THE EFFECT OF HEAT-MOISTURE TREATMENT ON THE STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF TUBER AND ROOT STARCHES

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by

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ABSTRACT

Starch from the tubers potato (*Solanum tuberosum*), taro (*Alocassia indica*), new cocoyam (*Xanthosoma sagitifolium*), true yam (*Dioscorea alata*), and root cassava,(*Manihot esculenta*) crops was isolated and its morphology, composition and physicochemical properties were investigated before and after heat-moisture treatment (HMT) [100^{0}C, for 10h at a moisture content of 30%). Native starch granules ranged in diameter from 3.0-110 μm and were round to oval to polygonal with smooth surfaces. The total amylose content ranged from 22.4 – 29.3%, of which 10.1 – 15.5% was complexed by native lipid. The phosphorus content ranged from 0.01 – 0.1%. The X-ray pattern of potato and true yam was of the "B " type. Whereas, that of new cocoyam and taro was of the "A" type. Cassava exhibited a mixed "A+B" type X-ray pattern. The relative crystallinity, swelling factor, amylose leaching, gelatinization temperature range and the enthalpy of gelatinization of the native starches ranged from 30– 46%, 22– 54%, 5 –23%, 13 –19^{o}C and 12 – 18 J/g, respectively. Susceptibility of native starches towards hydrolysis by 2.2N HCl and porcine pancreatic ∝-amylase were 60–86% (after 12 days), and 4 – 62% (after 72h), respectively. Retrogradation was most pronounced in the "B" type starches. Granule morphology remained unchanged after HMT. The X-ray pattern of the "B" type starches was altered ("B"→ "A+B") on HMT. However, that of the other starches remained unchanged. HMT decreased swelling factor, amylose leaching, gelatinization enthalpy and susceptibility towards acid hydrolysis, but increased gelatinization temperatures and enzyme susceptibility. Extent of retrogradation and relative

crystallinity decreased on HMT of true yam and potato starches, but remained unchanged in the other starches. The foregoing data showed that changes in physicochemical properties on HMT are influenced by the interplay of crystallite disruption, starch chain associations and disruption of double helices in the amorphous regions.

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LIST OF ABBREVIATIONS

ΔH	Enthalpy of gelatinization
ΔH_R	Enthalpy of retrogradation
AACC	American association of cereal chemists
ACS	American chemical society
AM	Amylose
AML	Amylose leaching
AMP	Amylopectin
CL	Chain length
$\bar{C}Ln$	Number average chain length
CP/ MAS - NMR	Cross polarization magic angle spinning nuclear magnetic resonance
Da	Dalton
db	Dry weight basis
DMSO	Dimethylsolfoxide
DNS	Dinitrosalicylic acid
DP	Degree of polymerization
DP_n	Degree of polymerization-number average
DSC	Differential scanning calorimetry
ECL	External average chain length
F-AM	Lipid free amylose
FFA	Free fatty acids
GL	Glycolipids
GPC	Gel permeation chromatography
HMT	Heat-moisture treatment
ICL	Interior average chain length
LPL	Lysophospholipids
L-AM	Lipid amylose
MW	Molecular weight
PPA	Porcine pancreatic α-amylase
PL	Phospholipids
PW	Propanol water
SF	Swelling factor
SAXS	Small angle X-ray scattering
TG	Triglycerides
T_o	Onset of gelatinization temperature
T_p	Peak gelatinization temperature
T_c	Conclusion of gelatinization temperature
T_c-T_o	Gelatinization transition temperature range
v/v	Volume/volume
WAXD	Wide angle X-ray scattering and diffraction
w/v	Weight / volume

INTRODUCTION

Tuber and root crops are grown throughout the world in hot and humid regions, where with sun and rain, and little or no artificial inputs, they are able to grow in great abundance. They are the plants yielding starchy roots, rhizomes, corns, stem and tubers. Tuber and root crops contain 70-80% water, 16-24% starch and trace quantities (<4%) of proteins and lipids. Some of the root and tubers that are grown for edible purposes are: potato (*Solanum tuberosum*), sweet potato (*Ipomea batatas*), true yams [(*Dioscorea*) species (*D. alata*, *D. cayenensis*, *D. spicata*, *D. bulbifera*, *D. esculenta*, *D. abyssinia*)] arrowroot [West Indian arrowroot (*Maranta arundinacea*), Indian arrowroot (*Hutchenia caulina*), East Indian arrowroot (*Tacca leonto petaloides*), buffalo gourd (*Cucurbita foetidissima*), Kuzu (*Pueraria hirsuta*), Cassava (*Manihot esculenta*) and edible aroid root crops belonging to the family *araceae* which include five genera (*Colocassia, Xanthosoma*, *Amorphallus*, *Alocassia* and *Cytosperma*) (Hoover, 2001). The agronomic and phenotypic properties of tropical tuber and root starches are well documented, however, their structure and physicochemical properties have not been studied extensively. Therefore, intensive research and product development such as physical modification (heat-moisture treatment, annealing) and chemical modification (cross-linking, substitution, cationization) are needed to exploit tuber and root starches.

Heat-moisture treatment of starches is defined as a physical modification that involves treatment of starch granules at low moisture levels (< 35% moisture w/w) during a certain time period (15min –16h) and at a temperature (84 –120°C)

above the glass transition temperature (Tg) but below the gelatinization temperature. Under the above conditions, changes in X-ray pattern, crystallinity, starch chain interactions, granule swelling, amylose leaching, viscosity, gelatinization parameters, retrogradation, acid and enzyme hydrolysis have been shown to occur in cereal (Sair, 1967; Fukui and Nikuni, 1969; Lorenz and Kulp,1981, 82, 83; Donovan et al., 1983; Hagiwara et al., 1991; Radosta et al., 1992; Kobayashi, 1993; Maruta et al., 1994; Kawabata et al., 1994; Schierbaum and Kettliz, 1994; Franco et al., 1995; Hoover and Manuel, 1996; Takaya et al., 2000), tuber (Sair, 1967; Lorenz and Kulp, 1981,82; Donovan et al., 1983, Kuge and Kitamura, 1985, Stute, 1992, Kobayashi, 1993, Abraham,1993, Hoover and Vasanthan, 1994, Hoover et al., 1994., Schmiedl et al., 1998, Stoof et al., 1998 Collado and Corke, 1999, Collado et al., 2001) and legume (Hoover et al., 1993, Hoover and Vasanthan, 1994) starches. Changes to starch structure and properties on heat-moisture treatment have been found to vary with the source. For instance, tuber starches have been shown to be more susceptible than legume or cereal starches towards heat-moisture treatment (Hoover and Vasanthan, 1994, Jacobs and Delcour, 1998). Most of the studies on heat-moisture treated tuber and root starches have been on potato and sweet potato starches. Thus, it is difficult to ascertain whether changes observed during heat-moisture treatment of the above starches are truly representative of tuber and root starches. Furthermore, tuber and root starches exhibit different types of unit cell structures (A, B, A+B) [Hoover, 2001] Consequently, the magnitude of starch

chain realignment and/or interactions during heat-moisture treatment may vary widely among these starches.

The objective of this study was, therefore, to examine changes to starch structure and physicochemical properties on heat-moisture treatment (under identical time / temperature / moisture combinations) of some selected tuber and root starches. This study is of significance, since there is a growing interest in physical modification of starches for food and non-food applications.

2. Review of Literature

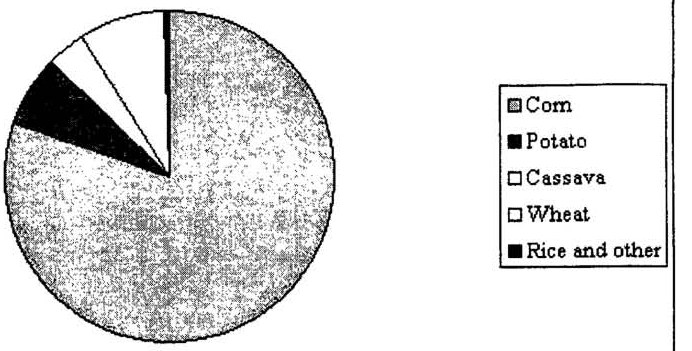
2.1 Starch – General introduction

Starch is the major reserve polysaccharide material of photosynthetic tissues and of many types of storage organs such as seeds, swollen stems, tubers and roots Starch is the second largest biomass, next to cellulose produced on earth. The most important source of starches are cereal grains, (40 to 90% of their dry weight), pulses (30 to 70%) and tubers (65 to 85%). Starch is predominantly produced in highly industrialized countries like the USA, EU and Japan (**Fig. 2-1 A and B**). Other starch containing raw materials from which starches are separated in small production units (mainly in Asia) are sago, palm, sweet potato, arrowroot, amaranth, sorghum, lotus, smooth pea, taro, cassava, mung bean, lentils, and wild rice.

Tuber and root starches such as cassava, arrowroot, taro, sago, sweet potato and yam have served as staple foods for people throughout the hot and humid regions of the world. These crops are naturally suited to tropical agro-climatic conditions, and they grow in great abundance with little or no artificial inputs. A recent study by the Post Harvest Management Services (Satin, 2001) found that, while exhaustive research has been carried out on their agronomic and phenotypic properties, tropical tuber and root crops have not benefited from the kind of value added research required for competitiveness on an international scale (Satin, 2001), and consequently, corn, potato, rice continue to dominate lucrative world markets for starches in food and non-food industries. At the present time, there is a dearth of information on tuber and root starches

Fig. 2-1 World starch production and distribution (Adapted from International Starch Institute, (1999)

Starch distribution world wide by raw materials (1997)



World starch production by region
1980: Inner wreath ~ 16,000,000t
1997: Outer wreath ~ 35,000,000t

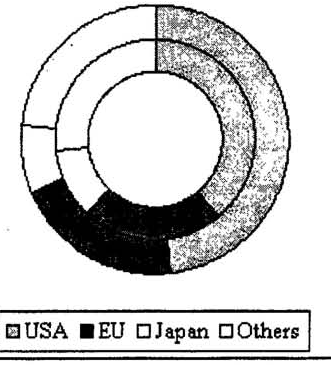
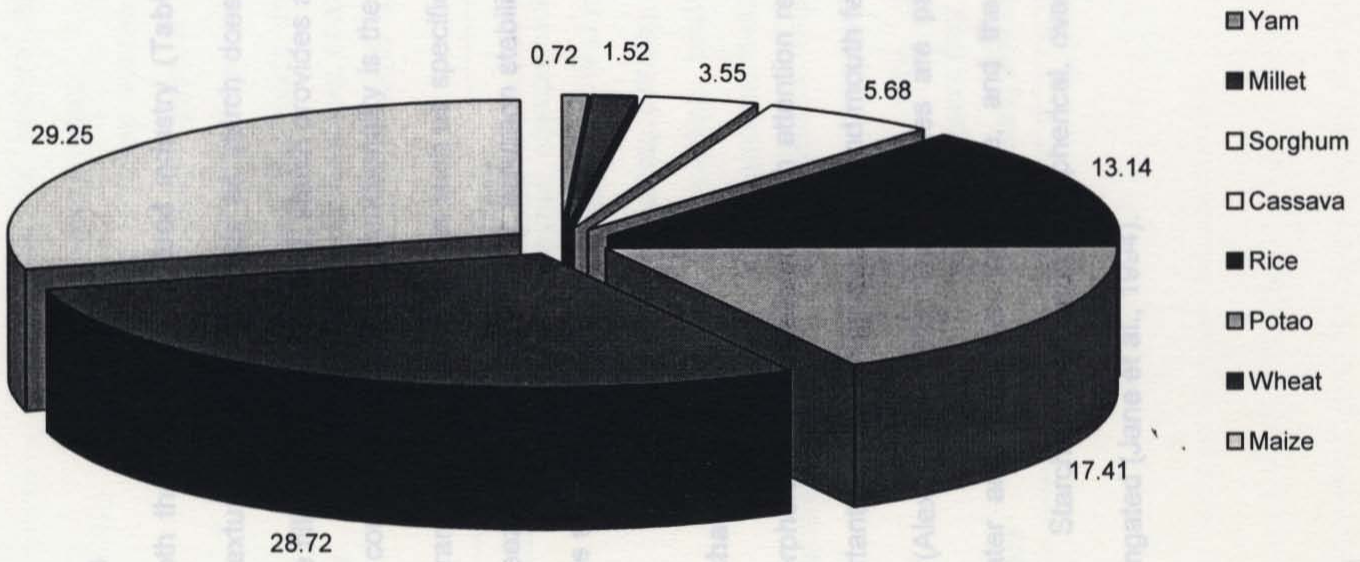


Fig. 2-2 Information available (% of publications) on various starches (Adapted from Satin, 2001)


0.72
1.52
3.55
5.68
13.14
17.41
28.72
29.25
Yam
Millet
Sorghum
Cassava
Rice
Potao
Wheat
Maize

(**Fig. 2-2**). It is therefore, clear that extensive research must be carried out on these starches if they are ever to become competitive with corn, wheat, rice, and potato.

