	58.1±0.3	63.0±0.2	69.2±0.5	10.7±0.3
	Native complexed	65.2±0.5	68.8±0.1	74.6±0.5	9.8±0.5
	Defatted complexed	65.9±0.2	68.0±0.4	75.1±0.2	10.5±0.4
Lentil	Native	55.0±0.1	60.6±0.5	67.5±0.3	7.7±0.4
	Defatted	60.3±0.2	67.0±0.4	75.1±0.2	8.0±0.3
	Native complexed	63.0±0.3	67.0±0.5	71.0±0.3	8.0±0.3
	Defatted complexed	68.0±0.4	73.1±0.3	79.0±0.4	7.6±0.2
Potato	Native	54.0±0.1	58.8±0.2	64.5±0.3	16.4±0.5
	Defatted	59.2±0.5	65.0±0.3	72.0±0.5	15.9±0.2
	Native complexed	58.0±0.4	62.0±0.2	67.0±0.5	16.4±0.4
	Defatted complexed	60.0±0.3	67.0±0.2	75.0±0.3	15.7±0.5

[a] Values are means of three replicates ± SD.

[b] Complexed with 5% dimodan starch dry weight basis. Non-interacting dimodan was removed with chloroform/methanol (2:1, v/v) at 25°C.

[c] Soxhlet extraction with hot n-propanol/water (3:1, v/v) for 7 h.

and defatted starches of wheat and oat (Table 4.10) were fairly close. However, those of potato and lentil were widely different, with defatted complexed starches exhibiting higher gelatinization temperatures than native complexed starches. This is indicative of increased (12-13.5% increase) amylose complexing (Table 4.6) (due to release of amylose chains) in defatted starches of potato and lentil.

4.3.8 Retrogradation

The thermal characteristics of retrograded amylopectin was studied by differential scanning calorimetry. The transition temperatures (T_o, T_p, T_c) and the enthalpy (ΔH_R), representing the fusion of retrograded amylopectin (of native and defatted starches) at various time intervals (0.5 h to 20 days) of storage are presented in Table 4.11. As seen in Table 4.10, T_o, T_p and T_c of the gelatinization endotherm of native starches were different from those of defatted starches. The reasons for this have been discussed above.

The retrogradation endotherm appeared after 3 days of storage (25°C) in native wheat, potato and lentil starch gels, whereas, for native oat starch gel, the corresponding time was 15 days (Table 4.11). Defatting did not hasten or delay the onset time of the retrogradation endotherm in wheat, potato and lentil starches. However, the retrogradation endotherm appeared after 3 days storage in defatted oat starch. The differences in T_o, T_p and T_c of the retrogradation endotherm of native and defatted starch gels were only marginal

Table 4.11 Thermal characteristics of native and defatted starch gels[a]

Starch source	Enthalpy[b] (ΔH) (J/g) Storage time (days)							Transition temperature[c] (°C)		
	2	3	4	6	8	15	20	To	Tp	Tc
Wheat										
Native	-	0.9	1.9	3.9	5.3	7.1	7.1	47.5	57.2	60.7
Defatted[d]	-	2.6	4.0	5.3	7.3	11.0	11.8	47.1	57.9	60.4
Oat										
Native	-	-	-	-	-	1.3	1.9	46.8	57.6	60.9
Defatted[d]	-	1.0	1.8	2.5	2.7	3.8	5.5	47.1	57.9	60.4
Lentil										
Native	-	0.6	1.3	2.5	3.4	6.0	8.4	47.1	57.9	62.8
Defatted[d]	-	1.2	2.5	3.8	4.3	8.4	10.1	47.4	57.9	63.2
Potato										
Native	-	1.0	2.4	6.0	7.5	9.8	11.2	50.6	57.2	69.2
Defatted[d]	-	1.8	3.8	8.0	10.0	12.6	13.4	50.3	58.4	69.5

[a] Values are averagesof three determinations.
[b] Average standard deviation = 4% of the value.
[c] Average standard deviation = 0.5°C.
[d] Hot n-propanol-water (3:1 v/v). for 7 h.

and remained practically unchanged during the time course of retrogradation. Furthermore T_o, T_p and T_c of the retrograded starch gels were lower than that for the gelatinization endotherm (Table 4.10). In native and defatted starch gels, the transition temperature range (Tc - To) of the retrogradation endotherm was broader (Table 4.11) than that of the gelatinization endotherm (Table 4.10). This was most pronounced in potato starch. Unlike T_o, T_p and T_c, the enthalpy of the retrogradation endotherm (ΔH_R) was greatly influenced by defatting. After 20 days of storage, the ΔH_R (J/g) of gels from native starches were 7.1 (wheat), 1.9 (oat), 11.2 (potato) and 8.4 (lentil). These values increased respectively by 4.7, 3.6, 2.2 and 1.7 J/g on defatting.

In wheat, potato and lentil starches the chain length of the outer branches of amylopectin have been reported to be respectively, 17 (Hizukuri, 1985), 20 (Hizukuri, 1985) and 16 (Biliaderis *et al.*, 1981a). Whereas amylose chain lengths are: respectively, 570 (wheat) (Takeda *et al.*, 1984), 4920 (potato) (Hizukuri and Takagi, 1984) and 1400 (lentil) (Biliaderis *et al.*, 1981a). Corresponding values for oat starch components have not been reported in the literature. In potato and lentil starches amylose and amylopectin are intermingled within the granule, while in cereal starches they are present in separate domains. Thus, during starch chain reassociation (within the gelatinized granule), interactions between amylose and amylopectin are theoretically more likely to occur to a larger extent in potato and lentil, than in wheat and oat starches. Since in all four starches, the transition temperature range (Tc - To) for retrogradation (Table 4.11) was broader than that for gelatinization (Table 4.10), it implies, that the retrogradation endotherm reflects melting of crystallites formed by different types of starch chain associations (amylose - amylopectin and amylopectin - amylopectin) during gel storage. Whereas the gelatinization endotherm represents mainly melting of crystallites formed by associations (formed during granule development) among or between amylopectin chains. It is likely that the bonding forces within crystallites of retrograded starches are weaker (due to improper alignment of the reassociating starch chains) than those in crystallites of native granules. This is based on the observation that transition temperatures of retrograded starches (Table 4.11) are lower than those of gelatinized starches (Table 4.10). The gradual increase in ΔH_R during storage of native and defatted starch gels suggests an increase in double helical content.

Interaction between native lipids and the starch components, and their implication on starch retrogradation have been discussed earlier. Complex formation between amylose and native lipids have been shown to occur during gelatinization (Eliasson and Larsson, 1993). However, controversy still exists with regard to the interaction of native lipids with amylopectin. Evidence from recent calorimetric studies have shown that lipids could interact with the short linear (15-20 glucose units) portions of the outer branches of amylopectin

(Eliasson and Ljunger, 1988). The interactions between reassociating starch chains would be of a stronger order of magnitude in defatted than in untreated starches, since removal of free and complexed lipids would increase the mobility of interacting starch chains. This would then explain the increase in ΔH_R on lipid removal, and the time of onset of the retrogradation endotherm in native (15 days) and defatted (3 days) starches from oat (Table 4.11). The above increase in ΔH_R is more pronounced in wheat and oat starches (Table 4.11) due to their higher content of free and complexed lipids (Table 4.1).

4.3.9 Gel strength

Gel strength (after a storage period of 1 day) of defatted starches of wheat, oat, potato and lentil were higher than those of their native counterparts (Table 4.12). As seen in Table 4.7, the swelling factor (at 95°C) of all starches decreased on defatting. Amylose leaching (at 95°C) increased in defatted wheat and oat starches, but decreased in potato and lentil starches (Table 4.8). Since the short term development of the structure, crystallinity and firmness in starch gels are dominated by irreversible (melting $\geq$ 100°C) gelation and crystallization of amylose within the gel matrix, an increase in amylose leaching or a decrease in swelling factor (highly swollen granules occurring between adjacent amylose chains would hinder their association during retrogradation) would theoretically be expected to increase gel firmness.

Table 4.12 Gel strength of native and defatted starch gels (40%) after storage for one day at 25°C

Starch source	Treatment	Gel strength[a] (g)
Wheat	Native Defatted[b]	25±2.0 42±3.0
Oat	Native Defatted	11±1.0 22±0.8
Lentil	Native Defatted	101±4.0 182±3.0
Potato	Native Defatted	23±1.0 41±2.0

[a] Values are averages of three determinations ± SD.
[b] Hot n-propanol-water (3:1 v/v) for 7 h.

Therefore, in wheat and oat starches the increase in gel firmness is more pronounced on defatting, since both the decrease in SF and increase in AML would favour aggregation between amylose chains. However, in low lipid containing starches such as lentil and potato (Table 4.1), the increase in gel firmness on defatting can be attributed to the large decrease in granular swelling (Table 4.7), which overrides the influence of decreased amylose leaching (Table 4.8) on gel firmness.

4.4 EFFECT OF HEAT-MOISTURE TREATMENT ON THE STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES OF STARCH

4.4.1 Granule morphology

Heat-moisture treatment did not alter the size or shape of the granules used in this study. Similar observations have also been made by Kulp and Lorenz (1981), on potato and wheat starches. However, in oat starch, many of the granules which were aggregated in the native state (Fig. 4.6.A) were less compactly packed after heat treatment (Fig. 4.6.B).

4.4.2 X-ray diffraction

The X-ray diffraction patterns and the intensities of the d-spacings of the major diffraction peaks of native and heat-moisture treated starches are presented in Fig. 4.7 and Table 4.13 respectively [yam starch (has B-type crystals like potato starch) included in this study in order to better understand the effect of heat-moisture treatment on B-type starch crystals). The d-spacings of all starches shifted slightly on heat-moisture treatment. However, none of the treated starches exhibited major new d-spacings (Table 4.13). Heat treatment at 30% moisture increased the intensities of the major peaks of wheat, lentil and oat starches (wheat > lentil > oat), but decreased those of potato and yam starches (potato > yam) (Table 4.13). The noted changes in intensities in potato and wheat starches were influenced by the level of moisture content during heat-treatment (Table 4.13). However, intensity changes in the other starches were only marginally affected by variations in

Fig. 4.6. Scanning electron micrographs of native (A) and heat-moisture treated (B) (100°C, 30% moisture for 16 h) oat starch granules.

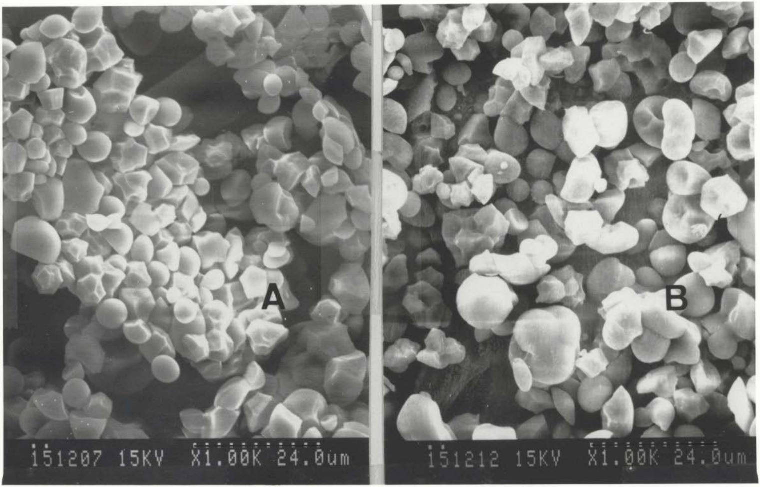

A
151207 15KV X1.00K 24.0um
B
151212 15KV X1.00K 24.0um

Fig. 4.7. X-ray diffraction patterns of native and heat-moisture treated (HMT) (100°C, 30% moisture for 16 h) starches.

(A), native yam (moisture content (MC), 9.6%); (B), HMT yam (MC 9.8%);
(C), native potato (MC 9.7%); (D), HMT potato (MC 9.5%);
(E), native lentil (MC 9.6%); (F), HMT lentil (MC 9.9%);
(G), native oat (MC 9.4%); (H), HMT oat (MC 9.9%);
(I), native wheat (MC 9.9%); (J), HMT wheat (MC 10.0%).

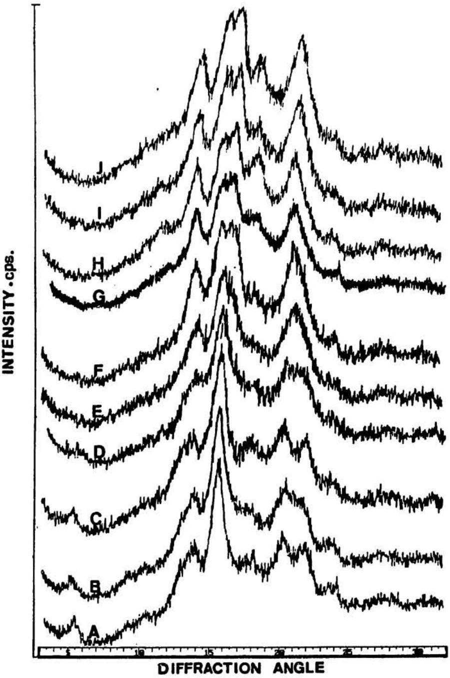

INTENSITY . cps.
J
I
H
G
F
E
D
C
B
A
5
10
15
20
25
30
DIFFRACTION ANGLE

Table 4.13 X-ray diffraction spacings and intensities of major peaks of native and heat-moisture treated starches

Starch source	Treatment	Interplanar spacings (d) in Å with intensities (cps)^a						
Wheat	Native^b		5.94 (489)	5.24 (523)		4.90 (712)		3.82 (522)
	HMT^c		5.94 (554)	5.19 (759)		4.91 (818)		3.83 (761)
	HMT^d		5.95 (615)	5.15 (1115)		4.90 (1191)		3.88 (925)
Oat	Native^b		5.87 (624)	5.22 (576)	5.13 (695)	4.90 (704)		3.83 (438)
	HMT^c		5.91 (689)	5.15 (686)		4.87 (744)		3.88 (551)
Lentil	Native^b		5.91 (404)	5.23 (523)	5.10 (539)	4.99 (527)		3.83 (409)
	HMT^c		5.90 (700)	5.26 (636)		4.99 (1079)		3.88 (447)

Continued

Table 4.13 Continued

Potato	Native[b]	16.0 (265)	5.98 (400)	5.26 (875)	4.50 (306)	4.00 (385)	3.70 (385)	
	HMT[c]	16.8 (155)	5.98 (380)	5.25 (703)	3.92 (424)	3.89 (445)	3.71 (310)	
	HMT[d]		5.96 (320)	5.20 (569)	4.50 (300)	3.98 (331)	3.74 (290)	
Yam	Native[b]	16.5 (288)	5.94 (482)	5.20 (1067)	4.53 (343)	4.00 (412)	3.75 (430)	
	HMT[c]	16.5 (150)	5.94 (370)	5.20 (905)	4.55 (345)	4.00 (352)	3.74 (344)	

Values are average of two determinations.