2.2 Uses of starches

Starch is used in both the food and non-food industry (**Table 2-1**). No other ingredient provides texture to as many foods as starch does. Whether it is a soup, stew, gravy, pie filling, sauce or custard, starch provides a consistent shelf-satiable product that consumers rely upon. Functionality is the key to marketing starches in the wide range of food applications such as specific viscosity, mouth feel, plate coating, freeze-thaw stability, clarity, emulsion stability capacity, color, film-forming properties and anti-caking.

2.3 Starch granule characteristics

Granule size and morphology have received much attention recently, since size of granules are important in determining the taste and mouth feel of some starch based fat mimetics (Alexander, 1992). The granules are partially crystalline, insoluble in cold water and their size, the shape, and the composition are essentially genetical. Starch granules may be spherical, oval, polygonal, disk, kidney shaped or elongated (Jane et al., 1994).

Table 2-1. Some of the food and non-food applications of starch and its derivatives (Adapted Satin, 2001; and Galliard, 1987)

Food applications	Examples
Canning	filling viscosity aid, suspension aid, body or texture agent, aseptically canned product
Cereals and Snack	hot extruded snacks, chips, ready-to-eat cereals, pretzels
Bakery	pies, tarts, fillings, glazes, cakes
Batters and bread	coated fried foods, dry mix coating
Dressings and Soups	low-fat dressing, soups and chowder
Cooked Meat binder	pet foods, smoked meat

Non-food application	Examples
adhesives	hot-melt, stamps, wood adhesives
paper industry	internal sizing, filler retention
textile industry	wrap sizing, printing, fabric finishing
metal industry	sand casting binder, foundry core binder, sintered metal additive
cosmetic and pharmaceutical industry	dusting powder, make-up, face cream
mining industry	ore flotation, ore sedimentation
construction industry	asbestos, clay, plywood/chipboard adhesive, paint filler
Biodegradable plastics	Films, coating and food packaging

In general, cereal starch granules are small and polyhedric, where as, tuber starch granules are large and spherical or ellipsoid. Most of the tuber and root starches are simple granules, the exception being cassava and taro starches, which appear to be a mixture of simple and compound granules (Hoover, 2001). The sizes and shapes of tuber and root starch granules are presented in (**Table 2-2**).

2.3.1 Surface of starch granules

The outer surface of the starch granule plays an important part in many applications of starch, but there is a lack of definitive information on the nature of starch surface (Galliard and Bowler, 1987). When observed under a scanning electron microscope the surfaces of all granules of root and tuber starches appear smooth with no evidence of any fissures (Hoover, 2001). However, Fannon et al. (1992) discovered pores on the surface of corn, sorghum, and millet starch granules which are real anatomical features of the native granule structure and not artifacts of drying, specimen preparation or observation techniques . Surface pores on granules of corn, sorghum and millet are openings to channels that penetrate in a radial direction through the granule (Fannon et al., 1993; Baldwin et al., 1994; Huber and miller, 1997). Several researchers (Gallant, 1973, Fuwa et al., 1997; Planchot et al., 1997) have postulated that pores on the granule surface increase the accessibility of ∝-amylase into the granule interior. Planchot et al. (2000) have shown by dynamic light scattering, HPAEC-PAD and polyethylene glycol molecular probes, that wet starch granules

Table 2-2 Size and shape of starch granules from different botanical origin

Starch source	Shape of granules	Size of granules	References
1.Tuber and root			
Potato	oval , spherical	15 - 110	Sivak and Preiss, (1998); Moorthy, (1994)
Potato (waxy)	round, oval	14-44	McPherson and Jane, (1999)
Sweet potato	round, oval and polygonal	2 - 42	Lim et al., (1994); Seog et al., (1987)
Sweet potato (Diploid hybrid)	round, angular	8.5	Shiotani et al., (1991b)
Sweet potato (tetraploid hybrid)	round, angular	14.6	Shiotani et al., (1991b)
True yam (*Dioscorea alata*)	round , oval	6 - 100	Moorthy, (1994)
True yam (*Dioscorea rotundata*)	round , oval	10 - 70	Moorthy, (1994), Emiola and Delarossa, (1981)
True yam (*Dioscorea esculenta*)	round ,oval	1 - 5	Yu et al., (1999)
True yam (*Dioscorea abyssinica*)	round	29.2	Mariam and Schmidt, (1998)
True yam (*Dioscorea cayenesis*)	round, oval	28.5-30.6	Emiola and Delarossa, (1981)
True yam (*Dioscorea dumetorum*)	round oval	28.5-30.6	Emiola and Delarossa, (1981)
Elephant yam	round, polygonal	3-30	Moorthy, (1994)
Cassava	round	5 - 40	Moorthy, (1994)
Queensland arrowroot	oval , elliptical	13 - 57.6	Soni, et al., (1999)
West Indian arroroot	round, oval, polygonal	10-16	
Kuzu	polygonal	3 - 23	Soni and Agarwal, (1983); Suzuki et al., (1981)
Taro	polygonal	3 - 3.5	Lim et al., (1994)
Cont.,			
New cocoyam	Polygonal	10-50	Moorthy,(1994)
Lotus	rod like-round	15-40	Suzuki et al., (1992)
Buffalo gourd	oval to eliptical	2-24	Dreher and Berry, (1983)
Lily	elliptical, polygonal	30-35	Takeda et al., (1983); Jane et al, (1994)
2. Cereals			
Oat	compound	2-15	Jane et al., (1994)
Wheat	lenticular, polyhedral	20-35	Lineback, (1984)
Maize (normal)	round ,polyhedral	3-26	Blanshard, (1987)
Rice	polugonal	3-8	Jane et al., (1994)
3. Legume			
Lentil	ellipsoidal	10-20	Jane et al., (1994)
Mung bean	oval, irregular	10-27	Jane et al., (1994)
Chick pea	spherical, oval	10-27	Jane et al., (1994)

can be considered as a porous substrate permeable to low molar mass solutes such as malto oligosaccharides or small polyethylene glycols, Whereas, molecules with a hydrodynamic radius greater than 0.6nm cannot penetrate such a substrate.

2.4. Starch structure

2.4.1. Major components

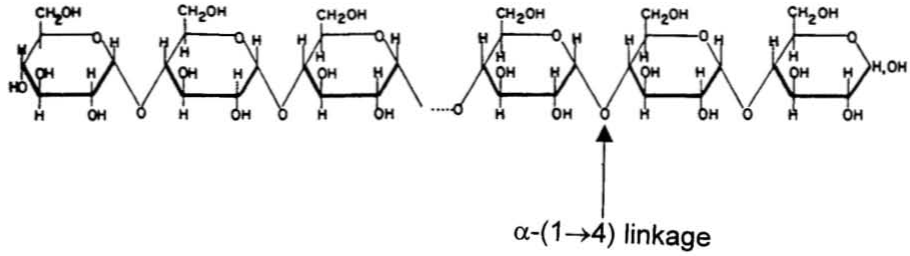
2.4.1.1. Amylose

2.4.1.1.1. Structure and conformation of amylose

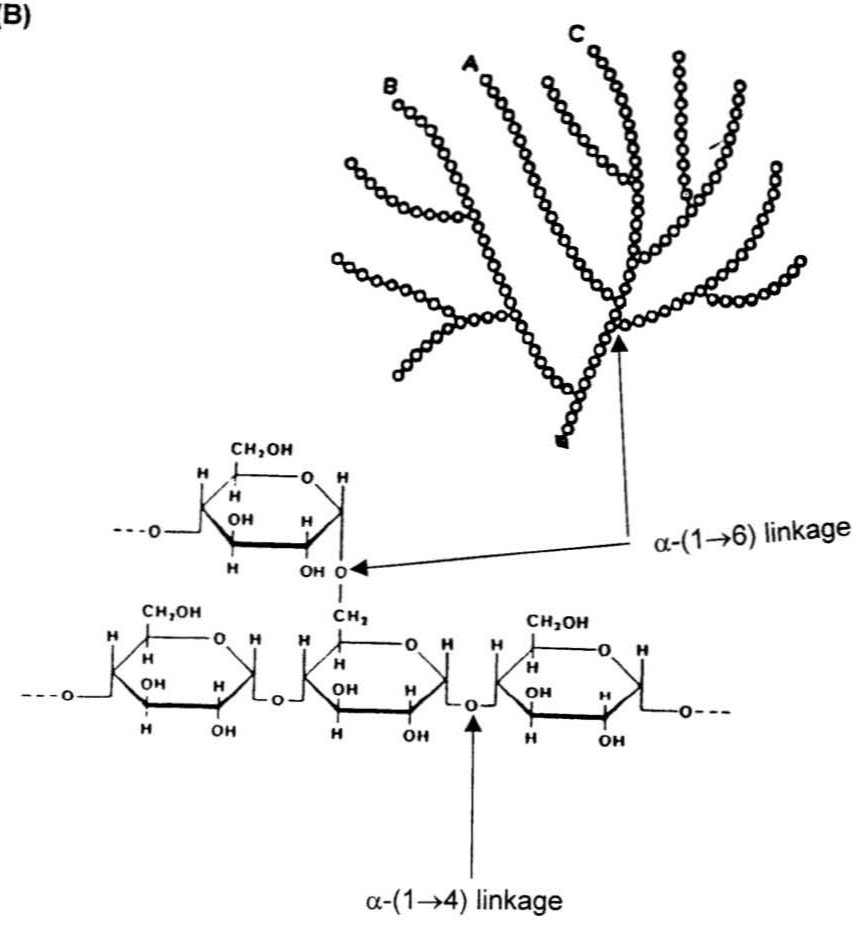
The two major components of starch are amylose and amylopectin. Amylose, the minor component, consists mainly of α-(1→4) linked glucose units (**Fig. 2-3 A**). The degree of polymerization (DP) of this linear polymer is usually in the range of 500-600 units (Jacobs and Delcour, 1998). However, a slight degree of branching (9-20 branch [α-(1→6] points per molecule) has been reported in amylose from various starches (Hizukuri et al., 1981). The side chains range in chain length from 4 to over 100 (Hizukuri et al., 1981; Takeda et al., 1987) and the extent of branching has been shown to increase with the molecular size of amylose (Greenwood and Thompson, 1959). Evidence of the occurrence of branching points in amylose is its incomplete conversion into maltose by β amylase: β amylolysis has been shown to vary from 73 to 95% (Morrison and Karkalas, 1990). The molecular weight of amylose has been reported to vary between 10^5 and 10^6 Da (Morrison and Karkalas, 1990; Hizukuri et al.,1989).

Fig.2-3 Structural representation of amylose (A) and amylopectin (B)

(A)



(B)



Amylose isolated from tuber and root starches, such as potato and tapioca have larger molecular sizes than those isolated from cereal starches, such as maize, rice, and wheat (Takeda et al., 1986). The conformation of amylose has been the subject of controversy and has been shown to vary from helical to an interrupted helix, to a random coil. In alkaline solutions (KOH) and in dimethyl sulfoxide (DMSO) amylose probably has an expanded coil conformation, while in water and neutral aqueous potassium chloride solutions it is a random coil with short, loose helical segments (Banks and Greenwood, 1971). Jane and Robyt, (1985) identified (using ^{13}CNMR) expanded and compact helical conformations in aqueous amylose solutions in the absence and presence of complexing agents, respectively. Physicochemical characteristics of amyloses of different botanical origin are presented in (**Table 2-3**).

2.4.1.1.2 Location and co-crystallization of amylose in the starch granule

Comparison of the amylose content in starch of different maturities has suggested that amylose is more concentrated at the periphery of the starch granule (Boyer et al., 1976). Blanshard, (1986) postulated that amylose is separated from amylopectin in the granules of maize and wheat starches and is partly co-crystallized with amylopectin in potato starch. Cross-linking of maize and potato starches and characterization of the products by molecular sieve chromatography showed that amylose was cross linked with amylopectin and that there was no cross-linking between amylose molecules (Jane et al., 1992;

Table 2-3 Physicochemical characteristics of amylose of different starches (Adapted from Hoover, 1995)

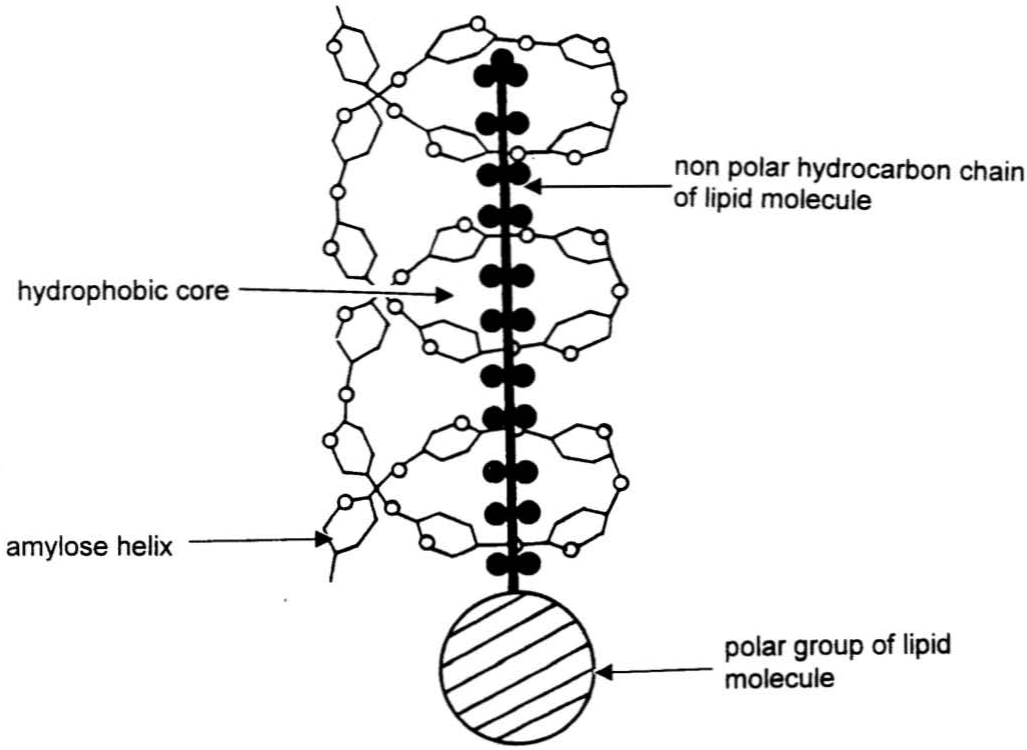
Starch source	Iodine binding capacity g/100g)	Limiting viscosity number (ml/g)	Degree of polymerization	β amylolysis limit (%)
1. Tuber and roots				
Potato	20.0	266-507	-	-
Cassava	20.5	-	-	-
Sago	19.9	-	-	-
Sweet potato	18.4-18.8	384	-	-
Yam	19.9	-	-	-
Buffalo gourd	20.2	384	-	-
2. Cereals				
Maize	20.1	179	930	84
Rice	20.0-21.1	180-216	990-1110	73-84
Barley	19.1-19.9	-	1700-1900	-
Sorghum	14.3-15.3	-	-	-
Wheat	20.5	-	300	82
3.Legume				
Lentil	19.6	258-264	-	-
Mung bean	19.4	188	-	-
Smooth pea	18.8-19.2	251	-	-
Wrinkled pea	17.9-19.2	180-194	-	-
Navy bean	18.5	136-150	-	-
Black bean	22.0	174	-	-

Kasemsuwan and Jane, 1994). These data suggested that in granular starch, amylose molecules do not exist in the form of bundles at the amorphous region but, rather, are interspersed among the amylopectin molecules. Biliaderis, (1998) has postulated that some amylose molecules participate in double helices with amylopectin and thereby become less prone to aqueous leaching or complexation with iodine. Jenkins and Donald, (1995) have shown by studies on normal, waxy and amylomaize starches that although the amylopectin cluster size remained constant, increasing the amylose content had the effect of increasing the size of the crystalline portion of the cluster. The above authors postulated that amylose acts to disrupt the packing of amylopectin chains within the crystalline lamella. Supporting evidence for this hypothesis was provided by the apparent reduction in crystalline lamella electron density with increasing amylose content.

2.4.1.1.3. Complex formation of amylose and V-polymorph

Amylose can form film and complexes with ligands. When amylose forms complexes with various ligands another crystallographically distinct structure of starch, the V-polymorph is formed (Biliaderis, 1998). Rappenecker and Zugenmaier, (1981) and Hinrichs et al., (1987) have shown that the chain conformation in V-amylose is a left handed single helix with six residues per turn and for complexes with aliphatic alcohols and monoacyl lipids the rise per monomer residue is approximately 1.32-1.36A° (**Fig. 2-4**). However, when the

Fig. 2-4 Schematic illustration of amylose - lipid inclusion (Adapted from Carlson et al., 1979)


non polar hydrocarbon chain of lipid molecule
hydrophobic core
amylose helix
polar group of lipid molecule

ligand is bulkier than a hydrocarbon chain, helices of seven or eight glucose residues per turn are also feasible (French and Murphy, 1977). X-ray diffraction diagrams of granular starches do not usually show the presence of V-structures, with the exception of wrinkled pea starch, amylomaize, and some other maize genotypes (dull, su) [all with amylose contents greater than 30% (Zobel,1988a; Gernat, et al., 1993; Zobel, 1992)]. The lack of V-type characteristics peaks upon X-ray analysis does not necessarily prove the absence of amylose-lipid complexes. It merely indicates the absence of organized helices into well-defined three -dimensional structures (Biliaderis, 1998). Recently, ^{13}CCP/MAS-NMR studies provided the proof for the presence of V-conformation in granules of maize, oat, barley, and wheat starches (Morgan et al., 1995; Morrison et al., 1993). The features in the CP/MAS-NMR spectrum indicative of single V-amylose helices were: (1) the presence of a broad resonance peak at 3I ppm (corresponds to mid chain CH_2 carbons of monoacyl lipids), which reflects a solid state structure of lipids due to steric constrains in the helical cavity, and (2) a signal of C-1 at 103-104 ppm attributed to V conformation. These resonances are enhanced in linterized starches (Morrison et al., 1993). Gernat et al. (1993) also showed the existence of V structure (indicative of amylose-lipid complex in native starch granules) by X-ray scattering studies on enzymatically degraded wheat starch. Development of V-type polymorph can be induced by heat-moisture treatments of starch (18-45% moisture, 90-130^{0}C for 1-16h) [Zobel, 1988a], and by extrusion cooking (Mercier, et al., 1979 and 1980) or simply by gelatinization and cooling of starch dispersions. Under such hydrothermal

conditions there is increased chains mobility which leads to complex formation between amylose chains and naturally occurring monoacyl lipids. Calorimetry and X-ray diffraction have been widely used to study the amylose-lipid complex (Hoover and Hadziyev, 1981; Biliaderis et al., 1993; Karkalas et al., 1995; Biliaderis et al., 1986; Biliaderis and Senaviratne, 1990; Galloway et al., 1989).

The formation of a helical complex between amylose and iodine gives rise to the typical deep blue color of starch dispersions stained with iodine and forms the basis for quantitative determination of amylose content (Hoover, 2001). Understanding the supermolecular structure, stability, and transformations between the various forms of amylose-lipid complexes is of great fundamental and technological importance, considering the multifunctional role of lipids in starch based products. For, example, incorporation of monoglycerides in the dough is known to retard starch retrogradation and bread firming (Biliaderis et al., 1991; Krong, 1971). Similarly, monoglycerides added to dried potato granules prevent stickiness (Hoover and Hadziyev, 1981), Improvements in structural integrity of cereal kernels (eg, rice parboiling) [Biliaderis et al., 1993] as well as decreased swelling, solubilization, and thickening power (Galliard and Bowler, 1987).

2.4.1.2 Amylopectin

Amylopectin is the major component of starch granules with an average molecular weight of the order 10^7- 10^9 (Aberle, 1994). It is composed of linear chains of (1→4)-α-D-glucose residues connected through (1→6)-α-linkages (5-

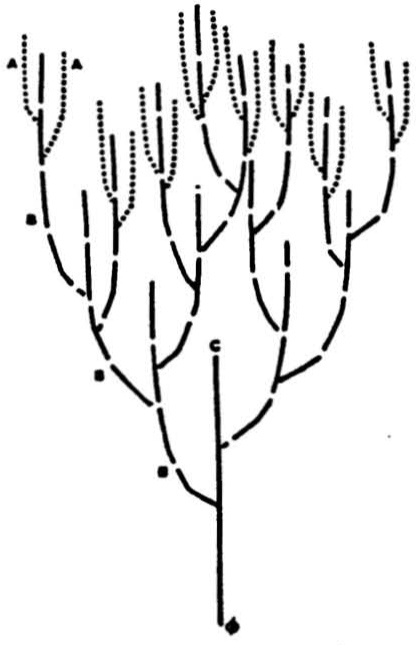
6%), leading to highly branched, compact, structure (**Fig. 2-3 B**). The average unit size of chains of amylopectin is 20-25 (Hizukuri, 1985). Kobayashi et al. (1986) have shown that amylopectin molecules contain several distributions of chains (A, B, and C) which differ in their chain length. The unbranched A-chains are linked to B- chains and do not carry any other chains, the B-chains (B1-B4), carry one or more A-chains and /or B-chains, while the C-chain contains the reducing end group of the molecule (**Fig. 2- 5**). The chain length of A and B1 chains and that of B2-B4 are 14-18 and 45-55, respectively (Hoover, 2001). The molar ratio of short to long chains varies between 3:1 and 12:1, depending on the botanical origin of starch (Hizukuri, 1985). Cereal starches generally have shorter chains in both long and short chain fraction and larger amounts of the short chain fractions, compared with those of tuber and root starches (Hizukuri, 1985; Hizukuri, 1986). Furthermore, Hizukuri et al. (1989) have shown that the branching points of amylopectin molecules are not randomly distributed but are clustered and the inner adjacent linear segments form thin crystalline lamella domains (5 - 7 nm width). Due to the short length of the unit chains, amylopectin does not form a stable complex with amylopectin and binds only trace amounts (< 0.6%) forming a reddish brown complex (λ_{max} at 530-540nm). Calorimetry studies have provided indirect evidence for weak interaction between amylopectin and lipids (Eliasson and Ljunger, 1988). The β-amylolysis limit of amylopectin (55 - 60%) is significantly less than that of amylose, since the activity of β-amylase is sterically hindered by the branch points in amylopectin. Morrison

Fig. 2-5 Chain segment designations and chain clusters projected for amylopectin

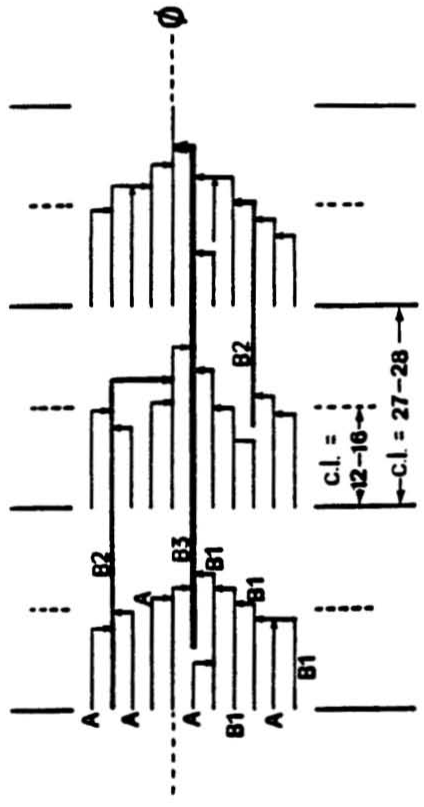
(A) Zobel, (1988b - with permission) ϕ = reducing end

(B) Hizukuri, (1986 - with permission), ϕ = reducing chain-end, — = (1→4)- α-D-glucan chain, → = α-(1→6) linkage

A



B



and Karkalas, (1990) have shown that there are three types of amylopectins: (1) high molecular weight amylopectin with A and B chains 5 - 15 glucose residues longer than normal: (2) amorphous amylopectin with similarly extended A and B chains: (3) normal amylopectin which contains very long chains (CL 85 - 180) with frequent branching.Several legume and tuber starches contain type 1 and 2 (Banks and Greenwood, 1975), while Hizukuri, (1996) has shown that tuber and root starches contain fewer A and B1 chains and more B2 and B3 chains than cereal starches. The blue value, iodine affinity, organic phosphorous,β-amylolysis, and average chain length amylopectin in tuber and root starches have been shown to be in the range 0.104 - 0.245, 0.06 - 1.1, 21 - 900, 43.8 - 64.8 and 19- 44, respectively (Hoover, 2001).