[a] Counts per second.

[b] Native starches had moisture contents ranging from 9.6 to 10.0%.

[c] Heat-moisture treated at 20% moisture for 16 h at 100°C.

[d] Heat-moisture treated at 30% moisture for 16 h at 100°C.

moisture levels (Table 4.13). The X-ray diffraction patterns of native wheat, oat and lentil starches was retained after treatment. However, alterations ('B' to 'A' + 'B')occurred in potato and yam starches (Fig. 4.7). The 'B' to 'A' + 'B' transformation in potato starch has also been reported by other researchers (Lorenz and Kulp, 1983; Stute, 1992).

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 diffraction 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 (Imberty *et al.*, 1991; Leach *et al.*, 1959). However, in crystallites of B-starches, adjacent double helices are mainly linked by hydrate water bridges and to a limited extent by direct hydrogen-bonding (Leach *et al.*, 1959). French (1984), has suggested that long range molecular order (crystallinity) in starch granules is the result of regular packing of double helices formed from adjacent clusters of the short DP chains of amylopectin.

The absence of new d-spacings and the slight shifting of the existing d-spacings on heat-treatment of wheat, oat, lentil, potato and yam starches (Table 4.13) suggest that the increases in X-ray intensities (Table 4.13) are largely due to structural changes within the crystalline domains of the granule. This seems plausible since the increase in d-spacing intensities on heat-moisture treatment of waxy corn starch (99% amylopectin) is greater than in native corn (73% amylopectin) starch (unpublished results). The increase in X-ray intensities with increase in moisture content on heat-treatment of wheat starch (Table 4.13) suggests that thermal energy and moisture may have caused double helices to shift within the crystallites and to assume a crystalline array that is more closely packed and ordered (due to an increase in the number of direct hydrogen bonds linking adjacent helices) than that in native starch. The decrease in X-ray intensities on heat-treatment of potato and yam starches (Table 4.13) suggests a loss of crystalline order. This probably occurs due to rupture of the hydrate water bridges linking adjacent double helices. Although direct hydrogen bonds between adjacent helices may have remained intact, the overall decrease in magnitude of the bonding forces between adjacent helices would cause them to move apart and assume orientations that are not in perfect parallel crystalline array. As a result, diffraction of X-rays would be less intense after heat treatment. The results for potato starch (Table 4.13) show that moisture level during heat-treatment influences the extent of decrease in X-ray intensities. This could be attributed to greater chain flexibility (increases with increase in moisture content) of the loosely packed double helices of heat-moisture treated potato starch. An increase in chain flexibility would hinder parallel alignment of the double helices.

The transformation of the X-ray pattern of potato and yam starches from B to A + B (Fig. 4.7) is probably initiated by rupture of the hydrate water bridges, which enable helices to rearrange themselves into a crystalline array that contains an amylosic helix in the central channel of the unit cell. The gradual reduction in intensity of the d-spacing at 16.8 Å in potato and yam starches (Table 4.13) with increase in moisture content (this peak disappears at 30% moisture in potato starch) and the disappearance (at 20% moisture) of the doublet centred at 4.0 Å and 3.7 Å in potato and yam starches (Fig. 4.7) clearly demonstrates that the extent of rearrangement of the helices is influenced by the moisture level during heat treatment.

Gernat *et al.* (1990), have shown that the legume starch 'C' crystalline polymorph is a mixture of A- and B- unit cells, and that these starches contain pure A- and B- polymorphs in varying proportions. The X-ray pattern of native lentil starch did not exhibit any spacings that were characteristic of the B-pattern. This meant that native lentil starch contained mainly A-unit cells. This would then explain the similar responses (increase in X-ray intensities) shown by lentil and cereal starches on heat treatment. The X-ray patterns of wheat, oat and lentil starches are not altered (Fig. 4.7) after heat treatment, since the direct hydrogen bonds linking adjacent double helices within the crystalline domains of these starches are resistant to disruption at the moisture level (30%) prevailing during heat-treatment. Consequently, only limited helical rearrangement is possible, and this is probably confined only to those helices that are linked *via* hydrate water bridges (fewer than in tuber starches). It is, therefore, likely that it is the movement and interaction (*via* direct hydrogen bonds) of these helices within the unit cell that is responsible for increased X-ray intensities on heat treatment of wheat, oat and lentil starches.

Starch lipids are known to form amylose-inclusion complexes in which the ligand resides within the central hydrophobic core of the helix (Mikus *et al*., 1946; Morrison, 1981). A 'V'-X-ray pattern is seen when lipid containing starches are subjected to extrusion cooking (Mercier *et al*., 1980) and after addition of monoacyl lipids to starch under appropriate conditions (Hoover and Hadizyev, 1981; Biliaderis *et al*., 1986b). Native (untreated) starches do not exhibit a 'V'-X-ray pattern (Galliard and Bowler, 1987). This means that either the complexes do not exist in native starch and are formed only on heating or more probably that they do exist but only in partially helical or with insufficient degree of long-range order to generate the necessary X-ray diffraction pattern (Galliard and Bowler, 1987).

The decrease in apparent amylose content (Table 4.14) in wheat, oat and lentil starches indicates a change in amylose chain conformation (random

Table 4.14 Apparent amylose content of native and HMT[a] starches

Starch source	Apparent amylose content (%)[b]	
	Native	HMT[a]
Wheat	21.1±0.4	19.8±0.1
Oat	16.7±0.2	14.8±0.3
Lentil	36.7±0.3	35.4±0.4
Potato	21.9±0.4	22.0±0.2
Yam	26.5±0.3	26.8±0.2

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

[b] Determined by I_2 binding without extraction of native lipids.

coil → partial helix) on heat-moisture treatment. This probably enables lipids which were unbound within the native granule to readily form 'V' amylose helices. The results indicate a close relationship between the amount of unbound lipids in the native granules (Table 4.1) and the magnitude of the decrease in apparent amylose content (Table 4.14). The apparent amylose content of potato and yam starches remain unchanged on heat-moisture treatment, due to their low content of unbound lipids (Table 4.1).

4.4.3 Swelling factor and amylose leaching

The swelling factor (SF) and amylose leaching (AML) of native starches increased with rise in temperature, but were reduced on treatment (16h, 100°C, 30% moisture) (Tables 4.15 and 4.16). The extent of decrease in SF among the starches followed the order: potato > lentil > yam > wheat > oat (Table 4.15). In contrast, the corresponding order in AML was: potato > yam > lentil > oat > wheat (Table 4.16). The apparent amylose content (determined by I_2 binding without lipid removal) of native and heat-moisture treated starches are presented in Table 4.14. The results showed that complex formation between native lipids and amylose increased on heat treatment.

Starch granule swelling is known to begin in the bulk, relatively mobile amorphous fraction, and in the more restrained amorphous regions immediately adjacent to the crystalline regions. Tester and Morrison (1990a),

Table 4.15 Swelling factor of native and heat-moisture treated starches

Starch source	Treatment	Swelling factor[a]			
		Temperature (°C)			
		50°	70°	80°	95°
Wheat	Native	6.0±0.6	14.4±0.2	21.5±0.6	27.6±0.3
	HMT[b]	1.7±0.2	4.5±0.4	10.6±0.8	17.2±0.5
Oat	Native	1.9±0.3	11.3±0.6	19.8±0.5	25.7±0.1
	HMT	1.3±0.2	7.1±0.8	13.0±0.8	16.1±0.9
Lentil	Native	6.9±0.5	14.7±0.7	19.5±0.6	24.4±1.0
	HMT	1.1±0.1	3.8±0.4	6.2±0.5	11.2±0.7
Potato	Native	10.6±1.1	31.5±1.5	50.5±1.3	93.1±1.8
	HMT	1.3±0.1	6.2±0.2	10.9±0.8	13.4±0.5
Yam	Native	3.9±0.5	15.1±1.0	27.1±1.2	38.9±1.4
	HMT	1.7±0.2	5.1±0.8	10.2±1.1	14.2±0.7

[a] Values are means of three replicates ± SD.

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

% Reduction = $(SF_{\text{native}} - SF_{\text{heat-moisture treated}})/SF_{\text{native}}$.

Table 4.16 Amylose leaching of native and heat-moisture treated starches

Starch source	Treatment	Amylose leaching[a] (% db)			
		Temperature (°C)			
		50°C	70°C	80°C	95°C
Wheat	Native	0	4.3±0.1	7.1±0.2	10.9±0.4
	HMT[b]	0	3.1±0.3	6.1±0.5	7.7±0.3
Oat	Native	0	4.9±0.2	7.8±0.1	12.3±0.3
	HMT	0	2.3±0.1	4.6±0.3	7.7±0.2
Lentil	Native	0.4±0.1	18.7±0.3	24.6±0.8	29.4±0.8
	HMT	0	8.6±0.1	14.1±0.4	19.5±0.5
Potato	Native	0.9±0.1	12.8±0.2	15.7±0.5	18.3±0.7
	HMT	0	2.0±0.1	4.5±0.1	7.1±0.3
Yam	Native	0	1.5±0.2	9.1±0.4	14.5±0.3
	HMT	0	0.5±0.1	3.7±0.2	8.7±0.5

[a] Values are means of three replicates ± SD.

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

% Reduction = $(AML_{native} - AML_{heat\text{-}moisture\ treated})/AML_{native}$.

have shown by comparative studies on normal and waxy barley starches that swelling is primarily a property of amylopectin and amylose is a diluent. These authors also showed that amylose and lipids in normal starches could also inhibit granule swelling under conditions where amylose-lipid complexes are likely to be formed.

The results from this study suggest that the extent of decrease in swelling factor on heat-moisture treatment of the various starches could be attributed to an interplay of three factors: 1) Changes in the packing arrangement of the starch crystallites (Table 4.13, Fig 4.7); 2) Interaction between or among starch components in the amorphous regions of the granule (Table 4.16) and 3) Amylose-lipid interactions (Table 4.14). Decreases in amylose leaching (Table 4.16) could be attributed to an interplay of factors 2 and 3.

4.4.4 Gelatinization

The gelatinization parameters of native and heat-moisture treated starches were studied by differential scanning calorimetry. The influence of heat treatment at moisture contents between 10 and 30% on gelatinization transition temperatures [onset (T_o), mid-point (T_p), and conclusion (T_c)] and gelatinization enthalpy (ΔH) are presented in Table 4.17. The T_o, T_p and T_c values of potato and oat starches (Table 4.17) increased on heat treatment (at all moisture levels). The increase was most marked at a moisture level of 30%. In both starches, the extent of increase in transition temperatures followed the order: $T_c > T_p > T_o$. At all moisture levels, the increase in T_o was more marked in potato than in oat starch. At 30% moisture, the gelatinization temperature range (T_c-T_o) increased by 5 and 6°C in potato and oat starches respectively. However, in wheat, lentil, and yam starches, significant increases in T_o, T_p and T_c were observed only at moisture levels beyond 20% ($T_c > T_p > T_o$). As in potato and oat starches, these increases were more marked at a moisture level of 30%. At 30% moisture, the increase in T_c - T_o in wheat, lentil and yam was respectively 2, 1 and 2°C. Increases in gelatinization temperatures on heat-moisture treatment has also been observed by other researchers in potato (Donovan *et al.*, 1983; Lorenz and Kulp, 1982; Lorenz and Kulp, 1983; Stute, 1992) and wheat (Donovan *et al.*, 1983; Kulp and Lorenz, 1981; Lorenz and Kulp, 1982, 1983) starches. The ΔH of potato and yam starches progressively decreased with an increase in the level of moisture during heat-treatment (Table 4.17). At 30% moisture, this decrease amounted to 5.1 and 6.3 J/g in potato and yam starches respectively. However, at all moisture levels of heat treatment, the ΔH of wheat, lentil and oat starches remained unchanged (Table 4.17). The foregoing changes in ΔH for potato starch agreed with those of Donovan *et al.* (1983) and Stute (1992). However, the former authors reported a decrease in ΔH for wheat starch (100°C, 16h, 27% moisture).

Table 4.17 DSC^a characteristics of native and HMT^b starches

Starch source	Treatment	To^c	Tp^c	Tc^c	ΔH^d (J/g)
Wheat	Native	56.2±0.4	61.1±0.2	66.6±0.4	9.7±0.4
	HMT^b				
	10%	56.1±0.2	62.0±0.1	67.3±0.5	9.2±0.3
	20%	57.2±0.3	62.3±0.2	67.1±0.2	9.2±0.2
	30%	65.1±0.2	70.4±0.4	78.0±0.6	9.7±0.4
Oat	Native	60.2±0.2	64.1±0.1	70.0±0.4	10.1±0.4
	HMT^b				
	10%	61.2±0.3	67.0±0.1	71.9±0.1	10.1±0.5
	20%	61.0±0.2	69.9±0.3	76.0±0.3	10.5±0.4
	30%	64.1±0.3	75.3±0.5	80.1±0.4	10.9±0.6
Lentil	Native	55.0±0.1	60.6±0.5	67.5±0.3	7.6±0.4
	HMT^b				
	10%	55.2±0.4	61.1±0.2	70.0±0.1	8.0±0.4
	20%	55.3±0.2	61.0±0.4	71.2±0.1	8.0±0.4
	30%	64.0±0.3	71.0±0.2	78.3±0.4	7.6±0.2
Potato	Native	54.0±0.1	58.8±0.2	64.5±0.3	16.4±0.5
	HMT^b				
	10%	59.2±0.1	63.8±0.3	71.0±0.1	14.7±0.3
	20%	60.1±0.3	64.0±0.5	73.1±0.5	12.6±0.4
	30%	64.9±0.1	71.0±0.6	79.8±0.3	11.3±0.5
Yam	Native	72.0±0.4	76.9±0.2	83.1±0.2	21.0±0.6
	HMT^b				
	10%	71.8±0.2	77.2±0.3	84.0±0.1	19.3±0.2
	20%	72.1±0.6	78.0±0.1	85.2±0.3	15.1±0.4
	30%	76.9±0.3	84.1±0.4	90.0±0.2	14.7±0.5

Values are mean of three determinations ± SD.

[a] Differential scanning calorimetry.

[b] Heat moisture treated (100°C, 16 h at moisture contents of 10, 20 and 30%).

[c] To, Tp and Tc indicates the temperature of the onset, midpoint and end of gelatinization.