2.4.2 Minor components of starch

The most abundant components of starch are amylose and amylopectin, which constitute almost of 100% starch dry matter. Apart from these main components, smaller amounts of other components such as proteins, free fatty acids, other lipids and phosphate groups may also be present in amounts depending on the botanical source and starch isolation procedure. (Morrison and Laignelet, 1983; Morrison and Karkalas,1990) and they impart dramatic effect on the physicochemical properties of starch.

Table 2-4 Proximate composition of starches from different botanical origin

Starch source	Starch yield	Amylose content	Total lipid (%)	Phosphorous (% db) organic	inorganic	Notrogen (%)	References
Potato	32	25.4	0.19	0.089	0.001	0.1	Kim et al., (1995); Lim et al., (1994)
Potato (Waxy)	-	-		0.069	0.001		McPhersona nd Jane, (1999)
Sweet potato	30	19.1	0.06-0.6	0.012	-	0.006	Collado et al., (1999); Lim et al., (1994);Tian et al., (1991)
Sweet potato (Diploid hybrid)	-	28	-	-	-	-	Shiotani et al., (1991b)
True yam (*Dioscorea abyssinica*)	-	29.7	0.05	-	-	0.08	Mariam and Schmidt, (1998)
True yam (*Dioscorea alata*)	84.6	22.8-30	0.03	-	-	0.33	Emiola and Delarossa, (1981); Gallant et., (1982)
True yam (*Dioscorea dumetorum*)	88.0	10-24.6	.04	-	-	0.29	Emiola and Delarossa, (1981); Gallant et al., (1982)
True yam (*Disocorea esculenta*)	-	30	-	-	-	0.013	Gallant et al., (1982); Rasper and Coursey, (1967)
Elephant yam	35	-	-	-	-	-	Rani et al., (1998)
Taro (old cocoyam)	55.1	21.4	.39	0.021	-	.0019	Lim et al., (1994)
New cocoyam	43.8	23.7	-	-	-	-	Moorthy, (19940; Lauzon et al., (1995)
Cassava	-	18.6-23.6	0.1	0.008	0.001	0.009-0.0131	Lim et al., (1994); Defloor et al., (1998);
Arrow root (W.Indian)	-	19.4	0.32	-		0.03	Erdman, (1986)
Cont.,							
Arrow root (Queensland)	60.3	38	0.3	-		0.01	Soni et al., (1990)
Kuzu	34.2	15.1-21.0	0.46	0.005	-	-	Soni and Agarwal, (1999); Suzuki et al., (1981)
Buffalo gourd	-	23.2	0.92-1.14	0.01-0.06	-	0.28--0.49	Dreher and Berry, (1983)
Lotus	-	15.9	-	48 (ppm)	-	-	Suzuki et al., (1992)
Lily	-	26.8	-	60(ppm)	33(ppm)	-	Takeda et al., (1983); Jane et al., (1994)
2. Cereals							
Oat	.	20.92	1.1-1.7	0.06-0.07 (total)		0.05	Gibinski et al., (1993)
Oat	-	21.1	1.13	-		0.03	Hoover and Vasanthan, 1992, 1994
Wheat	-		0.8	0.06 (total)		0.07	
Maize (normal)	-	23.7	0.7	0.02 (total)		0.10-0.13	
3. Legume							
Lentil	-	36.7	0.14	-		0.02	Hoover and Vasanthan, (1994)
Pea	33.7	42	0.31	-		0.04	Ratnayake et al.,(2001)

2.4.2.1 Lipids

The lipid composition of starches from a variety of sources has been described by Vasanthan and Hoover, (1992), while the lipid in cereal starches have been extensively reviewed by Morrison, (1988, 1995). In general, cereal starches contain 1-2% lipids (Tester, 1997) but the content is lower in waxy varieties and higher in high amylose starches (Morrison, 1995). Tuber and root starches contain generally low amounts of lipids (< 1%) (**Table 2-4**) [Hoover, 2001,]. Lipids associated with isolated cereal starch granules have been found to occur on the surfaces as well as inside the granules (Morrison and Laignelet, 1983; Morrison, 1981). The surface lipids are mainly triglycerides (TG), followed by free fatty acids (FFA), glycolipids (GL) and phospholipids (PL). 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). 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, 1997) or linked via ionic or hydrogen bonding to hydroxyl groups of the starch components (Hoover and Vasanthan, 1992). There has been some controversy whether amylose-lipid complexes exist in the intact granules or whether they are formed when granules are swollen or hydrated, but not necessaraly gelatinized. Studies using ^{13}CCP/MAS NMR and DSC have confirmed that lipid amylose complexes and lipid free amylose are both present in cereal starches (Morrison et al., 1993). Swinkels, (1985) summarized the effect of starch lipids as: (1) forming 'inert' complexes with amylose in starch pastes

and films, hence preventing part of the amylose from contributing the thickening power of gelatinized starch, (2) giving rise to undesirable flavors by oxidation of unsaturated lipids (found on the granular surface), (3) reducing granular swelling and amylose leaching.

2.4.2.2 Phosphate

Root and tubers contain significant amounts of mono phosphate esters covalently bound to starch (**Table 2-4**) [Lim et al., 1994; Kasemsuwan and Jane, 1994]. Many of the desirable qualities of potato starch such as enhanced paste clarity, high peak consistency, significant shear thinning and slow rate extent of retrogradation are attributed to its phosphate content (Jane et al., 1996; Galliard and Bowler, 1987). Starch phosphate - monoesters in native potato starch are mainly found on amylopectin (Jane et al., 1996). The distribution of the phosphate monoester content on the C_2, C_3 and C_6 of the glucose unit of potato starch has been reported to be 1, 38, and 61%, respectively (Hizukuri et al., 1970; Tabata and Hizukuri, 1971). Takeda and Hizukuri, (1982) have shown that potato amylopectin contains one phosphate monoester group per 317 glucosyl residues. The above authors also showed by isoamylase debranching and β - amylase treatment, that phosphate groups are present in the long branch chain (B chains with average degree of polymerization ~ 41). The phosphate group in potato starch has been reported to be located more than 9 glucosyl residues away from the branch point (Takeda and Hizukuri, 1981, 1982). Jane and Shen, (1993) have shown that phosphorous in potato starch is located densely in the

granule core together with amylopectin, while in cereal starches most, if not all of the phosphorous is in the lysophosphoplipid fraction (Morrison, 1995). Among the starches, potato and waxy potato contain the largest quantity of organic phosphate followed by taro (Hoover, 2001). The degree of phophorylation depends on the cultvar, the growth condition, fertilizer , temperature, and storage conditions (Anne et al., 1994; Hizukuri, et al., 1970; Nielsen et al., 1994; Muhrbeck and Tellier, 1991). An increase in phosphorylation results in a notable increase in the viscosity of gelatinized starch.

2.4.2.3 Protein

Nitrogen present in the starch is generally considered to be present as protein, but it may also part of the starch lipids (Lineback and Rasper, 1988). Nitrogen content of tuber and root starches generally range from 0.006 to 0.49% (**Table 2-4**) [Hoover, 2001]. The protein content of in purified starch is a good indicator of starch purity. Alkali extraction is very effective in solubilizing protein, therefore, careful washing of crude starch with diluted alkali can reduce protein values in purified starches. In wheat starch, the protein content has been estimated to be 0.1-0.25% (Eliasson and Larson, 1993), whereas a broader range has been reported for legume starch, 0.05-1.12% (Hoover and Sosulski, 1985). Approximately 10% of the starch protein appears to be associated with the granule surface (Galliard and Bowler, 1987).

2.4.3 Intermediate component of starch

Manners, (1985) has shown that some starches contain a third polysaccharide, usually referred to as an intermediate fraction, which has more or less branched materials. However, the average chain length and number of chains per molecule differ from those of amylose and amylopectin. Therefore, this intermediate fraction can not be categorized either as amylose or amylopectin (Colonna and Mercier, 1984; Hizukuri, 1996). Asaoka et al. (1986) and Inouchi et al. (1987) have observed such a intermediate fraction in high-amylose rice and maize. However, an intermediate fraction has not been observed in high-amylose barley starch (Tester et al., 1991; Salmonosson and Sundberg, 1994). The anomalous amylopectin of amylomaize starch was shown to be a mixture of short linear amylose (DP10) and normal amylopectin (average chain length 25) [Banks and Greenwood, 1968; Bank et al., 1974).

2.4.4 Semi crystalline structure of starch granule

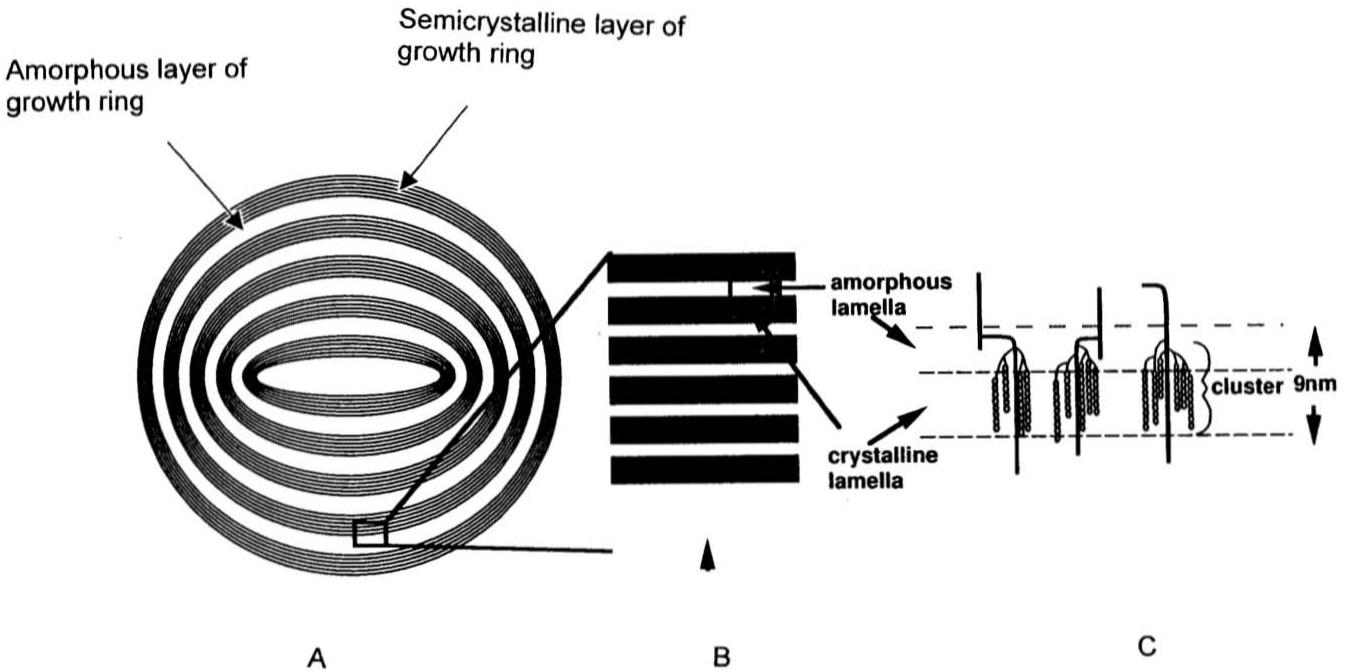
Different techniques have been employed to study the structural organization of the starch granule. Among these, electron microscopy, wide angle X-ray scattering and diffraction (WAXD), small angle X-ray scattering (SAXS), solid state ^{13}C-NMR, various viscometric techniques, and differential scanning calorimetry (DSC) are the most widely used (Biliaderis, 1998). However, each of these methods is sensitive to a different level of structure, and over a range of distances, present in a starch system. The molecular order of the starch granule (arrangement of amylose and amylopectin within the granule), which governs the

Fig. 2-6 Model for the semi crystalline structure of the starch granule (Donald, 1997 - with permission)

(A) Stacks of amylopectin lamellae are separated by amorphous growth ring

(B) A magnified view of one stack, showing that it is made up of alternating crystalline and amorphous lamellae

(C) Double helices formed from amylopectin branches in crystalline lamellae. Amylopectin branch points are located in the amorphous lamellae


Semicrystalline layer of growth ring
Amorphous layer of growth ring
amorphous lamella
crystalline lamella
cluster 9nm
A
B
C

physicochemical properties of native starches is still under investigation. The crystalline lamellae exist in the granule alternatively with amorphous lamellae (**Fig. 2-6)**. The combined thickness of crystalline lamellae plus amorphous lamellae is 9 nm and 9.2 nm for A-type starches and B-type starches respectively (Jenkins et al., 1993; Jane, 1997). Jenkins et al. (1994) postulated that most of the amylose is deposited in amorphous growth rings which represents the amorphous background. Yamaguchi et al. (1979) showed that clusters of amylopectin short chains occur within the crystalline domains of the granule.