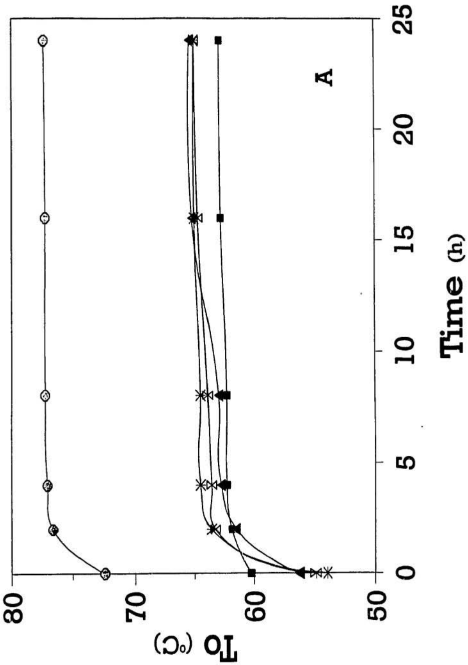
[d] Enthalpy of gelatinization.

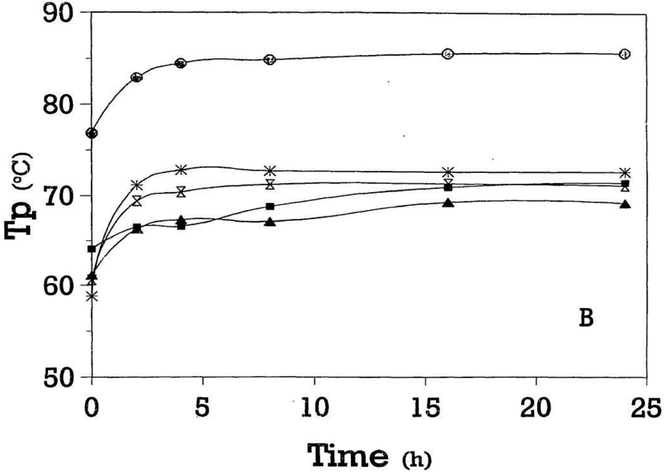
The changes in T_o, T_p, T_c, T_c-T_o, and ΔH as a function of time during heat treatment at 30% moisture content are presented in Figs. 4.8.A-E. During the first 5 h, rapid increases were observed in T_o, T_p and T_c (Figs. 4.8.A-C) for wheat, lentil, potato and yam starches (potato > lentil > wheat > yam). Thereafter, increases were less pronounced (Figs. 4.8.A-C). Oat starch behaved differently in exhibiting only gradual increases in T_o, T_p and T_c with time (less than those of the other starches). The increase in T_o ceased after 2 h of heat-treatment (Fig. 4.8.A). In contrast, T_p and T_c continued to increase gradually and then began to level off after 16 h of heat-treatment (Figs. 4.8.B,C).

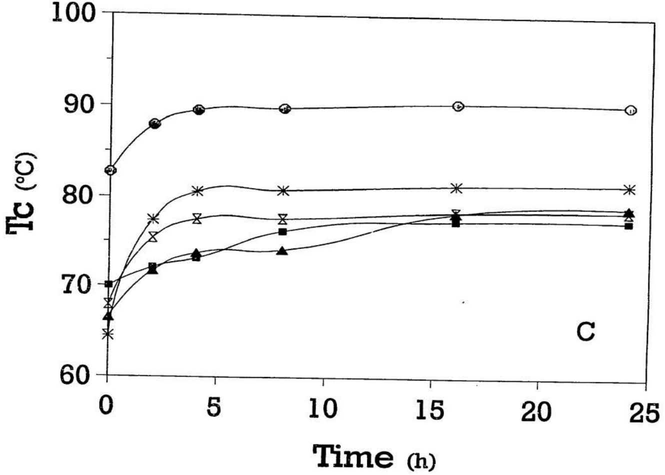
The gelatinization temperature range (T_c - T_o) of potato starch increased rapidly during the first 5h of heat-treatment (10.2 to 17°C) (Fig. 4.8.D), while increases during this time period were only marginal in the other starches. Heat-treatment beyond 5 h caused only marginal changes in (T_c - T_o) of potato, lentil and yam starches. However, (T_c-T_o) of oat and wheat starches showed rapid increases between the 8th and 16th h of heat treatment. Thereafter, changes were only minimal. Rapid decrease in ΔH of potato and yam starches occurred during the first 3 h and between the 8th and 16th h of heat-treatment (Fig. 4.8.E). However, no changes were observed after 16 h (Fig. 4.8.E). The changes in ΔH (decrease) were only marginal in wheat, whereas those of oat and lentil starches remained unchanged (Fig 4.8.E).

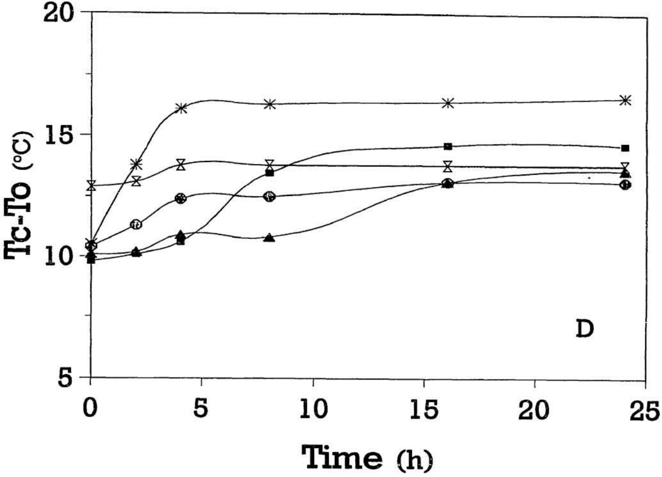
Fig. 4.8. Gelatinization transition temperatures (T_o, T_p, T_c), range (T_c-T_o) and enthalpy (ΔH) as a function of time of heat moisture treatment at 100°C,30% moisture. (—Δ—Δ—), wheat; (—■—■—), oat; (—Ι—Ι—), lentil; (—✱—✱—), potato and (—●—●—), yam.

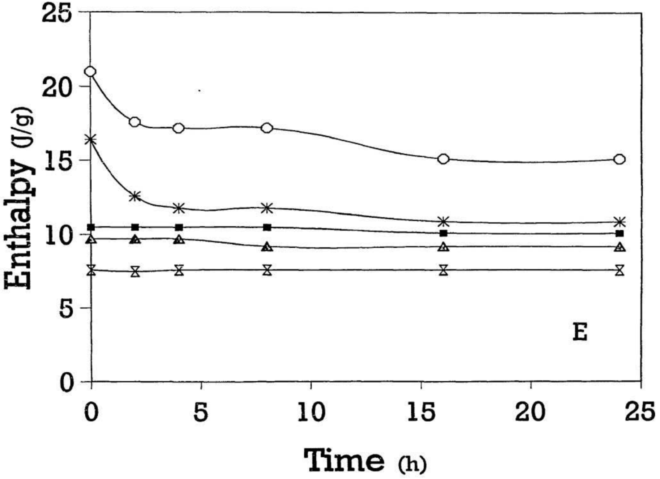
(A), T_o (onset) vs treatment time;
(B), T_p (peak) vs treatment time;
(C), T_c (conclusion) vs treatment time;
(D), (T_c-T_o) (range) vs treatment time;
(E), ΔH (enthalpy) vs treatment time.


A
80
70
60
50
To (°C)
0
5
10
15
20
25
Time (h)


B
Tp (°C)
90
80
70
60
50
0
5
10
15
20
25
Time (h)


100
90
80
70
60
Tc (°C)
0
5
10
15
20
25
Time (h)
C


20
15
10
5
Tc-To (°C)
0
5
10
15
20
25
Time (h)
D


E
Enthalpy (J/g)
0
5
10
15
20
25
Time (h)
0
5
10
15
20
25

In wheat, oat and lentil starches, the increase in T_o, T_p and T_c on heat-moisture treatment (Table 4.17) reflects a decrease in the destabilization effect of the amorphous regions on the melting of starch crystallites during gelatinization. This is probably due to interactions between amylose chains (within the bulk amorphous regions) and/or between amylose chains and the branched segments (within the intercrystalline regions) of amylopectin, rather than to any increase in crystalline stability. This seems plausible, since T_o, T_p and T_c of waxy maize starch are unaltered after heat-moisture treatment under similar conditions (unpublished results).

As seen from X-ray diffraction studies (Table 4.13), the crystallites of potato starch are disrupted on heat-moisture treatment. Consequently, if changes in crystalline stability were a factor influencing increases in thermal transition temperatures during heat-moisture treatment, then T_o, T_p and T_c of heat-moisture treated potato starch should have been less than that of its native counterpart. The results on potato starch (Table 4.17) therefore suggest that as in wheat, oat and lentil starches, the increase in T_o, T_p and T_c on heat-moisture treatment reflects starch interactions within amorphous regions of the granule. The foregoing increases are higher in potato owing to its longer amylose chain length (Takeda *et al.*, 1984; Biliaderis *et al.*, 1981a,b). As may be seen in Table 4.13, crystallite disruption also occurs on heat-moisture treatment of yam starch. Furthermore, the amylose chain length of yam and potato starches are fairly similar. Therefore, the marginal increases in T_o, T_p and T_c on heat-moisture treatment was rather surprising (Table 4.17). It is probable that starch chains in the amorphous regions of native yam starch granules may be highly associated [This is reflected in high values for T_o, T_p and T_c (Table 4.17)], as a result only minimal interactions are possible during heat-moisture treatment.

The changes in transition temperatures with time (Fig. 4.8.A-C) indicate that, in all starches, the interactions within the amorphous domains of the granules appear to occur entirely within the first 5 h of heat-moisture treatment. Cooke and Gidley (1992) have shown by DSC, ^{13}C-CP-MAS-NMR spectroscopy, and powder X-ray diffraction studies that ΔH values of gelatinization primarily reflect the loss of double helical order rather than loss of crystalline register. The lack of influence of heat-moisture treatment on ΔH of wheat, oat and lentil starches (Table 4.17) therefore suggests that double helices do not disrupt (do not unravel) under the conditions prevailing during heat-moisture treatment. This suggests that identical amounts of double helices unravel and melt during gelatinization of native and heat-moisture treated wheat, oat and lentil starches. Molecular (helical) order and not crystalline order thus may be the primary determinant of the endothermic enthalpy of gelatinization. If crystalline order had influenced ΔH, the ΔH values of wheat, oat and lentil starches should have then increased [because of increase in crystalline order (Table 4.13)] on heat-moisture treatment. The decrease in ΔH values on heat-moisture treatment of potato and yam starches (Table 4.17) suggests that some of the original double helices may have disrupted during the polymorphic transformation (B to A + B).

The progressive decrease in ΔH with increase in moisture content (Table 4.17) indicates that the extent of double helical disruption in potato and yam starches is influenced by double helical chain motions (which increases with increase in moisture content). The changes in ΔH with time of heat-moisture treatment (Fig. 4.8.E) shows that the movement and reorientation of double helices of potato and yam starches takes nearly 16h for completion.

4.4.5 Pasting Curves

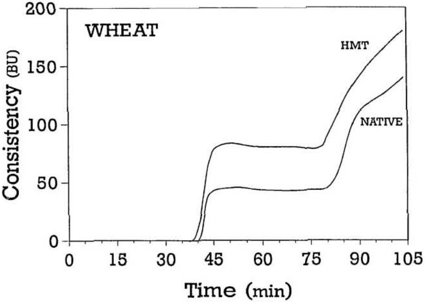
The pasting curves of native and heat-moisture treated starches are presented in Fig. 4.9.A-E. Heat-moisture treatment decreased the pasting temperature of wheat starch by 3°C (Fig. 4.9.A) and increased those of lentil (Fig. 4.9.E), potato (Fig. 4.9.C) and yam (Fig. 4.9.D) starches by 24, 30 and 16°C respectively. However, that of oat starch remained unchanged (Fig. 4.9.B). The 96°C consistency increased by 30 Brabender units (BU) on heat-moisture treatment of wheat starch, but decreased by 130, 220, 370 and 2100 BU respectively, in heat-moisture treated oat, lentil, yam and potato starches.

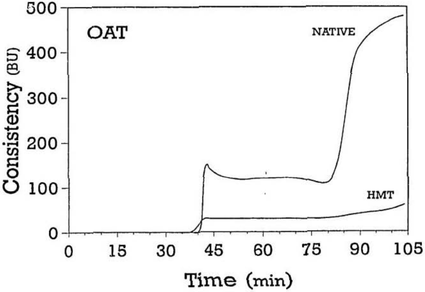
In all starches the thermal stability (during the holding cycle at 96°C) increased on heat-moisture treatment. The amylograms on potato starch are in general agreement with reported data (Kulp and Lorenz, 1981; Lorenz and Kulp, 1983). However, with respect to wheat starch, discrepancies were observed between the results of this study and those of Kulp and Lorenz (1981) and Lorenz and Kulp (1983). The former authors reported a gradual decrease in consistency (at 92°C) with increase in moisture level (21 to 27%) during heat-treatment (16h, 100°C), the decrease being 50 BU at 27% moisture. While the latter authors reported that during heat treatment (16h,100°C) the consistency (at 92°C) increased by 70 BU at a moisture level of 21% but decreased by 10 BU at a level of 27% moisture. These conflicting data are rather puzzling since the variety of wheat used in both studies was identical.

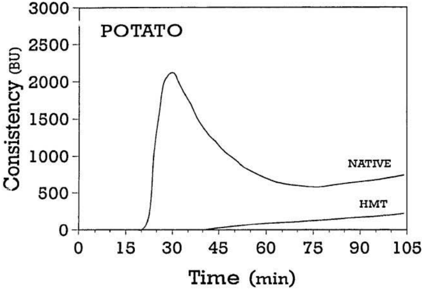
The increase in the Brabender 95°C viscosity on heat-moisture treatment of wheat starch (Fig. 4.9.A) could be attributed to an increase in granular rigidity resulting from an increase in crystalline order (Table 4.13) and starch chain interactions within the amorphous regions (Tables 4.15 and 4.17).

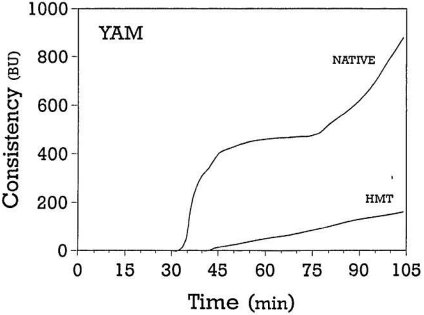
Although similar changes [somewhat reduced in magnitude (Tables 4.15 and 4.17)] also occur within the amorphous and crystalline regions during heat-moisture treatment of oat starch, the granules of native oat starch are better able to withstand the shear effects (higher 95°C viscosity) than those of their heat-moisture treated counterparts (Fig. 4.9.B). This could be due to oat

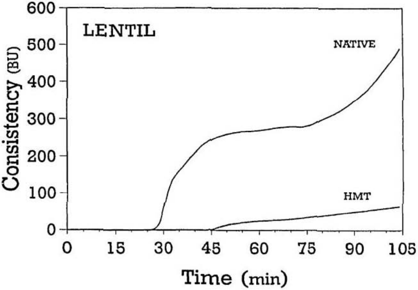
Fig. 4.9. Brabender amylograms of native and heat-moisture treated (HMT) (100°C, 30% moisture for 16 h) starches.