2.4.4.1 Amorphous region of starch granule

The amorphous region accounts for 70% of the starch granule (Oostergetel and Van Bruggen, 1993), and consists of free amylose, lipid-complexed amylose, and some branch points of amylopectin (Hizukuri, 1996). The conformation of chains in the amorphous domains appear to be mainly a single helix or random coil (Gidley and Bociek, 1985, 1988). The amorphous region has been shown to be very susceptible to chemical and enzymetic modification (Hood and Mercier, 1978; Robyt, 1984). Diffusion of small water soluble molecules (< 1000 Dalton) in the granule also occurs through the amorphous phase. At the present time, there are no techniques to distinguish between mobile amorphous region (lipid free amylose and branching regions of amylopectin) and solid like V-amylose chains. Biliaderis, (1998) has postulated that there is no sharp demarcation between crystalline and amorphous domains in granular starch. Instead, a range of structures is expected between well-developed crystallites and fully disordered

regions. In this type of super molecular organization, the amorphous and crystalline phases are interdependent. The argument provided by Gallant et al. (1997) on the organization of lamellae and their polymeric constituents in granular starch concurs with the above postulate.

2.4.4.2 Crystallinity

X-ray diffractometry has been used to reveal the presence and characteristics of crystalline structures of starch granules (Katz and Van Itallie, 1930; Zobel, 1988a; Hizukuri et al., 1983; Cheetham and Tao, 1998; Ratnayke et al., 2001). The crystallinity of starch is due to its amylopectin component (Banks and Greenwood, 1975; Blanshard, 1987; Hizukuri, 1996; Biliaderis, 1998), and the crystalline domains are constructed mainly of 'A' chains and outer 'B' chains of amylopectin (Hizukuri, 1996). Starch is classified accordingly to the packing arrangement of the amylopectin double stranded helices in the granules, namely A-, B. and C- type (**Fig.2-7**) as determined by differences in the X-ray diffraction pattern. The 'A' type crystallinty is found mainly in cereal starches and is characterized by peaks at 15^0, 17^0, 18^0, 20 and 23 2θ angles (Zobel, 1988a; Cheetham and Tao, 1998). Most of the tuber and root starches exhibit the typical 'B' type X-ray pattern (Zobel, 1988a) with peaks that are both broad and weak with two main reflections centered at 5.5^0 and 17^0 angles, the exception being *Ipomea batatus* (A and C, *Mainhot esculenta* (C_a, A, C), *Nelumbo nucifera* (Ca C_b), *Dioscorea dumetorum* (A) and *Rhizoma dioscorea* (C_b), (**Table 2-5)**. The C_a, C_b and C_c classification is based on the extent of their resemblance to the 'A' and

Fig. 2-7 X-ray diffraction pattern from different starches (Zobel, 1988a - with permission)

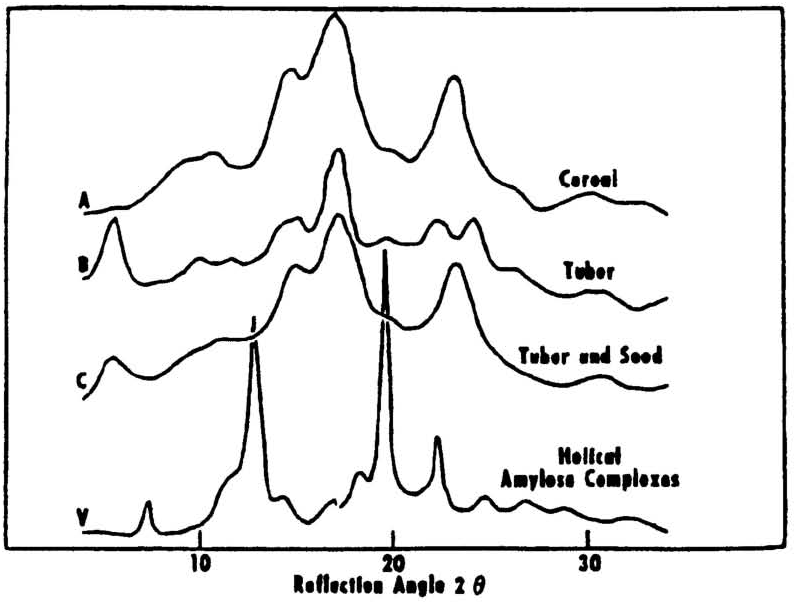

A
B
C
V
Cereal
Tuber
Tuber and Seed
Helical
Amylose Complexes
10
20
30
Reflection Angle 2 θ

Table 2-5 X-ray pattern and crystallinity of different starches

Starch Source	X-ray pattern	Crystallinity (%)	Reference
1.Tuber and root			
Potato	B	28	Zobel, (1998a)
Sweet potato	A, C, C_a	38	Zobel, (1998a), Moorthy, (1994), Lauzon et al., (1995)
True yam (*Dioscorea abyssinica*)	B	-	Mariam and Schmidt, (1998)
True yam (*Dioscorea alata*)	B	-	Moorthy, (1994)
True yam (*Dioscorea esculenta*)	B	-	Gallant et al., (1982)
Arrow root (queensland)	B	26	Rickard et al., (1991)
Taro	A	45	Zobel, (1998a)
New Coco yam	A	24	Moorthy, (1994), Takeda et al., (1993)
Cassava	A, C_a, C	38	Zobel, (1998a), Moorthy, (1994), Gallant et al., (1982)
Kuzu	C_a	-	Takeda et al., (1983)
Baffalo gourd	B	-	Dreher and Berry, (1983)
2. Cereals			
Rice			Zobel, (1998b)
Oat	A	33	Zobel,(1998b)
Wheat	A	36	Zobel, (1998b)
Rye	A	34	Zobel, (1998b)
Amylomaize	B	15-22	Zobel, (1998b)
Corn	A	40	Zobel, 1998b)
Waxy rice	A	37	Zobel, (1998b)
3. Legumes			
Field pea (Carnevel)		25.1	Ratnayake et al., (2001)
Field pea (Keoma		24.7	Ratnayake et al., (2001)

'B' types or between the two types, respectively (Hizukuri et al., 1960). Legume starches have been shown to exhibit a 'C' type pattern (Hoover and Sosulski, 1991; Cheetham and Tao, 1998), with prominent peaks at 2θ angles of 5.6^0, 15^0, 17^0, 20^0 and 23^0. Bogracheva et al. (1998) showed that in C-type starches the 'B' polymorphs are arranged centrally while the 'A' polymorphs are located peripherally within the granules. The amylopectin of 'A' type starches has a closer packing arrangement compared with that of 'B' type starches. The unit cell of amylopectin is estimated to hold 8 water molecules for the A-type and 36 water molecules for the B-type (**Fig. 2-8**) [Imberty et al., 1991; Zobel, 1988a). The C-polymorph is a mixture of A and B unit cells, and is thus intermediate between the A and B types in packing density. The type of crystalline polymorph has been shown to be mainly influenced (Hizukuri et al., 1983) by the chain length (CL) of amylopectin [A type CL < 19.7; B-type CL ≥ 21.6], and starches exhibiting CL between 20.3 and 21.3 exhibit A, B or C-type patterns. Other factors influencing polymorphism are growth temperature (Hizukuri, et al., 1961), alcohol's and fatty acids (Hizukuri, 1996). The degree of crystallinity and the double helical content (in the amorphous and crystalline domains) of tuber and root starches have not been thoroughly investigated. Consequently, the influence of these parameters on starch properties can not be ascertained. Jane et al. (1997) have shown that in A -type starches, the branch α (1→6) linkages are located within the crystalline and amorphous areas, whereas in B-type starches, the branches are located mainly within the amorphous area (**Fig, 2-9**).

Fig. 2-8 Double helical packing and arrangements in A and B-type unit cell structures (Adapted from Wu and Sarko 1978a, 1978b)

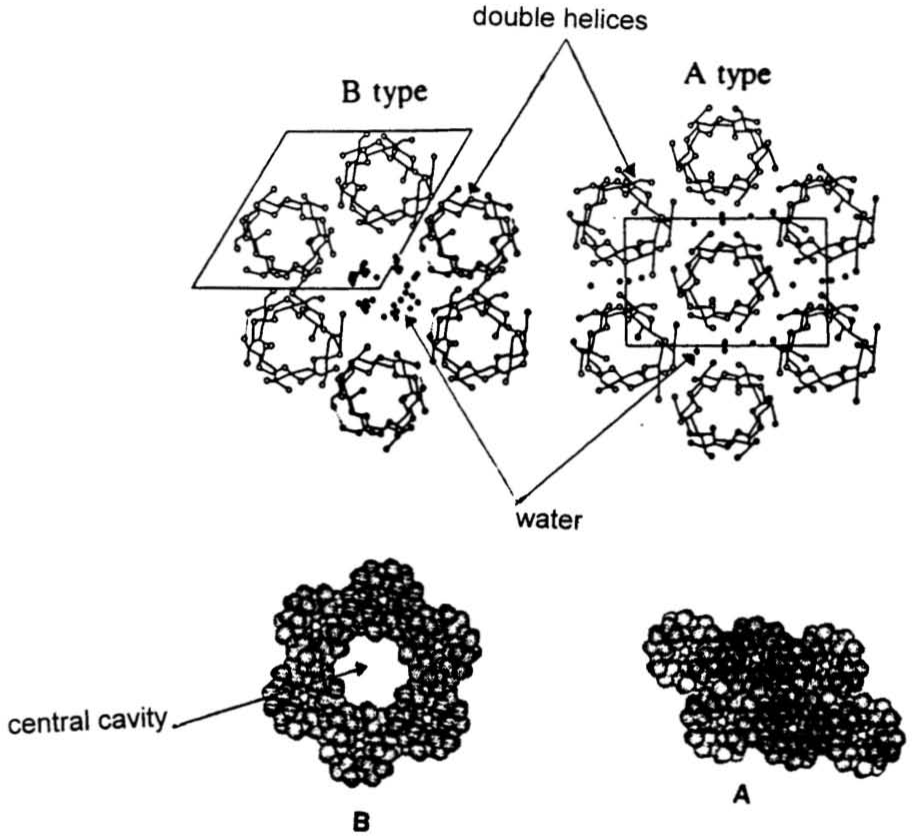
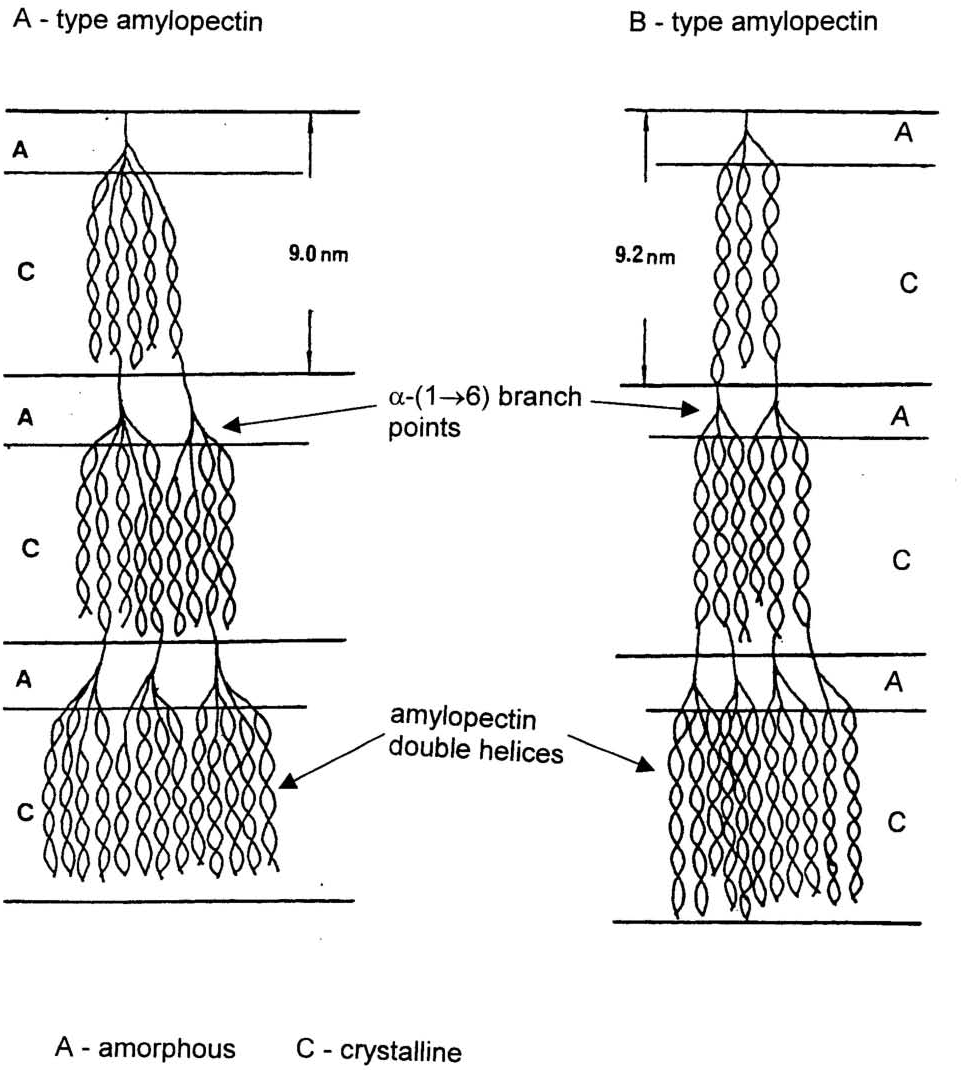