WHEAT
HMT
NATIVE
Consistency (BU)
200
150
100
50
0
0
15
30
45
60
75
90
105
Time (min)


OAT
NATIVE
HMT
Consistency (BU)
500
400
300
200
100
0
0
15
30
45
60
75
90
105
Time (min)


POTATO
Consistency (BU)
3000
2500
2000
1500
1000
500
0
0
15
30
45
60
75
90
105
Time (min)
NATIVE
HMT


YAM
Consistency (BU)
1000
800
600
400
200
0
0
15
30
45
60
75
90
105
Time (min)
NATIVE
HMT


LENTIL
NATIVE
HMT
Consistency (BU)
600
500
400
300
200
100
0
0
15
30
45
60
75
90
105
Time (min)

granules being less compactly packed (increases shear sensitivity) after heat-moisture treatment (Fig 4.6.A,B). These results suggest that the change in granule packing overrides the influence of increased granular rigidity on viscosity. The decrease in 95°C viscosity on heat-moisture treatment of lentil starch (Fig 4.9.E) could be attributed to a very high decrease in the volume occupied by the swollen granules (decreased SF) in the continuous phase (Table 4.15). This is not surprising, since the magnitude of the changes within the amorphous (Tables 4.15 and 4.17) and crystalline regions (Table 4.13) during heat-moisture treatment of lentil starch is much higher than in wheat and oat starches. The decrease in volume fraction apparently negates the influence of increased granular rigidity on viscosity. In potato and yam starches, the decrease in 95°C viscosity on heat-moisture treatment (Fig 4.9.C,D) is to a larger extent due to crystallite destruction (Table 4.13) which decreases both granular rigidity and the volume fraction occupied by the swollen granules.

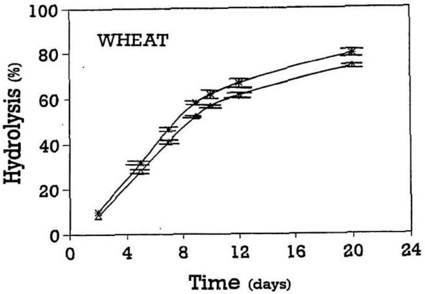
4.4.6 Acid hydrolysis

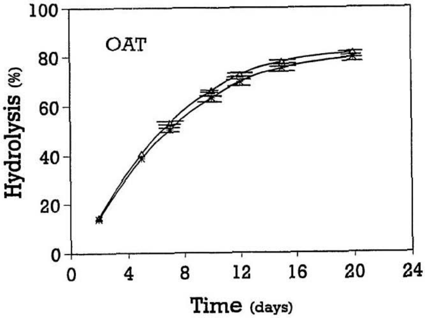
The solubilization patterns of native and heat-moisture treated (100°C, 30% moisture for 16 h) starches are shown in Figs. 4.10.A-E (values are presented in Appendix 4). The extent of hydrolysis of the amorphous and crystalline regions of wheat (Fig. 4.10.A) and lentil (Fig. 4.10.E) starch granules decreased on heat-moisture treatment. The extent of the decrease was nearly the same in both starches. However, heat-moisture treated granules of oat starch were hydrolysed to a slightly greater extent than those of native starch (Fig. 4.10.B). During the first 7 days of hydrolysis, heat-moisture treated starches of potato (Fig. 4.10.C) and yam (Fig. 4.10.D) were hydrolysed to a greater extent than their native counterparts. Thereafter, hydrolysis was more extensive in native starches.

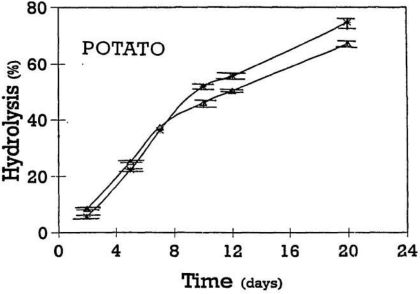
The extent of acid hydrolysis (after 48h) in potato and yam starches subjected to heat-treatment (at 30% moisture content) for 16h at 100°C and 75°C are presented in Table 4.18. The hydrolysis remained unchanged in starches treated at 75°C, but heat treatment at 100°C increased the susceptibility of both starches to acidic degradation.

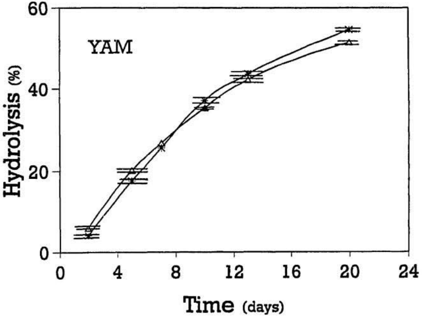
It has generally been accepted (Biliaderis *et al.*, 1981b; Cairns *et al.*, 1990) that heterogenous acid hydrolysis preferentially attacks the more amorphous regions of the granule, whether they be at the surface or interior. In contrast, crystalline regions are less accessible to hydrated protons (H_3O^+) and are attacked only after a period of 10-12 days. The decrease in acid (Figs. 4.10.A-E) and enzyme hydrolysis (Table 4.19) after heat-moisture treatment suggests that the structural changes within the amorphous and crystalline regions of the granule on heat-moisture treatment may have rendered these regions less accessible to penetration by H_3O^+ and α-amylase.

Fig. 4.10. Heterogeneous hydrolysis of native (—*—*—) and heat-moisture treated (—Δ—Δ—) (100°C, 30% moisture for 16 h) starches in 2.2 N HCl at 35°C.


WHEAT
Hydrolysis (%)
0
20
40
60
80
100
0
4
8
12
16
20
24
Time (days)


OAT
Hydrolysis (%)
0
20
40
60
80
100
0
4
8
12
16
20
24
Time (days)


POTATO
Hydrolysis (%)
0
20
40
60
80
Time (days)
0
4
8
12
16
20
24


YAM
Hydrolysis (%)
0
20
40
60
Time (days)
0
4
8
12
16
20
24

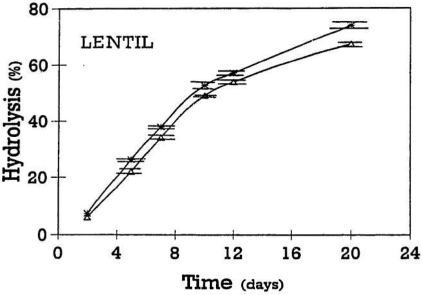

LENTIL
Hydrolysis (%)
0
20
40
60
80
Time (days)
0
4
8
12
16
20
24

Table 4.18. Acid hydrolysis of native and heat-moisture treated potato and yam starches.

Starch source	Treatment	Hydrolysis[b] (%)
Potato	Native	5.9±0.3
	[a]HMT at 75°C	5.4±0.2
	[a]HMT at 100°C	8.6±0.4
Yam	Native	4.3±0.1
	[a]HMT at 75°C	4.1±0.2
	[a]HMT at 100°C	5.7±0.3

[a] Heat-moisture treated at 30% moisture for 16 h.

[b] After 2 days in 2.2 N HCl at 35°C (n=3).

These decreases are higher in lentil than in wheat starch because of stronger starch chain associations within the amorphous regions of the former (Tables 4.16 and 4.17). This seems plausible, since in both starches the extent of increase in crystalline order on heat-moisture treatment were nearly of the same order of magnitude (Table 4.13). The susceptibility of oat starch to attack by H_3O^+ or α-amylase increases on high-moisture treatment (in spite of starch chain interactions within amorphous regions) due to a greater degree of contact of the individual granular surfaces with H_3O^+ or α-amylase (Figs. 4.6.A,B). Crystallite disruption in potato and yam starches during heat-moisture treatment may have been the causative factor responsible for differences in the extent of acid hydrolysis between native and heat-moisture treated starches (heat-moisture treated > native) during the first 7 days of hydrolysis (Figs. 4.10.C,D). It is plausible that the initial attack of H_3O^+ may have been on the disrupted crystallites present at or near the granular surface. The reversal (native > heat-moisture treated) in the trend of acid hydrolysis after the 7th day (Fig. 4.10.C,D) suggests that the action of H_3O^+ is now confined mainly to the amorphous regions in the granule interior (which are more accessible to hydrolysis in native than in heat-moisture treated starches).

The results in Table 4.18 show that crystallite disruption is influenced by the amount of thermal energy imparted to the starch crystallites during heat-moisture treatment.

4.4.7 Enzymatic hydrolysis

The extent of hydrolysis (after 72h) of native and heat-moisture treated starches (30% moisture) by porcine pancreatic α-amylase are presented in Table 4.19. Among native starches the extent of hydrolysis followed the order: wheat ≈ lentil > oat > potato > yam. The hydrolysis of wheat and lentil starches decreased by 2.1 and 6.5% respectively on heat-moisture treatment (Table 4.19). However, heat-moisture treatment increased the susceptibility of oat (56%), potato (300%) and yam (320%) starches towards α-amylase.

These results on wheat and potato starches are not in agreement with those of Lorenz and Kulp (1982) who reported that the extent of hydrolysis (in 24h) of wheat and potato starches by a commercial fungal α-amylase increased respectively, by 110 and 45% on heat-moisture treatment (16h, 100°C, 27% moisture). This discrepancy may have been due to differences in enzyme source or purity. The very high degree of susceptibility of potato starch towards α-amylase after heat-moisture treatment has also been shown by Kuge and Kitamura (1985). These authors showed that the extent of hydrolysis of potato starch by α-amylase from *Bacillus subtilis* increased on heat-moisture treatment (120°C, 27.3% moisture for 1 h) from 3% (in 24h) to 80% (in 6h). For corn starch the corresponding increase (in 24h) was only 11.1%.

Table 4.19 Hydrolysis[a] of native and heat-moisture treated starches by porcine pancreatic α-amylase

Starch source	Treatment	Hydrolysis[b] (%)	% Change
Wheat	Native HMT[c]	66.1±0.7 64.7±1.1	2.1 (decrease)
Oat	Native HMT	32.3±0.6 50.0±1.3	54.7 (increase)
Lentil	Native HMT	64.6±1.4 59.0±0.5	8.6 (decrease)
Potato	Native HMT	5.4±0.3 21.4±0.9	296.3 (increase)
Yam	Native HMT	1.5±0.1 6.2±0.4	313.3 (increase)

[a] Hydrolysis for 72 h in phosphate buffer (pH=6.9).

[b] Values are means of three replicates ± SD.

[c] Heat-moisture treated (at 100°C, 30% moisture for 16 h)

The four fold increase in enzyme hydrolysis on heat-moisture treatment of potato and yam starches (Table 4.19) clearly demonstrates that as in acid hydrolysis, the initial action of α-amylase could have been on the disrupted starch crystallites near the granule surface. Therefore, it is plausible that if the reaction between α-amylase and the above starches was monitored over time periods > than 72h, a stage may have been reached when the extent of enzyme hydrolysis would have been greater in native than in heat-moisture treated starches.

4.4.8 Scanning electron microscopy

Scanning electron micrographs of native and heat-moisture treated granules of potato starch after attack by α-amylase (72h) are presented in Figs. 4.11.A-D. While those of native and heat-moisture treated oat starch granules are presented in Figs. 4.11.E,F respectively. The attack of α-amylase on native potato starch (Figs. 4.11.A,B) manifested itself in only superficial erosion of the granules. Morphological changes were not discernible and the result is in agreement with a low amylolysis rate (Table 4.19). However, during the same time period, granules of heat-moisture treated potato starch were more extensively attacked by α-amylase (Figs. 4.11.C,D) than those of native starch (Fig. 4.11.A,B). Many of these granules showed deep cracks and fissures (Figs. 4.11.C,D). Granules of oat starch were also more degraded

Fig. 4.11. Scanning electron micrographs of native and heat-moisture treated (100°C, 30% moisture for 16 h) starches after attack (72h) by porcine pancreatic α-amylase:

(A) and (B), native potato starch;
(C) and (D), heat-moisture treated potato starch;
(E), native oat starch;
(F), heat-moisture treated oat starch.


A
000047 15KV X250 96um
B
000034 15KV X1.31K 18.3um
C
000041 15KV X250 96um
D
000036 15KV X1.50K 16.0um
E
000026 15KV X1.00K 24.0um
F
000022 15KV X1.00K 24.0um

after heat-moisture treatment (Fig. 4.11.F). However, unlike potato starch, the mode of attack of α-amylase on native (Fig. 4.11.E) and heat-moisture treated (Fig. 4.11.F) oat starch granules were identical.

4.4.9 Retrogradation

The thermal characteristics of retrograded amylopectin was studied by differential scanning calorimetry. The transition temperatures (T_o, T_p, T_c) and the enthalpy (ΔH_R), representing the fusion of retrograded amylopectin [of native and heat-moisture treated (16h, 100°C, 30% moisture) starches] at various time intervals of storage (0.5 h to 20 days) are presented in Table 4.20. As seen in Table 4.17, T_o, T_p and T_c of the gelatinization endotherm of native starches were different from those of heat-moisture treated starches (100°C, 30% moisture for 16 h). The reasons for this has been discussed earlier (section 4.4.4).

The retrogradation endotherm appeared after 3 days of storage in native wheat, potato and lentil starch gels. Whereas for native oat starch gel, the corresponding time was 15 days (Table 4.20). Heat-moisture treatment did not hasten or delay the onset time of the retrogradation endotherm in wheat, potato and lentil starches. However, the retrogradation endotherm appeared after 6 days of storage in heat-moisture treated oat starch. The differences in T_o, T_p and T_c of the retrogradation endotherm of native and heat-moisture

Table 4.20 Thermal characteristics of native and heat-moisture treated starch gels[a]

Starch source	Enthalpy[b] (ΔH) (J/g)							Transition temperature[c] (°C)		
	Storage time (days)									
	2	3	4	6	8	15	20	To	Tp	Tc
Wheat										
Native	-	0.9	1.8	3.9	5.3	7.1	7.1	47.5	57.2	60.7
HMT[d]	-	2.4	3.4	5.0	6.9	9.0	9.7	46.9	58.1	59.8
Oat										
Native	-	-	-	-	-	1.3	1.9	46.8	57.6	60.9
HMT[d]	-	-	-	1.9	2.2	2.5	3.5	48.0	57.4	60.3
Lentil										
Native	-	0.6	1.3	2.5	3.4	6.0	8.4	47.1	57.9	62.8
HMT[d]	-	1.7	3.4	5.4	6.8	11.3	12.2	46.8	57.5	63.3
Potato										
Native	-	1.0	2.4	6.0	7.5	9.8	11.2	50.6	57.2	69.2
HMT[d]	-	0.8	1.9	4.7	5.7	9.1	9.7	50.5	59.1	69.1

[a] Values are averagesof three determinations.
[b] Average standard deviation = 5% of the value.
[c] Average standard deviation = 0.5°C.
[d] Heat moisture treated at 100°C and 30% moisture for 16 h.

treated starch gels were only marginal and remained practically unchanged during the time course of retrogradation (Table 4.20). Furthermore T_o, T_p and T_c of the retrograded starch gels were lower than that for the gelatinization endotherm (Table 4.20). In native, and heat-moisture treated starch gels, the transition temperature range (T_c - T_o) of the retrogradation endotherm was broader (Table 4.20) than that of the gelatinization endotherm (Table 4.17). This was most pronounced in potato starch. Unlike T_o, T_p and T_c, the enthalpy of the retrogradation endotherm (ΔH_R) was greatly influenced by heat-moisture treatment. After 20 days storage, the ΔH_R (J/g) of gels (Table 4.20) from native starches were 7.1 (wheat), 1.9 (oat), 11.2 (potato) and 8.4 (lentil). These values increased, on heat-moisture treatment by 2.6, 1.7 and 3.8 J/g in wheat, oat and lentil respectively. Whereas in potato starch, ΔH decreased by 1.5 J/g.

As discussed earlier, during heat-moisture treatment, crystallinity is enhanced within granules of wheat oat and lentil starches (wheat > lentil > oat), but is disrupted in potato starch. Thus, after heat-moisture treatment the degree of separation between the outer chain branches of adjacent amylopectin chain clusters would decrease in wheat, oat and lentil starches, and increase in potato starch. Consequently, during gel storage, the formation and lateral association of double helices involving amylopectin chains, would be easier and much stronger in heat-moisture treated wheat, oat and lentil starches, but more difficult and weaker in heat-moisture treated potato starch.

This would then explain the observed increase (wheat, oat and lentil) and decrease (potato) in ΔH_R during retrogradation of heat-moisture treated starches (Table 4.20). If changes in crystallinity on heat-moisture treatment was the sole factor influencing ΔH_R, then, the extent of increase in ΔH_R on heat-moisture treatment may have followed the trend: wheat > lentil > oat. However, as seen in Table 4.20, the above order was reversed with respect to wheat and lentil (lentil > wheat). This suggests that interaction between amylose and amylopectin chains during gel storage may have also influenced changes in ΔH_R. The amylose and amylopectin chains of lentil starch have been shown to be longer than those of wheat starch. Furthermore, as shown in Table 4.16, the decrease in amylose leaching on heat-moisture treatment is higher in lentil than in wheat starch. Therefore, the probability of interaction between amylose and amylopectin chains (within gelatinized granules) during storage, would be greater in lentil starch. Similar interactions between amylose and amylopectin chains may have also contributed to changes in ΔH_R in heat moisture treated potato starch [since the chain length of the starch components and the decrease in amylose leaching (Table 4.16) is much higher than in the other starches]. However, the extent of the foregoing contribution cannot be properly assessed, since crystallite disruption in potato starch on heat-moisture treatment is so extensive, that it probably negates any influence that amylose-amylopectin interaction may have had on ΔH_R.

4.4.10 Gel strength

Gel strength (after a storage period of 1 day) of heat-moisture treated starches of wheat, oat, potato and lentil were higher than those of their native counterparts (Table 4.21). As seen in Table 4.15 and 4.16, the swelling factor (at 95°C) and amylose leaching (at 95°C) of all starches decreased upon heat-moisture treatment. As stated earlier, the short term development of the structure, crystallinity and firmness in starch gels is dominated by irreversible ($T < 100°C$) gelation and crystallization of amylose within the gel matrix, an increase in amylose leaching or a decrease in swelling factor (highly swollen granules occurring between adjacent amylose chains would hinder their association during retrogradation) would theoretically be expected to increase gel firmness. In heat-moisture treated starches, the aggregation between amylose chains during gel storage would be favored only by the decrease in SF, since the decrease in amylose leaching would tend to reduce the amount of amylose chains within the continuous amylose gel matrix. In all four native starches, the increase in gel firmness on heat-moisture treatment suggests that the large decrease in SF (potato > lentil > wheat > oat) overrides the influence of decreased amylose leaching on gel firmness.

Upon heat-moisture treatment, unlike in wheat and oat starches, the most pronounced increase in gel firmness occurred in potato and lentil starches (Table 4.21). This is probably due to the decrease in swelling factor

Table 4.21 Gel strength of native and heat moisture treated starch gels (40%) after storage for one day at 25°C

Starch source	Treatment	Gel strength[a] (g)
Wheat	Native HMT[b]	25±1.0 30±0.8
Oat	Native HMT	11±0.5 13±0.8
Lentil	Native HMT	101±2.0 230±1.5
Potato	*Native* HMT	23±1.0 80±2.0

[a] Values are averagesof three determinations ± SD.

[b] Heat-moisture treated at 100°C and 30% moisture for 16 h.

being of a very high order of magnitude in potato and lentil after heat-moisture treatment. Although, amylose leaching is also highly restricted after heat moisture treatment in these starches (Table 4.16), the decrease in swelling factor is so large (potato > lentil) (Table 4.15) that it overrides the effect of decreased amylose leaching on gel firmness. Therefore, the increase in gel firmness of heat-moisture treated starches reflect an interplay of granule swelling and amylose leaching.

4.5 EFFECT OF ANNEALING ON THE STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES OF STARCH

4.5.1 Effect of annealing on leaching of starch components from granules

No soluble carbohydrates were determined in the filtrate when annealing was performed at 50°C for 72 h. However, amylose leaching occurred in all starches at temperatures above 50°C.

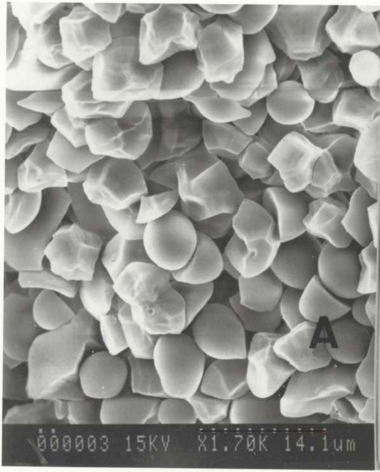
4.5.2 Granule Morphology

The size and shape of individual starch granules of wheat, oat, potato and lentil starches remained unchanged after annealing. However, granules of oat starch which appeared aggregated in the native state (Fig. 4.12.A), were less compactly packed after annealing (Fig. 4.12.B).

4.5.3 X-ray Diffraction

The X-ray diffraction spacings and intensities of the major peaks of native and annealed starches (75% moisture, 50°C, 72h) are presented in Table 4.22. The results showed that X-ray intensities of wheat, oat and lentil starches increased slightly on annealing, while those of potato starch showed a slight decrease. However, in all starches d-spacings remained unchanged (Table 4.22). This implies that only marginal changes in crystallinity occur on annealing.

Fig. 4.12. Scanning electron micrographs of native (A) and annealed (B) (50°C, 75% moisture for 72 h) oat starch granules.


A
000003 15KV X1.70K 14.1um

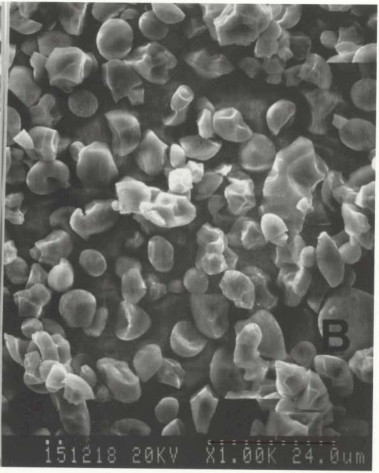

B
151218 20KV X1.00K 24.0um

Table 4.22. X-ray diffraction spacings and intensities of the major peaks of native and annealed starches

Starch source	Treatment	Interplanar spacings (d) in Å with intensities (cps)[a]						
Wheat	Native	5.94 (489)		5.24 (523)		4.90 (712)	4.45 (378)	3.82 (522)
	Annealed[b]	5.94 (599)		5.23 (669)		4.92 (715)	4.46 (407)	3.82 (557)
Oat	Native	5.86 (624)		5.24 (628)	5.13 (695)	4.98 (768)		3.80 (591)
	Annealed	5.98 (633)		5.29 (651)	5.19 (684)	4.95 (814)		3.90 (623)
Lentil	Native	5.91 (404)		5.23 (523)		4.99 (527)	4.40 (375)	3.83 (409)
	Annealed	5.95 (462)		5.25 (586)		4.99 (546)	4.41 (370)	
Potato	Native	16.0 (265)	5.98 (411)	5.26 (875)		4.00 (385)	3.70 (385)	
	Annealed	16.0 (260)	5.97 (405)	5.26 (864)		4.00 (364)	3.70 (464)	

Values are averagesof two determinations.
[a] Counts per second.
[b] Annealed at 50°C, 75% moisture for 72 h.

4.5.4 Gelatinization

The thermal transition temperatures (T_o, T_p, T_c) and the enthalpy (ΔH) of gelatinization of wheat, oat, potato and lentil starches subjected to an annealing temperature of 50°C for different time periods at a moisture content of 75% are presented in Table 4.23. The T_o, T_p and T_c of all starches increased on annealing. The increase was most pronounced in T_o and least in T_c. Similar observations have been made by Knutson (1990) and Larsson and Eliasson (1991) on maize, wheat and potato starches. The rate of increase in T_o, T_p and T_c was gradual in wheat and oat starches, but rapid in potato (during the first 30 min) and lentil (during the 30th and 60th min) starches (Table 4.23). Annealing beyond 24h did not significantly increase T_o, T_p and T_c of oat, potato and lentil starches. However, those of wheat starch became more pronounced as the annealing time exceeded 24h. At the end of 72h, the increase in T_o for wheat, oat, potato and lentil starches was respectively, 13.1, 3.8, 17.2 and 15°C, (Table 4.23). Annealing decreased the transition temperature range (T_c -T_o) and increased the ΔH of all starches, (Table 4.23). In wheat starch, the decrease in T_c - T_o continued throughout the time course of annealing. However, in oat and lentil starches the decrease in T_c - T_o ceased after 48h, and for potato starch the corresponding time was 12h. At the end of 72h, the decrease in T_c - T_o amounted to 5.1, 2.5, 3.3 and 4.9°C respectively, in wheat, oat, potato and lentil starches.

Increases in ΔH were evident in wheat, oat, potato and lentil starches

Table 4.23. The influence of annealing[a] time on the thermal characteristics of starches

Starch source	Transition temperature[b] and Enthalpy[c]	Annealing time (h)								
		0	0.5	1.0	2.0	6.0	12.0	24.0	48.0	72.0
Wheat	To	56.2	58.2	60.1	60.5	61.0	62.0	63.1	66.8	69.6
	Tp	61.1	63.0	64.4	64.5	64.5	64.5	65.3	69.5	72.0
	Tc	66.6	68.0	68.5	68.6	68.5	68.7	68.8	72.0	74.7
	Tc-To	10.4	9.8	8.4	8.1	7.5	6.8	5.7	5.7	5.1
	ΔH	9.6	9.7	9.7	9.6	9.7	9.7	9.8	12.2	12.1
Oat	To	60.4	60.4	60.8	61.0	61.8	61.8	62.5	64.0	64.0
	Tp	64.1	64.7	65.2	64.7	64.9	65.0	65.9	67.3	67.1
	Tc	70.0	70.0	70.1	70.9	70.3	70.7	70.9	71.2	71.3
	Tc-To	9.6	9.6	9.3	9.1	9.2	8.5	8.2	7.3	7.3
	ΔH	10.2	10.1	10.1	10.2	10.1	10.3	10.1	12.2	12.3
Lentil	To	55.0	55.9	62.3	63.1	65.2	66.0	68.0	69.0	70.0
	Tp	60.6	61.4	66.8	67.9	68.7	68.8	70.0	71.7	72.2
	Tc	67.5	67.7	73.2	73.4	73.5	74.0	76.1	76.5	77.4
	Tc-To	12.5	11.8	10.9	10.3	8.3	8.0	8.0	7.5	7.4
	ΔH	7.6	7.5	9.2	9.2	10.5	10.5	10.6	10.4	10.5
Potato	To	54.0	62.5	62.6	64.1	67.4	68.1	70.0	70.0	71.2
	Tp	58.8	66.3	66.7	67.0	70.9	71.5	72.3	72.4	74.2
	Tc	64.5	71.4	71.5	72.0	74.8	75.3	77.27.	77.4	78.4
	Tc-To	10.5	8.9	8.9	7.9	7.4	7.2	2	7.4	7.2
	ΔH	16.7	16.5	16.8	18.9	20.2	20.2	20.1	20.0	20.2

[a] Annealing moisture and temperature were respectively, 75% and 50°C.

[b] To, Tp and Tc represent respectively the onset, midpoint and end of gelatinization (°C). Volume fraction of water (V_w) = 0.85; Average standard deviation (SD) = 0.4°C (n = 3).

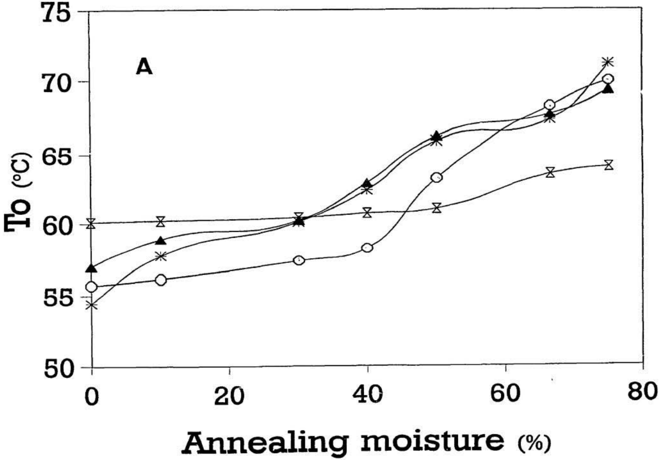
[c] Enthalpy (ΔH) of gelatinization (J/g of dry starch); Average SD = 0.3 (n = 3).