double helices
B type
A type
water
central cavity
B
A

Fig. 2-9 Proposed models for branching patterns of (A) A-type starch and (B) B-type starch (Jane et al., 1997 - with permission)

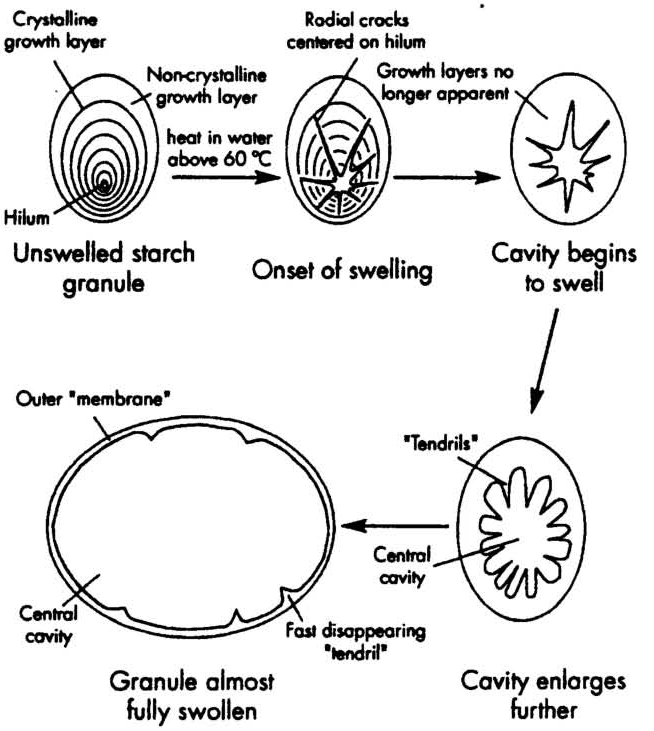

A - type amylopectin
B - type amylopectin
A
C
9.0 nm
A
C
A
C
9.2 nm
A
C
A
C
α-(1→6) branch points
amylopectin double helices
A - amorphous
C - crystalline

2.5 Properties of starch

2.5.1 Granular swelling

Most starch granules are insoluble in water. When dry starch granules are placed in water, a small amount of water is absorbed (exothermic process), and the heat of immersion decreases to zero at a water content of 0.18g water/g dry starch for wheat and 0.20g water/g dry starch for potato (French, 1984). If the temperature is increased, the amount of absorbed water increases, until a certain temperature (the onset of gelatinization) the water uptake is reversible, but then the changes are irreversible. The sequence of events during swelling of potato starch is presented in (**Fig. 2-10**). Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction is influenced by the amylose/amylopectin ratio and by the characteristics of amylose and amylopectin in terms of molecular weight distribution and conformation. Granular swelling has been shown to be influenced by granular size (Vasanthan and Bhathy, 1996), amylose content (Eliasson, 1985; Tester and Morrison, 1990), starch damage (Karkalas et al., 1992), temperature (Colonna and Mercier, 1985), bound lipid content (Gudmundsson and Eliasson, 1987, Tester, 1997a) and crystallinity (Robin et al., 1975). Jenkins et al. (1994) showed that the initial absorption of water and the location of swelling occurs primarily within the amorphous growth ring rather than the amorphous lamellae.

Fig. 2-10 The process of swelling of a potato starch granule in hot water (Hancock and Tarbet, 2000 - with permission)


Crystalline growth layer
Non-crystalline growth layer
Hilum
heat in water above 60 °C
Radial cracks centered on hilum
Growth layers no longer apparent
Unswelled starch granule
Onset of swelling
Cavity begins to swell
Outer "membrane"
"Tendrils"
Central cavity
Central cavity
Fast disappearing "tendril"
Granule almost fully swollen
Cavity enlarges further

2.5.2 Gelatinization

Starch, when heated in the presence of excess water, undergoes an order-disorder phase transition called gelatinization over a temperature range characteristic of the starch source. The above phase transition is associated with the diffusion of water into the granule, water uptake by the amorphous background region, hydration and radial swelling of the starch granules, loss of optical birefringence, uptake of heat, loss of crystalline order, uncoiling and dissociation of double helices (in the crystalline regions) and amylose leaching (Stevens and Elton, 1971; Lelievere and Mitchell, 1975; Donovan, 1979; Biliaderis, et al., 1980; Hoover and Hadziyev, 1981; Evans and Haismann, 1982; Jenkins,1994). Jenkins, (1994) showed by means of small angle neutron scattering studies that the mechanisms proposed by Evans and Haismann, (1982), Blanshard, (1987) and Biliaderis et al. (1986) were not compatible with his results, but were in broad agreement with the gelatinization mechanism proposed by Donovan, (1979). According to Jenkins, (1994), gelatinization in excess water is a primarily a swelling driven process. This swelling acts to destabilize the amylopectin crystallites within the crystalline lamellae, which are ripped apart (smaller crystallites are destroyed first). This process occurs rapidly for an individual crystallite, but over a wide range for the whole granule. The same mechanism occurs in conditions of limiting water, however, there is insufficient water for gelatinization to proceed to completion. At higher temperatures the remaining crystallites simply melt. Recently, Waigh et al. (2000a) have proposed a model for gelatinization based on the side-chain liquid

crystalline model for starch. In this model, the lamellae in starch are considered in terms of 3 components: 1) backbone, 2) side-chain and 3) double helices (**Fig. 2-11**). It is the degree of mobility of those three components, coupled with the helix-coil transition, which gives starch its distinctive properties. Waigh et al. (2000a, b) used this model to explain the phenomena involved during hydration and gelatinization. Their postulate is as follows: (1) at low water contents (< 5% w/w) the amylopectin helices are in a glassy nematic state (**Fig. 2-12A**). Upon heating in a DSC a single endotherm is observed due to the helix to coil transition (**Fig 2-12A**); 2) Intermediate water (**Fig. 2-12B**) contents (> 5%, < 40% w/w) have two steps in their breakdown and there are correspondingly two DSC endotherms The first is thought to be due to the rearrangement of dislocation between constituent amylopectin helices leading to a semectic -nematic transition (Waigh et al., 2000b). The second is the helix to coil transition as the amylopectin helices unwind in an irreversible transition; 3) In excess (**Fig. 2-12C**) water (40% w/w) lamellae break up and the helix to coil transition occurs at the same point, since free unassociated helices are unstable. Gelatinization has been shown to be influenced by a number of factors: 1) species, 2) growth conditions, 3) extraction procedures, 4) water content, 5) added solutes 6) heating rate, 6) thermal history and 7) the malevolent influence of thermodynamic irreversibility (Waigh et al., 2000a). Many methods are presently available for the determination of the gelatinization, such as Kofler hot stage microscope (Watson, 1964), X-ray diffraction (Zobel, 1988a), DSC (Donovan, 1979), pulsed nuclear magnetic resonance (Lelievre and Mitchel, 1975), enzymatic digestibility

Fig. 2-11 Schematic representation of side-chain liquid crystalline model for starch (Waigh, et al., 2000a, with permission)

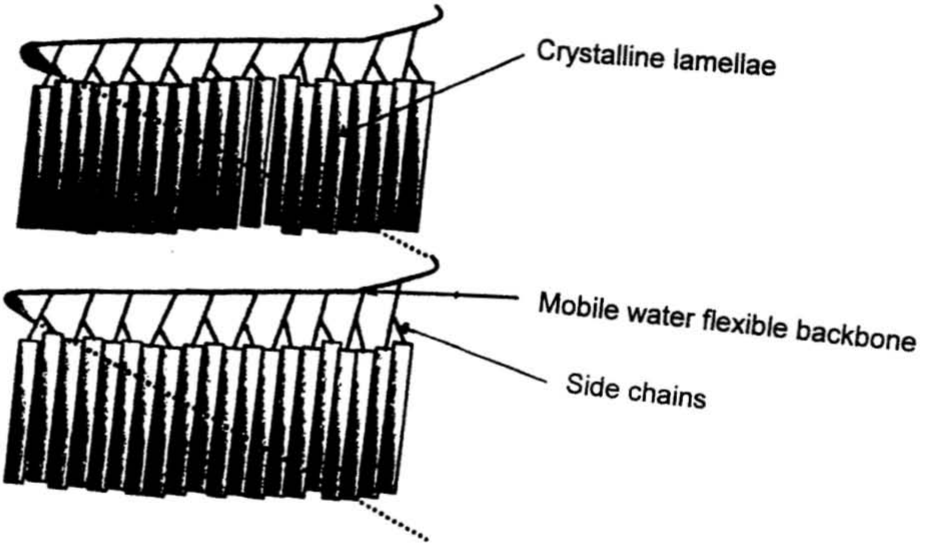

Crystalline lamellae
Mobile water flexible backbone
Side chains

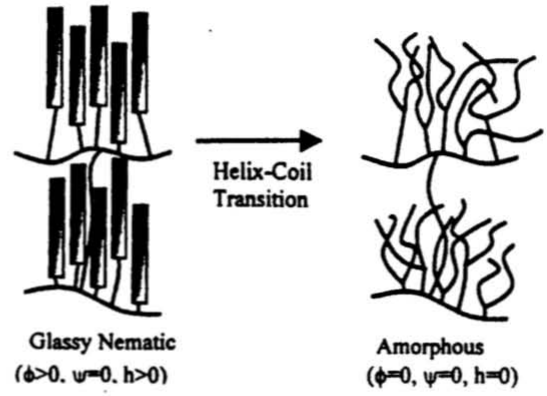
Fig. 2-12 Models for gelatinization process based on water content available during gelatinization (Waigh et al., 2000a - with permission)

(A) The single stage process in the gelatinization of starch at low water contents

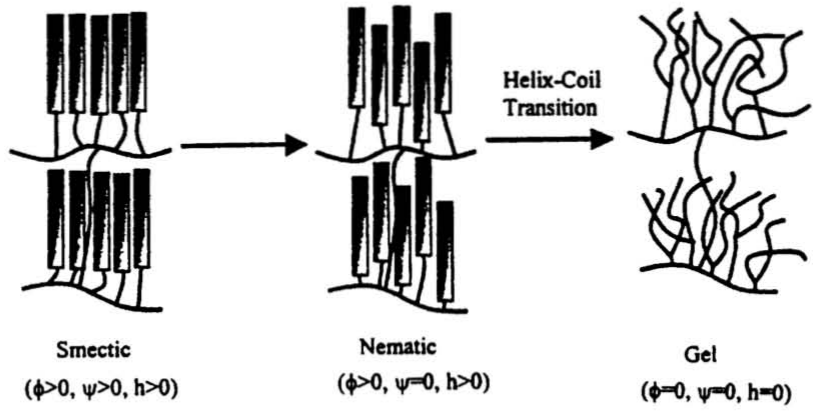
(B) The two-stage process involved in the gelatinization of starch in limiting water (intermediate water content)

(C) The two stage process involved in the gelatinization of starch in excess water: relative values of the orientational = ϕ, lamellar = ψ, and helical order parameter = h

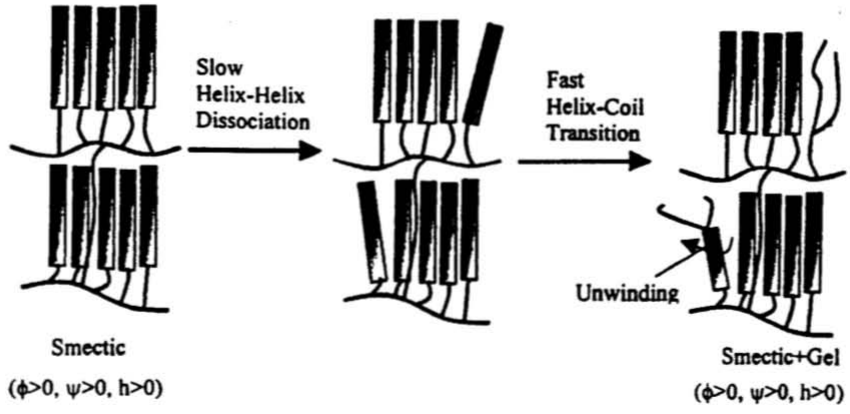
(A) Low water content gelatinization



(B) Intermediate water content gelatinization



(C) Excess water content gelatinization



(Shiotsuba, 1983), small angle X-ray scattering (Jenkins, 1994), and small angle neutron scattering (Jenkins, 1994). However, only the Kofler hot stage microscope and DSC have been widely used to study the gelatinization temperatures of root and tuber starches (**Table 2-6**). Kofler hot stage microscopy is limited by the subjective nature of the observations (loss of birefringence) and only temperature measurements are obtained (**Table 2-6**). DSC measures the gelatinization transition temperatures [onset [T_o], midpoint [T_p], conclusion [T_c], and the enthalpy (ΔH)] of gelatinization. Noda et al., (1998) have postulated that DSC parameters (T_o, T_p, T_c, ΔH) are influenced by the molecular architecture of the crystalline region, which corresponds to the distribution of amylopectin short chains (DP 6-11) and not by the proportion of crystalline region which corresponds to the amylose to amylopectin ratio. The above authors have shown by studies on sweet potato and wheat starches, that a low T_o, T_p, T_c and ΔH reflect the presence of abundant short amylopectin short chains. Tester, (1997b) has postulated that the extent of crystalline perfection is reflected in the gelatinization temperatures, whereas, the ΔH reflects the overall crystallinity (quality and amount of starch crystallites) of amylopectin (Tester and Morrison, 1990a). Cooke and Gidley, (1992) have postulated that ΔH primarily reflects the loss of double helical order. Gernat et al. (1993) have stated that the amount of double helical order in native starches can be strongly correlated with the amylopectin content, and that granule crystallinity increases with amylopectin content. This suggests that ΔH values should preferably be calculated on an

Table 2-6 Gelatinization parameters of starches from different botanical origin (S;W = starch to water ratio)

Starch source	Methodology	T_C-T_0(gelatinization temperature range	ΔH (j/g) (Gelatinization enthalpy)	Reference
1. Tuber and root				
Potato	DSC:S:W 1:3	70.2-62.5	18.2	Kim et al., (1995)
Potato (42 genotypes)	DSC:S:W 1:2.3	71.7-63.5	17.3	Kim et al., (1995)
Sweet potato(44 genotypes)	DSC:S:W 1:3	84.6-64.6	12.9	Collado et al., (1999)
Sweet potato(RCB selection)	DSC:S:W 1:6	81.4-61.3	15.7	Garcia and Walter, (1998)
Sweet potato (6DLP selections)	DSC:S:W 1:6	83.5-60.0	17.2	Garcia and Walter, (1998)
Sweet potato (6 Diploid strains)	DSC:S:W 1:3	86.9-72.4	7.4	Asante et al., (1993)
Old cocoyam(varieties)	DSC:S:W 1:3	63.3-43.0	6.8	Jane et al., (1992)
True yam (*Dioscorea abyssinica*)	DSC:S:W 1:2	74.8-64.2	19.2	Mariam and Schmidt, (1998)
True yam (*Dioscorea alata*)	Kofler hot stage	71.5-65.0	-	Emiola and Delarossa, (1981)
True yam (*Dioscorea rotundata*)	Kofler hot stage	71.0-63.5	-	Emiola and Delarossa, (1998)
True yam (Dioscorea cayenesis)	Kofler hot stage	74.5-68.0	-	Emiola and Delarossa, (1998)
True yam (Dioscorea dumetorum)	Kofler hot stage	72.5-65.5	-	Emiola and Delarossa, (1998)
New cocoyam	DSC:S:W 1:4	81.8-66.0	12.9	Valetudie et al., (1995)
New cocoyam	DSC:S:W 1:2	87.0-74.0	4.0	Perez et al., (1998a)
Cassava (5 varieties)	DSC:S:W 1:2	76.1-57.0	12.9	Moorthy, (1994)
Cassava	Kofler hot stage	70.0-58.5	-	Srivastava et al., (1970)
Cassava	DSC:S:W 1:2	84.1-62.4	4.8	Perez et al., (1998b)
West Indian arrowroot	DSC:S:W 1:2	85.8-61.0	19.2	Erdman, (1986)
Queensland arrow root	Kofler hot stage	70.0-65.0	-	Soni et al., (1990)
Buffalo gourd	Kofler hot stage	68.8-61.2	-	Dreher and Berry, (1983)
2. Cereals				
Barely	DSC S:W 1:2)	74-59	8.3-13.3	Fujita et al., (1992)
Maize	DSC S:W 1:2	72-61	3.6	Inouchi et al., (1991)
Cont.,				
Oat	DSC S:W 1:3	73-61	10.4	Hoover and Vasanthan, (1992)
Wheat	DSC S:W 1:2	82-53	13.7	Shamekh et al., (1994)
3.Legume				
Lentil	DSC S:W 1:3	13-55	7.6	Hoover and Vasanthan, (1994)
Black bean	DSC S:W 1:3	82-62	3.0 (cal/g)	Hoover and Manuel, (1996)
Pinto bean	DSC S:W 1:3	82-59	2.9 (cal/g)	Hoover and Manuel, (1996)
Field pea	DSC S:W 1:3	76-61	11.5	Ratnayake et al., (2001)

amylopectin basis. However, ΔH values for tuber and root starches (**Table 2- 6**) have not been calculated in this manner.

The gelatinization and swelling properties are controlled in part by the molecular structure of amylopectin (unit chain length, extent of branching, molecular weight, and polydispersity), starch composition (amylose to amylopectin ratio, lipid complexed amylose chains, and phosphorous content), and granular architecture (crystalline to amorphous ratio) [Tester, 1997a]. The molecular structure, amylopectin and granule architecture of many tuber and root starches have not been determined. Thus, it is not possible to discuss structure-gelatinization relationships in these starches. Furthermore, gelatinization parameters have been determined at different starch water ratio's (**Table 2-6**) and at different rates. This makes it difficult to make a meaningful comparison of the gelatinization properties of these starches.

2.5.3 Retrogradation

Starch granules when heated in excess water above their gelatinization temperature, undergo irreversible swelling resulting in amylose leaching into solution. In the presence of high starch concentration this suspension will form an elastic gel on cooling. The molecular interactions (mainly hydrogen bonding between starch chains) that occur after cooling have been called retrogradation. These interactions are found to be time and temperature dependent. Starch gels are metastable and nonequilibrium systems and therefore undergo structural changes during storage (Ferrero et al., 1994). Miles et al. (1985) and Ring et al.

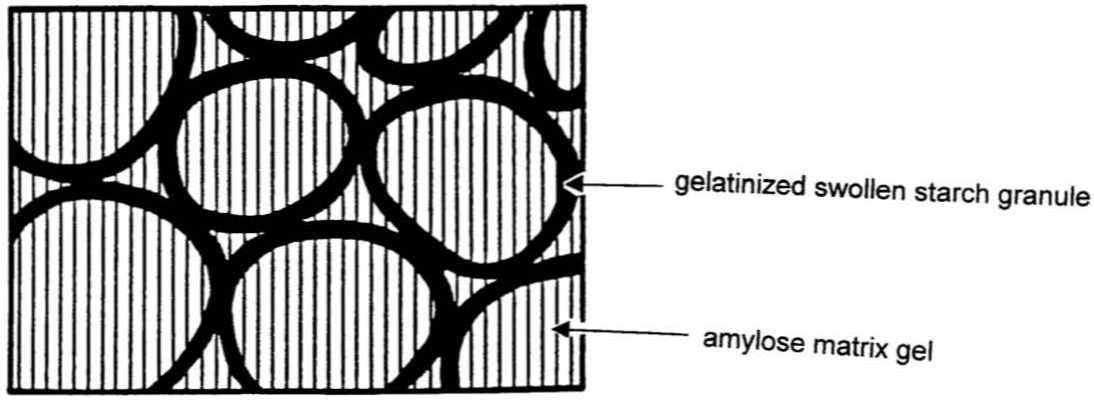
(1987) attributed the initial gel firmness during retrogradation to the formation of an amylose matrix gel (**Fig.2-13A**) and the subsequent slow increase in gel firmness to reversible crystallization of amylopectin. During retrogradation, amylose forms double -helical associations (**Fig. 2-13B**) of 40-70 glucose units (Jane and Robyt, 1984; Leloup et al., 1992), whereas amylopectin crystallization occurs by association of the outermost short branches (DP= 15) [Ring et al., 1987]. The retrograded starch, which shows a B-type X-ray diffraction pattern (Zobel, 1988a) contains both crystalline and amorphous regions.