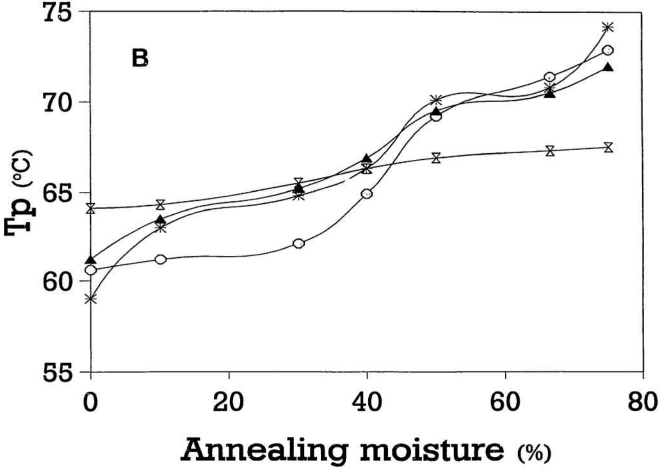
only after annealing had been in progress for 18, 6, 2 and 1h, respectively. At the end of 72h, increases in ΔH were respectively, 2.5, 2.1, 3.8 and 2.9 J/g in wheat, oat, potato and lentil starches. The magnitude of the increase in ΔH for potato and wheat starches was higher than that reported by Larsson and Eliasson (1991). The above authors showed that ΔH of the above starches increased by 0.8 J/g (wheat) and 0.2 J/g (potato) when subjected to an annealing temperature of 50°C for 24h (starch:water, 1:1). Whereas, Stute (1992) observed no changes in ΔH on annealing of potato starch (starch: water, 1:5, 96 h, 52°C). However, significant increases in ΔH have been reported on annealing of normal and waxy maize genotypes (excess water, 50°C, 48h) (Krueger *et al.*, 1987b), and high amylose maize starches (starch: water, 1:3, 50°C, 24 h) (Knutson, 1990). It was interesting to observe that increases in T_o and ΔH did not begin concurrently on annealing, and furthermore, the rate of increase in ΔH was slower in comparison to that of T_o (Table 4.23).

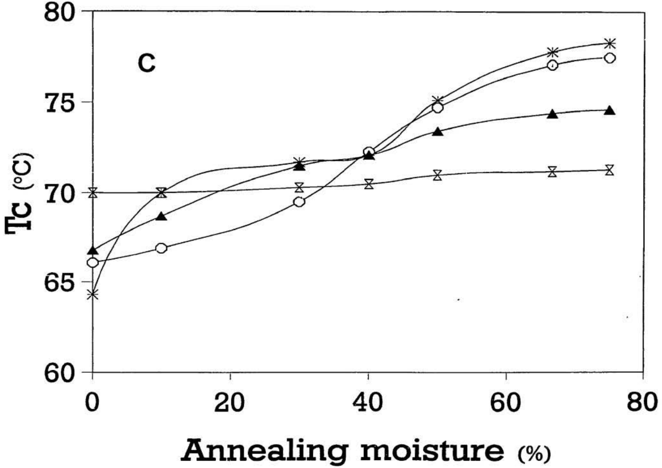
The effects of moisture content (during annealing) on T_o, T_p, T_c and ΔH of the starches are presented in Figs. 4.13.A-D. The effect of annealing moisture was most pronounced in potato starch and least in oat starch. Steep increases in T_o, T_p and T_c were observed at an annealing moisture of 50% in wheat and lentil starches whereas in potato and oat starches this occurred at moisture contents of 10 and 70% respectively. A steep increase in ΔH

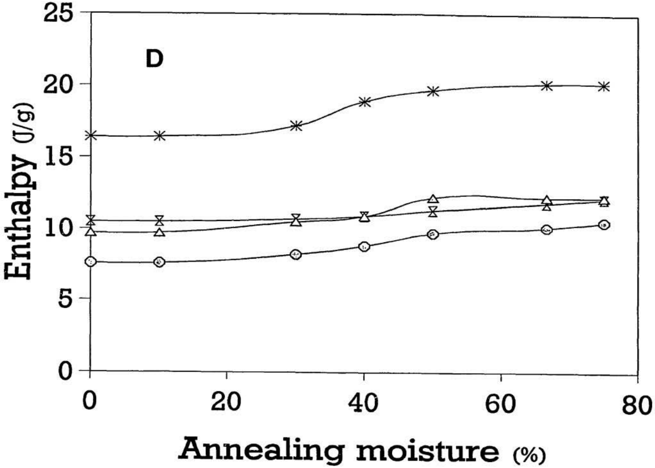
Fig. 4.13. Gelatinization transition temperatures (T_o, T_p, T_c) and enthalpy (ΔH) as a function of moisture content during annealing, at 50°C for 72h. (—Δ—Δ—), wheat; (—⧗—⧗—), oat; (—●—●—), lentil; and (—✳—✳—) potato.

(A), T_o (onset) vs annealing moisture;
(B), T_p (peak) vs annealing moisture;
(C), T_c (conclusion) vs annealing moisture;
(D), ΔH (enthalpy) vs annealing moisture.


A
To (°C)
75
70
65
60
55
50
0
20
40
60
80
Annealing moisture (%)


B
Tp (°C)
75
70
65
60
55
0
20
40
60
80
Annealing moisture (%)


C
Tc (°C)
80
75
70
65
60
0
20
40
60
80
Annealing moisture (%)


D
Enthalpy (J/g)
25
20
15
10
5
0
0
20
40
60
80
Annealing moisture (%)

occurred at moisture contents of 40% and 50% in potato and wheat starches respectively; whereas changes in ΔH for oat and lentil starches were gradual (Fig. 4.13.D). Cooke and Gidley (1992), showed by X-ray spectroscopy and ^{13}C solid-state NMR that ΔH reflects mainly the loss of double helical order. The difference in ΔH between native and annealed starches (Table 4.23) suggests that the amount of double helices that unravel and melt during gelatinization is higher in annealed (indicated by higher ΔH values) than in native starch. This suggests that the double helical content of starch granules increase on annealing. Formation of double helices during annealing may be the result of interactions between: A) The outer branches of adjacent amylopectin chain clusters; B) amylose and the outer branches of amylopectin and C) amylose chains. It is plausible that the rate of formation of double helices via type **A** interaction would be much slower than either via type **B** or **C** due to steric hindrance between adjacent branches of amylopectin chains. The slow increase in ΔH and the rapid increase in T_o during the time course of annealing (Table 4.23) suggests that the former is influenced mainly by type **A** interactions, while types **B** and **C** contribute to changes in T_o. It is likely that type **C** interactions also makes a significant contribution to both changes in ΔH and T_o on annealing. However, their influence cannot be properly ascertained since amylose-amylose crystallization cannot be reversed by heating to 100°C in the DSC.

The differences in the rate and extent of increase in ΔH during annealing of wheat, oat, potato and lentil starches (Table 4.23) is probably due to differences in amylopectin chain length and on the degree of proximity of the annealing temperature to T_o. In the starches studied, the average chain length of the outer branches (A-chains) of amylopectin have been reported to be respectively, 20 (Hizukuri, 1985), 14-18 (Biliaderis *et al.*, 1981a), and 13-17 (Hizukuri, 1985) in potato, lentil and wheat starches (corresponding data for oat starch is not available). Thus, the double helices formed between outer branches of adjacent amylopectin chains (during annealing) would be longer in potato than in the other starches. Furthermore, double helical stability would also be higher in potato starch, due to closer proximity of the annealing temperature to T_o (Table 4.23). Consequently, on gelatinization the amount of thermal energy associated with the unravelling and melting of double helical structures (formed during annealing) would be most pronounced in potato starch. Thus, the differences in the rate and magnitude of increase in ΔH between potato and the other starches is explained.

As discussed earlier, the main type of interaction influencing increases in T_o, T_p and T_c is that between amylose and the outer branches of amylopectin. Further evidence for this has come from studies on starches from waxy maize (contains no amorphous amylose regions) and normal corn. In these starches, T_o, T_p and T_c of waxy maize showed only marginal changes on annealing, while those of normal corn showed significant increases (Knutson, 1990).

As discussed earlier, in potato and lentil starches, amylose and amylopectin are intermingled within the native granule, while in cereal starches they are found in separate domains. Consequently, the opportunity for interaction between amylose and amylopectin chains during annealing would be higher in potato and lentil than in wheat and oat starches. Furthermore, the extent of interaction between amylose and amylopectin would also be influenced by the degree of proximity of the annealing temperature to T_o (Table 4.23) and the chain lengths of the starch components. The chain length of amylose in wheat, potato and lentil starches have been reported to be respectively, 570 (Takeda *et al.*, 1984), 4920 (Takeda *et al.*, 1984) and 1400 (Biliaderis *et al.*, 1981a). Thus, in the above starches, the magnitude of interaction between amylose and amylopectin during annealing would follow the sequence: potato > lentil > wheat > oat. This would then explain the rapid increases in T_o, T_p and T_c during the initial stages of annealing (< 2h) in potato and lentil starches and the slow rate of increase in wheat and oat starches (Table 4.23).

The rate of increase in T_o, T_p and T_c with increase in annealing moisture (Figs. 4.13.A-C), suggests that the moisture level at which significant interactions occur between amylose and amylopectin chains, (due to increase in chain mobility) is largely influenced by the degree of separation of the starch components within the granule and on their chain lengths. Significant interactions between amylose and amylopectin chains occur at lower annealing moisture levels (<30%) in potato than in the other starches (Figs. 4.13.A-C), due to their closer association (within the native granule) and longer chain lengths in the former. The steep increase in ΔH (due to interaction between adjacent amylopectin chains) at an annealing moisture of 30% in potato starch (Fig. 4.13.D) could also be explained in a similar manner.

It is necessary at this stage to mention the arrangement of the double helices within the native crystalline polymorphs, which will allow a subsequent discussion of the decrease in gelatinization temperature range (T_c - T_o) on annealing.

As discussed earlier, in starch granules, amylopectin is considered to be responsible for crystallinity through the ordered arrangement of double helices formed by adjacent branches within the structure (French, 1984). Cooke and Gidley (1992) have shown that there are two types of double helices in starch granules, those involved in crystallites large/perfect enough to diffract X-rays and those which are not; together with substantial non-ordered material. Furthermore, these authors have postulated that the forces holding starch granules together are largely at the double-helical level, and that the observed crystallinity may function as a means of achieving dense packing rather than as a primary provider of structural stability. The reason for the decrease in the gelatinization temperature range (T_c - T_o) on annealing has been the subject of controversy. It has been suggested that the decrease in T_c - T_o may be due to alterations of the coupling forces between the crystallites and the amorphous matrix (Stute, 1992), perfection of starch crystallites (Larsson and Eliasson 1991), more crystalline orientation (Krueger *et al.*, 1987a), and interaction between amylose and amylopectin chains (Knutsen, 1990). The results of this study indicate that the decrease in T_c - T_o is mainly due to an increase in crystalline order resulting from a closer packing of the pre-existing crystallites within the starch granule. Gidley (1987), showed that in A-type (wheat, oat) starch structure there is a close packed array of double helices, whereas the B-type (potato) structure is more open with a greater amount of interhelical water. As discussed earlier the crystalline organization within granules of lentil starch closely resemble those of cereal starches.

The decrease in T_c - T_o is higher in wheat (Table 4.23) due to closer proximity of the double helices within the crystalline structure of the granule. (This permits stronger interaction between adjacent double helices which then increases crystalline order). However, X-ray diffraction data (Table 4.22) showed no significant increase in crystallinity on annealing. This implies that the magnitude of the increase in crystalline order may have been below the detection level of the X-ray diffractometer. The observed order of decrease in T_c - T_o (wheat > lentil > potato) suggests that it is not influenced by interchain associations between amylose and amylopectin. Since if it were, the magnitude of the above decrease should have followed the order: potato > lentil > wheat.

4.5.5 Swelling factor and Amylose leaching

The swelling factor (SF) and amylose leaching (AML) of native and annealed starches at various temperatures are presented respectively, in Tables 4.24 and 4.25. The SF and AML of native starches increased with rise in temperature, but were reduced on annealing. The percentage reduction (with respect to their native counterparts) among the starches (lentil > wheat > potato > oat) increased with starch amylose content [lentil (≈39%) > wheat (≈27%) > potato (≈23%) > oat (≈19%)] (Table 4.1). At 90°C, the decrease in SF (Table 4.24) in lentil, wheat, potato and oat starches were 39, 32, 30 and 23%, respectively. While, at the same temperature, decreases in AML (Table 4.25) for the above starches were 5.1, 1.7, 1.6 and 0.5%, respectively.

The decrease in SF and AML on annealing suggests interaction between amylose chains and/or between amylose and the outer branches of amylopectin. The latter interaction is probably not the major causative factor since the extent of decrease in SF and AML (among the starches) would have then followed the sequence: potato > lentil > wheat > oat, rather than that observed in this study (lentil > wheat > potato > oat). This suggests that the magnitude of the above decrease is mainly influenced by interaction between amylose chains (greater in lentil starch, due to its higher amylose content (Table 4.1). It is likely that interaction between amylose and amylopectin chains also contributes to the above decrease. However, unlike with DSC their influence cannot be properly ascertained by measurements on SF and AML.

Table 4.24. Swelling factor of native and annealed starches

Starch source	Treatment	Swelling factor[a]			
		Temperature (°C)			
		50°	70°	80°	95°
Wheat	Native	6.0±0.6	14.4±0.2	21.5±0.6	27.6±0.3
	Annealed[b]	4.2±0.4	10.1±0.5	13.5±0.4	18.9±0.5
Oat	Native	1.9±0.3	11.3±0.6	19.8±0.5	25.7±0.1
	Annealed	1.7±0.2	9.6±0.4	15.5±0.2	19.8±0.7
Lentil	Native	6.9±0.5	14.7±0.7	19.5±0.6	24.4±1.0
	Annealed	4.5±0.1	9.7±0.4	12.5±0.3	15.0±0.2
Potato	Native	10.6±1.1	31.5±1.5	50.5±1.3	93.1±1.8
	Annealed	8.3±0.3	25.3±0.8	38.0±1.1	65.3±2.3

[a] Values are means of three replicates ± SD.

[b] Annealing was carried out at 50°C, 75% moisture for 72 h.

% Reduction = $(SF_{native} - SF_{annealed})/SF_{native}$.

Table 4.25. Amylose leaching of native and annealed starches

Starch source	Treatment	Amylose leaching[a] (% db)			
		Temperature (°C)			
		50°	70°	80°	95°
Wheat	Native Annealing[b]	0 0	4.3±0.1 3.5±0.2	7.1±0.2 6.0±0.4	10.9±0.4 9.3±0.1
Oat	Native Annealing	0 0	4.9±0.2 4.2±0.1	7.8±0.1 6.7±0.3	12.3±0.3 11.8±0.2
Lentil	Native Annealing	0.4±0.1 0	18.7±0.3 14.6±0.2	24.6±0.6 20.0±0.2	29.4±0.8 24.8±0.4
Potato	Native Annealing	0.5±0.1 0	12.8±0.2 10.7±0.3	15.7±0.5 13.6±0.3	18.3±0.2 16.6±0.4

[a] Values are means of three replicates ± SD.

[b] Annealing was carried out at 50°C, 75% moisture for 72 h.

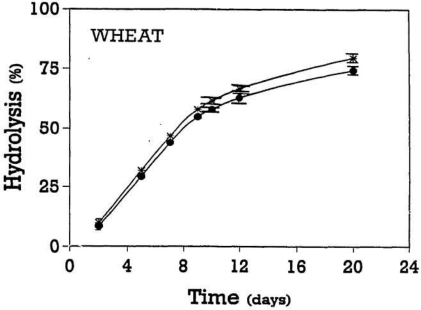
% Reduction = $(AML_{native} - AML_{annealed})/AML_{native}$.

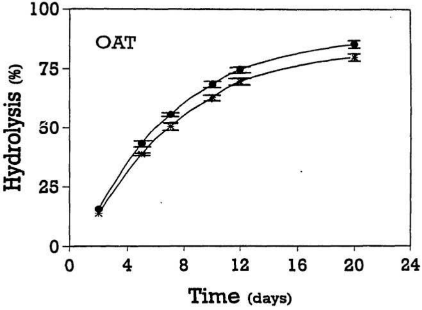
4.5.6 Acid hydrolysis

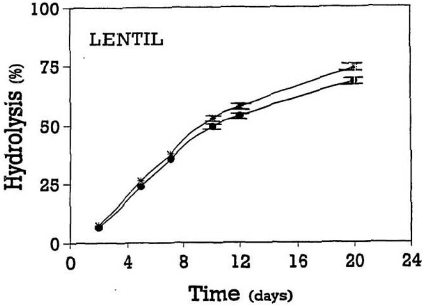
The solubilization patterns of native and annealed starches are shown in Fig. 4.14.A-D (values are presented in Appendix 5). Throughout the time course of hydrolysis, annealed starches of wheat, potato and lentil were degraded less readily than their native counterparts. However, annealing increased the susceptibility of oat starch granules to acidic hydrolysis (Fig. 4.14.B). At the end of 20 days, the differences in the extent of hydrolysis between native and annealed starches were 6.1, 5.4, 5.6 and 4.4 in wheat (Fig. 4.14.A), oat (Fig. 4.14.B), lentil (Fig. 4.14.C) and potato (Fig. 4.14.D) starches, respectively.

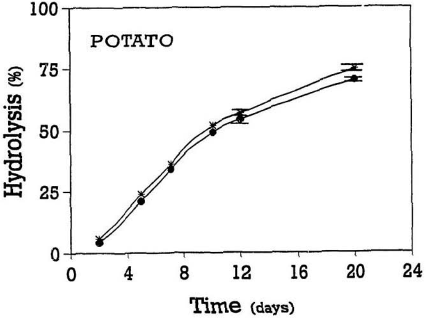
It has generally been accepted that hydrated protons (H_3O^+) preferentially attack the more amorphous regions of the granule, whether they be at the surface or in the interior. However, crystalline regions are less accessible to H_3O^+ and are attacked only after a period of 10-12 days (Robin *et al.*, 1974). The marginal differences in the extent of hydrolysis (during the first 10 days) between native and annealed starches suggests that although starch chain interactions occur within amorphous regions of the granule (during annealing), these do not lead to the formation of crystallites. This seems plausible, since crystallite formation within amorphous regions of the granule would have decreased the susceptibility of annealed starches towards H_3O^+ to a much greater degree than that observed in this study. French and Murphy (1977) have shown that models of double helices (either parallel or antiparallel) have all glycosidic links buried in the interior of the double helix where they are not

Fig. 4.14. Heterogeneous hydrolysis of native (—✳—✳—) and annealed (—●—●—)(50°C, 75% moisture for 72 h) starches in 2.2 N HCl at 35°C.


WHEAT
Hydrolysis (%)
0
25
50
75
100
Time (days)
0
4
8
12
16
20
24


OAT
Hydrolysis (%)
0
25
50
75
100
Time (days)
0
4
8
12
16
20
24


LENTIL
Hydrolysis (%)
100
75
50
25
0
0
4
8
12
16
20
24
Time (days)


POTATO
Hydrolysis (%)
100
75
50
25
0
0
4
8
12
16
20
24
Time (days)

accessible to H_3O^+. Therefore, the slight difference in hydrolysis (during the first 10 days) between native and annealed starches (Fig. 4.14.A-D) may have been due to formation of double helices (which do not form into a crystalline array) within the amorphous regions of annealed starches. The difference in the extent of hydrolysis between native and annealed starches are more prominent after the 10th day (Fig 4.14.A-D), since at this time, hydrolysis is mainly confined to the starch crystallites. It is likely that the increase in crystalline order on annealing may have rendered the starch crystallites more resistant to penetration by H_3O^+. It was interesting to observe, that the extent of decrease (wheat > lentil > potato) in hydrolysis (after the 10th day) closely paralleled the increase in crystalline order on annealing (wheat > lentil > potato). As discussed earlier, many of the aggregated granules of native oat starch become less compactly packed on annealing (Fig. 4.12.A,B). Consequently, the accessibility of H_3O^+ to the individual granular surfaces and its concentration within the granule would be much greater on annealing. This would then explain the greater susceptibility of the amorphous and crystalline regions of annealed oat starch to degradation by H_3O^+ (Fig. 4.14.B). It is plausible that, if the difference between the annealing temperature and T_o was of the same order of magnitude as in wheat starch, then the difference in acid hydrolysis between native and annealed oat starches may have been either less pronounced than that shown in Fig. 4.14.B or annealed oat starch may have been hydrolyzed to a lesser extent than its native counterpart. However, it is difficult to test this hypothesis, since amylose leaching occurs when annealing temperature exceeds 50°C.

4.5.7 Enzymatic Hydrolysis

The extent of hydrolysis (after 72 h) of native and annealed starches by porcine pancreatic α-amylase are presented in Table 4.26. Among native starches, the extent of hydrolysis after 72 h was respectively 63, 32, 5.4 and 65.0% in wheat, oat, potato and lentil starches.

Annealing decreased the extent of hydrolysis of wheat, potato and lentil starches by 3, 1.3 and 5%, respectively, but increased that of oat starch by 14% (Table 4.26). The action of α-amylase on raw starch granules depends on the penetration of the enzyme inside the granule and particularly on its capacity to attack the granular surface. The initial attack is mainly on the amorphous regions of the granule, which then increases the susceptibility of the exposed crystalline regions to the enzyme. Consequently, any changes in the degree of susceptibility of native starches towards hydrolysis by α-amylase on annealing would be influenced by changes in: 1) the amount of granular surface exposed to the action of α-amylase; 2) the degree of interaction between starch components in the amorphous regions and 3) the degree of packing of double helices within the starch crystallites. Therefore, the observed decrease in hydrolysis (lentil > wheat > potato) is probably influenced by an interplay of factors 2 and 3 (since the amount of granular surface exposed to α-amylase remains unchanged on annealing). However, the increased susceptibility of annealed oat starch granules towards hydrolysis by α-amylase is largely due to the greater degree of contact between α-amylase and the surfaces of the separated granules (Fig. 4.12.A,B).

Table 4.26 Hydrolysis[a] of native and annealed starches by porcine pancreatic α-amylase

Starch source	Treatment	Hydrolysis[b] (%)	% Change
Wheat	Native Annealed[c]	66.1±0.7 63.3±0.5	4.2 (decrease)
Oat	Native Annealed	32.0±0.6 46.2±1.4	44.4 (increase)
Lentil	Native Annealed	64.6±1.4 59.7±0.5	7.6 (decrease)
Potato	Native Annealed	5.4±0.3 4.1±0.2	24.1 (decrease)

[a] Hydrolysis for 72 h in phosphate buffer (pH=6.9).

[b] Values are means of three replicates ± SD.

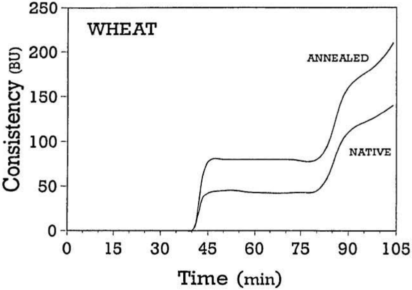
[c] Annealing was carried out at 50°C, 75% moisture for 72 h.

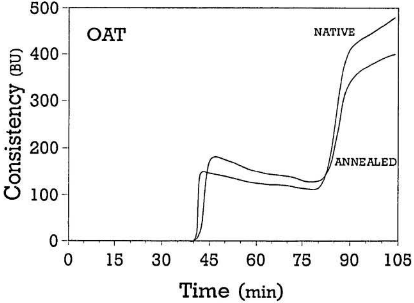
4.5.8 Pasting curves

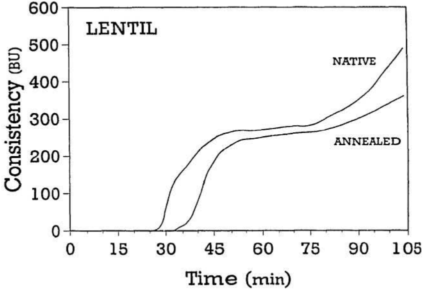
The pasting curves of native and annealed (50°C, 75% for 72 h) starches are presented in Fig. 4.15.A-D. The pasting temperature of wheat, oat, lentil and potato starches increased, respectively, by 0.5, 1.0, 9.0 and 7.0°C. The maximum viscosities of wheat (40 BU) and oat (150 BU) starches were attained at 95°C. The corresponding values on annealing were 80 BU (wheat) and 180 BU (oat). However, maximum viscosities of native and annealed potato starches occurred respectively, at 75 and 90°C. The maximum viscosity of annealed potato starch (2020 BU) was less than that of its native (2120 BU) counterpart. However, at 95°C the viscosity of native and annealed potato starches were, respectively, 1180 and 1680 BU. Native and annealed lentil starches behaved differently from the above starches in exhibiting continuously rising viscosities throughout the heating cycle. Furthermore, at 95°C the viscosity of annealed lentil starch was lower than that of its native counterpart by 70 BU. The stability of the starch granule towards shear (during the 30 min holding period at 95°C) increased on annealing (Fig. 4.15.A-D). The increase was more pronounced in potato than in the other starches.

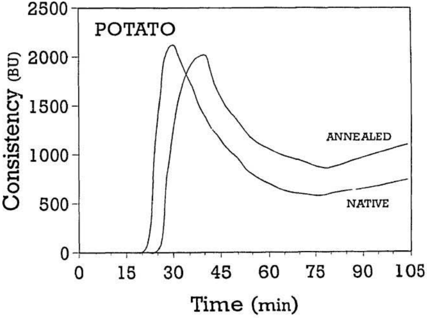
These results on native and annealed potato starch (Fig. 4.15.D) agree with those of Stute (1992) with respect to the temperatures at maximum viscosities and also with the higher viscosity of annealed starch (both at 95°C and at the end of the 95°C holding period). However, the difference in maximum viscosities between native and annealed potato starches in the

Fig. 4.15. Brabender amylograms of native and annealed (50°C, 75% moisture for 72 h) starches.


WHEAT
ANNEALED
NATIVE
Consistency (BU)
250
200
150
100
50
0
0
15
30
45
60
75
90
105
Time (min)


OAT
NATIVE
ANNEALED
Consistency (BU)
500
400
300
200
100
0
0
15
30
45
60
75
90
105
Time (min)


LENTIL
NATIVE
ANNEALED
Consistency (BU)
600
500
400
300
200
100
0
0
15
30
45
60
75
90
105
Time (min)


POTATO
ANNEALED
NATIVE
Consistency (BU)
2500
2000
1500
1000
500
0
0
15
30
45
60
75
90
105
Time (min)

present study (145 BU) was much lower than that reported (1330 BU) by Stute (1992). The above results suggest that annealing increases granular stability, this being more pronounced in potato and lentil than that in wheat and oat starches.

4.5.9. Retrogradation

The thermal characteristics of retrograded amylopectin [from native and annealed (50°C, 75% moisture for 72 h) starches] were studied by differential scanning calorimetry. The transition temperatures (T_o, T_p, T_c) and the enthalpy (ΔH_R) of retrograded amylopectin of native and annealed (50°C, 75% moisture for 72 h) starches at various time intervals (0.5 h to 20 days) of storage are presented in Table 4.27. The T_o, T_p and T_c of the gelatinization endotherm of native starches were different from those of annealed starches (Table 4.23). The reasons for this have been discussed earlier.

The retrogradation endotherm appeared after 3 days of storage in native wheat, potato and lentil starch gels. Whereas, for native oat starch gel, the corresponding time was 15 days (Table 4.27). Annealing did not hasten or delay the onset time of the retrogradation endotherm in wheat, potato and lentil starches. However, the retrogradation endotherm appeared after 6 days of storage in annealed oat starch. The differences in T_o, T_p and T_c of the retrogradation endotherm of native and annealed starch gels were only marginal and remained practically unchanged during the time course of retrogradation (Table 4.27). Furthermore T_o, T_p and T_c of the retrograded

Table 4.27. Thermal characteristics of native and annealed starch gels[a]

Starch source	Enthalpy[b] (ΔH) (J/g)							Transition temperature[c] (°C)		
	Storage time (days)									
	2	3	4	6	8	15	20	To	Tp	Tc
Wheat										
Native	-	0.9	1.8	3.9	5.3	7.1	7.1	47.5	57.2	60.7
Annealed[d]	-	2.1	3.1	4.4	5.9	8.0	8.8	47.2	57.4	60.2
Oat										
Native	-	-	-	-	-	1.3	1.9	46.8	57.6	60.9
Annealed[d]	-	-	-	1.3	1.5	2.1	2.5	47.5	58.3	59.9
Lentil										
Native	-	0.6	1.3	2.5	3.4	6.0	8.4	47.1	57.9	62.8
Annealed[d]	-	1.4	3.0	4.7	5.5	10.2	11.8	47.2	57.6	63.0
Potato										
Native	-	1.0	2.4	6.0	7.5	9.8	11.2	50.6	57.2	69.2
Annealed[d]	-	3.4	4.7	10.5	12.7	16.5	16.5	49.8	58.3	70.1

[a] Values are averages of three determinations.

[b] Average standard deviation = 5% of the value.

[c] Average standard deviation = 0.6°C.

[d] Annealed at 50°C and 75% moisture for 72 h.

starch gels were lower than that for the gelatinization endotherm. In native and annealed starch gels, the transition temperature range (Tc - To) of the retrogradation endotherm was broader (Table 4.27) than that of the gelatinization endotherm (Table 4.23). This was most pronounced in potato starch. *Unlike* T_o, T_p and T_c, the enthalpy of the retrogradation endotherm (ΔH_R) was greatly influenced by annealing. After 20 days of storage, the ΔH_R (J/g) of gels (Table 4.27) from native starches were 7.1 (wheat), 1.9 (oat), 8.4 (lentil) and 11.2 (potato). These values increased on annealing by 1.7, 0.6, 3.4 and 5.3 J/g in wheat, oat, lentil and potato respectively (Table 4.27).

It has been shown that annealing induces double helical formation between the outer branches of amylopectin in starch (however, crystallinity changes were only marginal). Furthermore, as shown in Table 4.25, annealing did not significantly affect the extent of amylose leaching in any of the starches (amylose leaching and its implication on the enthalpy of retrogradation has been discussed earlier). These results suggest that the increased ΔH_R in annealed starches (Table 4.27) probably reflect their double helical content prior to gelatinization. During retrogradation reassociation of starch chains (that were separated during gelatinization) would be more extensive among those that were originally a part of the double helix (due to closer proximity and better alignment), than those that may have been present as single chain helices or as extended chains within the ungelatinized granule.

4.5.10 Gel strength

Gel strength (after a storage period of one day) of annealed (50°C, 75% moisture for 72 h) starches were higher than those of their native counterparts (Table 4.28). However, in wheat and oat starches this difference was only marginal. The extent of this increase among the starches followed the order: potato > lentil > wheat > oat. As discussed earlier (Section 4.4.10), the gel strength is influenced by the interplay between the extent of decrease in swelling factor and amylose leaching. In all starches the amylose leaching was only marginally affected by annealing (Table 4.25). However, the decrease in swelling factor was significant (Table 4.24), being more pronounced in potato and lentil than with wheat and oat starches. Thus, the increase in gel strength on annealing would be mainly influenced by the extent of decrease in swelling factor. This would then explain the order of increase in gel strength among the starches.

Table 4.28. Gel strength of native and annealed starch gels (40%) after storage for one day at 25°C

Starch source	Treatment	Gel strength[a] (g)
Wheat	Native	25±0.5
	Annealed[b]	27±1.0
Oat	Native	11±0.7
	Annealed	12±1.0
Lentil	Native	101±4.0
	Annealed	225±3.0
Potato	Native	23±2.0
	Annealed	56±3.0

[a] Values are averagesof three determinations ± SD.

[b] Annealed at 50°C and 75% moisture for 72 h.

CONCLUSIONS

An evaluation of the quantity and composition of lipids associated with wheat, oat, lentil and potato starches revealed that there was a great variation among the starches with respect to the major components of the lipid classes (NL, GL and PL) in both CM (2:1, v/v) and PW (3:1, v/v) extracts. Cereal (wheat and oat) starches showed higher TSL content than those of legume (lentil) and tuber (potato) starches. The combined action of CM (2:1, v/v) and PW (3:1, v/v) resulted in almost complete removal of starch lipids from oat, lentil and potato. However, in wheat starch, the solvent extraction efficiency (%TSL) being 96.3%., suggested the presence of solvent un-extractable, strongly bound lipids. Neutral lipids (NL) formed the major lipid class in the CM extracts of all starches investigated, while in PW extracts these were NL, phospholipids (PL) and glycolipids (GL) in lentil; NL and PL in potato and PL in wheat and oat. Palmitic, linoleic and oleic were the major fatty acids in NL, GL and PL fractions of both CM and PW extracts. The monoacyl lipid content in both CM and PW extracts of wheat, oat, lentil and potato starches amounted to, respectively 88.2%, 60.0%, 29.5% and 49.6%. Since it is the monoacyl lipids that interact most strongly with the amylose helix, their removal by defatting would therefore result in greater functionality changes in starches.

Defatting of starches with PW (3:1, v/v) altered their physicochemical properties. The effect was pronounced to various degrees depending on the starch source. Increased relative crystallinity and better lipid complexing ability of potato and lentil starches after defatting suggested that the moisture and thermal energy increased the mobility of entrapped amylose chains, resulting in their release in to the amorphous regions of the granule and facilitated the development of a better molecular order within the granule. The change in the X-ray pattern of potato starch from a B-type to a combination of A- and B-types suggested that a clustering of the outer "A" chains of amylopectin may have occurred, resulting in the formation of a close-packed arrangement of double helices. Defatting increased the thermal stability, susceptibility towards α-amylolysis and retrogradation of all native starches. These changes in physicochemical properties were more pronounced in starches from potato and lentil than wheat and oat. Overall, it was evident that the effect of defatting with PW (3:1, v/v) on the starch granule structure and physicochemical properties depends on the type of crystalline structure (A, B or C), nature and composition of the extracting solvent system, maximum temperature experienced by the starch granules, extent of association between amylose and amylopectin chains in the native granule, and on the lipid content.

Heat-moisture treatment did not cause any significant changes on starch granule size and shape. However, in oat starch (occurs as compound granules), granules were less compactly packed after heat treatment. In wheat, oat and lentil starches, the above treatment increased X-ray intensities without causing any significant changes in the d-spacings. Furthermore, the enthalpy of gelatinization (ΔH) remained unchanged after treatment. This suggested that thermal energy and moisture may have caused the existing double helices to shift within the crystallites and to assume a crystalline array that is more closely packed and ordered. However, B-type starches such as those from potato and yam were found to be highly sensitive to heat-moisture treatment, and they underwent crystal disruption (decreased X-ray intensities and ΔH) and transformation ($B \rightarrow B + A$) during treatment. This probably occurred due to rupture of the hydrate water bridges linking adjacent double helices, which form the unit cells of B-type crystals. Although, in B-type crystals, direct hydrogen bonds between adjacent helices may have remained intact. The overall decrease in magnitude of the bonding forces between adjacent helices would cause them to move apart and assume orientations that are not in perfect parallel crystalline array. The X-ray pattern of native lentil starch (generally classified as C-type starch) did not exhibit any spacings that were characteristic of the "B" pattern. This meant that native lentil starch contained mainly A-type unit cells. Hence, lentil starch showed similar responses as observed in cereal starches. In tuber starches (B-type), the increase in gelatinization temperatures (T_o, T_p and T_c) in spite of the crystal destruction indicated interactions within amorphous regions of the granules. Physical separation of compound granules in oat starch and the disruption of crystals in potato and yam starches after heat-treatment may explain the increased susceptibility of these starches towards α-amylolysis. Heat-moisture treatment increased thermal and shear stability of all native starches. Furthermore, in cereal starches (wheat and oat), heat-moisture treatment induced the formation of amylose-lipid complexes. Overall, the results showed that heat-moisture treatment modifies the physicochemical properties of starch by altering the extent of starch chain associations within the amorphous regions and the degree of crystalline order. The magnitude of these changes were found to be dependent upon treatment conditions (starch moisture content, temperature and duration of treatment) and on starch structure and composition (crystal type, amylose content, lipid content, chain lengths of amylose and amylopectin molecules and the degree of proximity between these starch molecules).

Annealing (50°C, 75% moisture for 72h) did not affect the shape and size of the native granules from wheat, oat, potato and lentil. However, granules of oat starch which appeared aggregated in the native state were less compactly packed after annealing. In all starches, annealing caused only marginal changes in X-ray pattern and intensities. It was interesting to note that upon annealing the increase in T_o and ΔH did not begin concurrently, and the rate of increase in ΔH was slower in comparison to that of T_o. Furthermore, the gelatinization temperature range (T_c-T_o) decreased on annealing. The results suggested that crystalline perfection and double helical content within the starch granules increase on annealing. These increases were found to be influenced by the interplay of the following factors: 1) annealing moisture, 2) proximity of the annealing temperature to T_o, 3) chain lengths of the starch components, 4) the degree of intermingling of amylose and amylopectin within the native granule and 5) the lipid and amylose contents.

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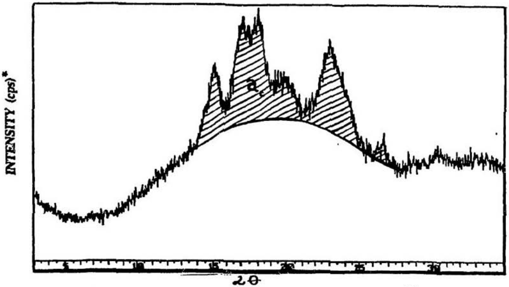
APPENDIX

APPENDIX 1



$$X = a_c / A_c$$

X = Relative crystallinity.

a_c = Area under the crystalline peaks (shaded ▨) of starch sample

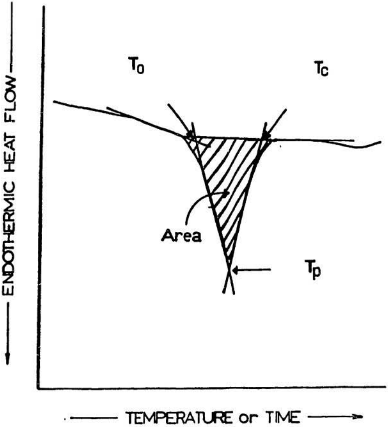
A_c = Area under the crystalline peaks of a 100% crystalline (quartz) sample (not shown in the diagram).

*Counts per second

d-spacing of a crystal is calculated by the following equation:
$n\lambda = 2d\sin\theta$ ——→ Bragg's equation.

Where: n = Phase number (usually 1); λ = Wave length (1.5478 nm);
d = Crystal d-spacing (Å) and θ = Diffraction angle.

APPENDIX 2



T_o = Onset temperature of gelatinization

T_p = Peak temperature of gelatinization

T_c = Conclusion temperature of gelatinization

AREA : Used for enthalpy calculation

Appendix 3 - Heterogeneous hydrolysis of native and defatted starches in 2.2N HCl at 35°C.

Starch source	Treatment	Hydrolysis (%, w/w)					
		Time (Days)					
		2nd	5th	7th	10th	12th	20th
Wheat	Native Defatted[a]	9.7±0.2 9.3±0.5	31.2±0.5 30.7±0.7	45.4±0.6 45.1±0.5	60.8±0.7 61.4±1.0	66.0±0.6 65.2±0.9	77.8±0.8 78.5±1.5
Oat	Native Defatted	13.5±0.1 13.2±0.4	37.3±0.3 36.5±0.6	49.7±0.4 48.9±0.9	62.5±0.5 61.7±1.2	69.5±0.5 68.8±1.3	74.6±0.6 73.9±0.9
Lentil	Native Defatted	7.1±0.2 10.3±0.2	26.3±0.2 27.3±0.8	37.5±1.3 37.1±0.8	52.5±0.3 50.4±0.7	58.1±0.5 55.4±0.6	75.0±0.4 70.5±0.7
Potato	Native Defatted	5.2±0.1 9.3±0.3	22.4±0.4 26.2±0.2	36.6±0.5 37.8±0.2	52.3±0.2 50.4±0.6	57.8±0.7 55.2±1.0	75.4±0.5 72.2±0.8

Values are averages of three determinations ± SD.

[a] Soxhlet extraction with hot n-propanol/water (3:1, v/v) for 7 h.

Appendix - 4: Heterogeneous hydrolysis of native and heat-moisture treated starches in 2.2N HCl at 35°C.

Starch source	Treatment	Hydrolysis (%, w/w)							
		Time (Days)							
		2nd	5th	7th	9th	10th	12th	15th	20th
Wheat	Native	9.6±0.2	31.9±0.7	46.8±0.6	58.0±1.2	63.4±1.3	67.6±0.8	--	79.4±1.3
	HMT[a]	8.1±0.4	28.1±0.5	44.2±0.4	51.6±0.7	56.4±0.9	61.2±1.1	--	73.3±1.0
Oat	Native	12.8±0.6	38.8±0.7	50.3±0.5	--	63.6±1.3	69.5±0.9	76.2±1.0	79.7±0.7
	HMT	13.3±0.4	41.2±0.9	52.4±0.6	--	66.8±1.0	73.4±1.2	78.5±0.8	82.1±0.5
Potato	Native	5.9±0.3	23.1±0.1	36.7±0.5	--	52.4±0.8	56.1±0.9	--	74.8±1.0
	HMT	8.6±0.4	25.4±0.3	37.2±0.9	--	45.7±1.3	50.6±0.5	--	66.4±0.8
Yam	Native	4.3±0.1	17.2±0.4	25.7±0.7	--	37.4±0.6	43.5±0.4	--	55.2±0.6
	HMT	5.7±0.3	20.5±0.2	27.0±0.3	--	35.3±0.3	41.8±0.4	--	51.3±0.9
Lentil	Native	7.3±0.2	25.7±0.5	37.9±0.6	--	52.9±1.1	57.3±0.5	--	73.9±1.4
	HMT	5.1±0.3	22.8±0.8	34.8±0.9	--	49.1±0.6	54.0±0.8	--	67.4±1.0

Values are meansof three determinations ± SD.

[a] Heat moisture treated (HMT) at 100°C and 30% moisture for 16h.

Appendix 5 - Heterogeneous hydrolysis of native and annealed starches in 2.2N HCl at 35°C.

Starch source	Treatment	Hydrolysis (%, w/w)						
		Time (Days)						
		2nd	5th	7th	9th	10th	12th	20th
Wheat	Native	10.3±0.3	32.1±0.5	46.7±0.6	58.3±0.7	62.4±1.0	67.1±0.6	79.1±0.9
	Annealed[a]	8.8±0.4	29.6±0.3	44.1±1.1	55.0±0.4	58.1±0.8	64.2±1.1	73.0±0.7
Oat	Native	14.0±0.3	38.6±0.6	51.1±0.4	–	64.1±0.6	69.8±1.0	79.9±0.9
	Annealed	16.8±0.2	43.5±0.2	55.8±1.0	–	68.2±0.9	75.1±1.1	85.3±1.2
Lentil	Native	8.1±0.2	26.6±0.5	38.4±0.6	–	53.6±0.5	57.5±0.7	73.1±1.0
	Annealed	7.0±0.1	23.8±0.3	35.8±0.5	–	50.1±0.9	54.7±0.5	67.5±1.3
Potato	Native	7.4±0.1	24.5±0.8	36.2±0.4	–	52.3±0.6	57.7±0.9	76.2±0.6
	Annealed	6.1±0.1	22.0±0.3	34.8±0.5	–	49.8±0.4	55.1±0.8	71.8±0.9

Values are meansof three determinations ± SD.

[a] Annealed at 50°C, 75% moisture (w/w) for 72 h.