Many factors have been shown to influence starch retrogradation: 1) starch concentration (Longton and LeGrys, 1981; Orford et al., 1987; Gudmudsson and Eliasson, 1990; Biliaderis and Tonogai, 1991; Liu and Thompson, 1998), 2) storage temperature (Clowell et al., 1969; Slade and Levine, 1987), 3) initial heating temperature (Liu and Thompson, 1998), chain length distribution of amylopectin (Yuan et al., 1993; Shi and Seib, 1995; Liu and Thompson, 1998), 4) molecular size of amylose (Lu et al., 1997), 5) salts (Ward et al., 1994), 6) lipids (Biliaderis and Tonogai, 1991; Ward et al., 1994), 7) sugars (Biliaderis and Prokopowich, 1994; Seow et al., 1996), 8) physical modification (Hoover et al., 1994), 9) chemical modification (Hoover and Sosulski, 1986; Hoover et al., 1988; Wu and Seib, 1990; Perera and Hoover, 1998), 10) starch source (Hoover and Sosulski, 1985; Orford et al., 1987; Hoover, 1995). Cereal retrogradation has been investigated by wide variety of methods such as: 1) enzymic hydrolysis (Matsunaga and Kainuma, 1986), 2) differential scanning calorimetry (Eberstein et al., 1980; Eliasson, 1983; Russel, 1983; Roulet et al., 1988; Ishii et al, 1988;

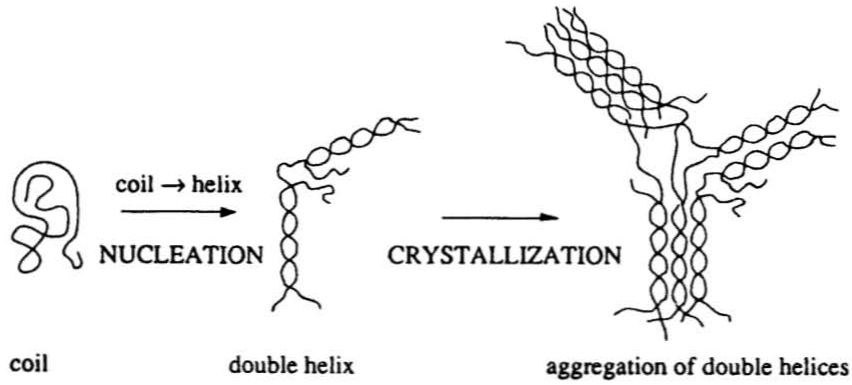
Fig. 2-13 (A) Schematic representation of starch gel. Swollen granules fill the amylose gel (Morris, 1990 - with permission)

(B) Conformational changes occurring during amylose gelation (Colonna, et al., 1992 - with permission)

(A)


gelatinized swollen starch granule
amylose matrix gel

(B)


coil → helix
NUCLEATION
CRYSTALLIZATION
coil
double helix
aggregation of double helices

Cairns et al., 1991; Perera and Hoover, 1988), 3) nuclear magnetic resonance (Teo and Seow, 1992; Wu et al., 1992; Morgan et al., 1992). 4) Raman spectroscopy (Bulkin et al., 1987) 5) Fourier transform infrared spectroscopy (Wilson et al., 1987; Wilson and Belton, 1988; VanSoest et al, 1994; Lizuka and Aishima, 1999), 6) turbidity (Jacobson et al., 1997; Perera and Hoover, 1998) and 7) rheological techniques (Kim et al., 1976; Wong and Lelievre, 1992; Inaba et al., 1994).However, retrogradation of tuber and root starches have not been monitored using different physical probes.

2.5.4 Starch hydrolysis

2.5.4.1 Acid hydrolysis

Acid hydrolysis has been used to modify starch granule structure and produce "soluble starch" for many years Nageli, (1874) reported the treatment of native potato starch in water with 15% H_2SO_4 for 30 days at room temperature. He obtained an acid - resistant fraction readily soluble in hot water, which has come to be known as Nageli amylodextrin and has been shown to be mixture of low molecular-weight, linear and branched dextrins, with an average degree of polymerization (DP) of 25-30. Subsequently, Lintner, (1886) described an acid modification of native potato starch in which granules were treated in an aqueous suspension with 7.5% (w/v) HCl for 7 days at room temperature. The product was a high-molecular weight starch, which formed a clear solution in hot water. This is used as an indicator in iodometric titration and for enzyme analysis. In industry acid-modified starches (maize, waxy maize, wheat, and cassava) are

prepared by treating a starch slurry (40%) with dilute HCl or H_2SO_4 at 25-55^{0}C for various time periods. The conditions used during acid hydrolysis are influenced by the ratio of the cold to hot paste viscosity and by the required gel texture. When the desired viscosity or fluidity is attained, the starch slurry is neutralized, and the granules are recovered by washing, centrifugation, and drying. Industrial uses of acid hydrolyzed starches are as follows: 1) as a premodification step for the production of cationic and amphoteric starches (Solarek, 1987); 2) as a wrap sizing agent to increase yarn strength and abrasion resistance in the weaving operation (Solarek, 1987); 3) for preparation of starch gum candies (Solarek, 1987) ; 4) for manufacture of gypsum board for dry wall construction (Solarek, 1987); and 5) for paper and paperboard manufacture (Solarek, 1987). Recently, Chun et al. (1997) have shown that rice amylodextrins prepared by hydrolyzing rice starch in acidic (4% HCl) alcohol (70%) solutions at 78-80^{0}C were readily soluble in warm water (50^{0}C). An emulsion prepared by replacing a portion of the oil (used in the formulation of a mayonnaise-type emulsion) with rice amylodextrin, exhibited small and uniform droplets and displayed high viscosity and stability. This suggests that amylodextrins could be used as fat replacers (Chun et al., 1987).

2.5.4.1.1 Mechanism of acid hydrolysis

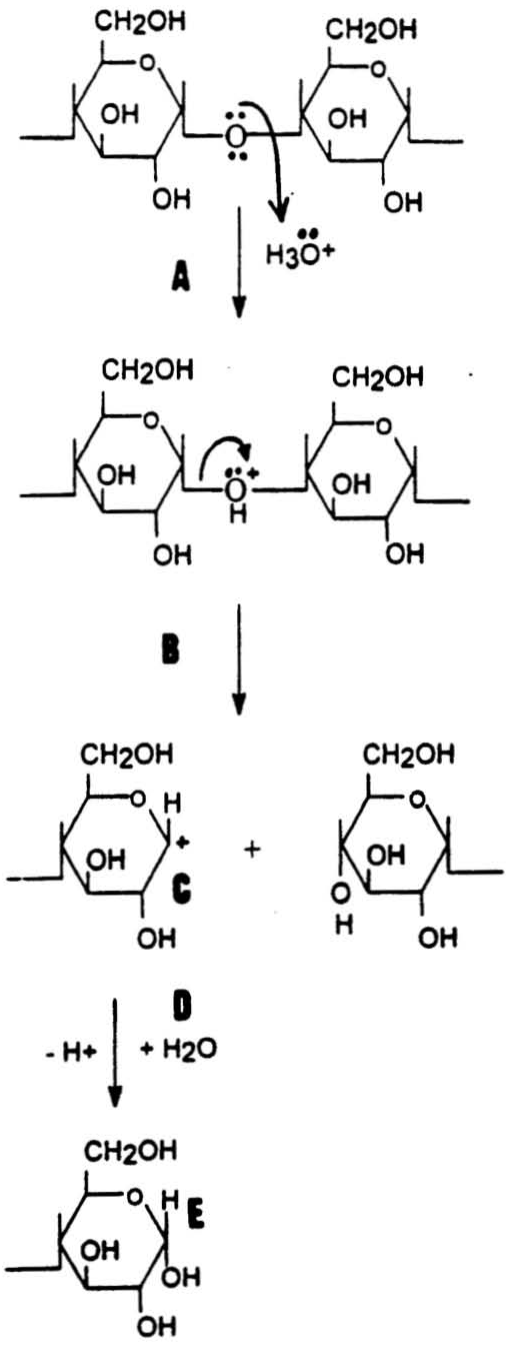
In acid hydrolysis, the hydronium ion (H_3O^+) carries out an electrophilic attack on the oxygen atom of the $\alpha(1\rightarrow4)$ glycosidic bond (**Fig. 2-14A**). In the next step, the electrons in one of the carbon-oxygen bonds move onto the oxygen atom

(**Fig. 14-B**) to generate an unstable, high-energy carbocation intermediate (**Fig. 2-14C**). The carbocation intermediate is a Lewis base, leading to regeneration of a hydroxyl group (**Fig. 2-14E**) [Hoover, 2000].

2.5.4.1.2 Solubilization patterns of starches

All starches exhibit a two-stage hydrolysis pattern. A relatively fast hydrolysis rate during the first 8 days followed by a lower rate between 7 and 12 days has been reported for corn, waxy corn, high amylose corn, wheat, potato, oat, rice, waxy rice, smooth pea, lentil, wrinkled pea, adzuki bean, mung bean, and red kidney bean (Robin et al., 1974; Mainngat and Juliano, 1979; Biliaderis et al., 1981; Hoover and Vasanthan, 1994; Hoover et al.,1993). The faster stage corresponds to the hydrolysis of the more amorphous parts of the starch granule. During the second stage, the crystalline material is slowly degraded (Kainuma and French, 1971). Evidence to suggest a preferential attack on amorphous domains within the granule comes from transmission electron microscopy observations of acid hydrolyzed starches (Mussulmam and Wagoner, 1968). These authors observed a preferential etching of amorphous growth rings from normal and waxy maize starches treated with 7% HCl at room temperature for 35 days. To account for the slower hydrolysis rate of the crystalline parts of the starch granule, two hypotheses have been proposed (Kainuma and French, 1971). First, the dense packing of starch chains within the starch crystallites does not readily allow the penetration of H_3O^+ into the regions. Second, acid hydrolysis of a glycosidic bond may require a change in conformation (chair $\rightarrow$half chair) of

Fig. 2-14 Mechanism of acid hydrolysis of starch (Hoover, 2000 - with permission)


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

the D-glycopyranosyl unit. Obviously, if the crystalline structure immobilizes the sugar conformation then this transition (chair→half chair) would be sterically impossible. The difference in the rate and extent of hydrolysis between the starches has been attributed to differences in granular size, extent of starch chain interactions, (within the amorphous and crystalline regions of the granule). and starch composition (extent of phosphorylation, amylose content, and lipid-complexed amylose chains).

2.5.4.2 Hydrolysis by α-amylase

Alpha-amylase (1,4-α-D glucan glucanohydrolase, EC 3.2.1.1) catalyzes the hydrolysis (endo attack) of the α(1→4) glycosidic bond in amylose, amylopectin and related oligosaccharides. Robyt and French, (1970) proposed that α-amylases have a multiple attack mechanism. In this mechanism, once the enzyme forms a complex with the substrate and forms the first cleavage, the enzyme remains with one of the fragments of the original substrate and catalyzes the hydrolysis of several bonds before it dissociates and forms a new active complex with another molecular substrate (Robyt, 1984). The direction of the multiple attack by porcine pancreatic α-amylase is from the reducing end towards the non-reducing end (Robyt, 1984). Robyt, (1984) has shown that the products of hydrolysis of porcine pancreatic α-amylase (PPA) are mainly maltose, maltotriose and maltotetraose. PPA has been shown to have 5-D glucose subsite from the reducing end subsite (Robyt and French, 1970).

Differences in the *in vitro* α-amylase digestibility of native starches among and within species have been attributed to the interplay of many factors such as starch source (Ring et al., 1988), granule size (Snow and O'Dea, 1981), amylose/amylopectin ratio (Hoover and Sosulski, 1985) and amylose lipid complexes (Hoover and Manuel, 1995; Holm et al., 1982). Furthermore, it has been reported (Marsden and Gray, 1986; Franco et al., 1987) that hydrolysis by α-amylase predominantly occurs in the amorphous regions of the granule. Planchot et al. (1997) have shown that there is a clear relationship between the hydrolysis rate of lintnerized starches and their crystalline type. Regardless of morphology, particles with 'A' type crystallinity were found to be more susceptible to amylolysis than those with 'B' type. "A' type lintners (waxy maize) showed the highest rates, whereas the rates for 'C' type lintners (mixtures of 'A' and 'B' type structures) was dependent on the 'A' type ratio. Jane et al. (1997) have explained the susceptibility differences between 'A' and 'B' type starches towards α-amylase in the following way: in 'A' type starches, the branch points are scattered in both amorphous and crystalline regions. Consequently, there are many short 'A' chains derived from branch linkages located inside the crystalline regions, which produces an inferior crystalline structure. This inferior crystalline structure containing α- (1→6) linked branch points and short double helices are more susceptible to enzyme hydrolysis leading to weak "points" in the 'A' type starches. These weak points are readily attacked by α-amylase. However, in 'B' type starches more branch points are clustered in the amorphous region and furthermore, there are fewer short branch chains. Consequently, the crystalline

Table 2-7 *In vitro* amylolysis of various starches

Starch source	∝-amylase source	Reaction time (h)	Hydrolysis (%)	Reference
1. Tuber and roots				
Potato	Porcine pancreatic	72	5.4	Hoover and Vasanthan, (1994)
	Pancreatic		8.5	Fuwa et al., (1997)
	Bacillus subtilis		5.0	Fuwa et al., (1997)
Sweet potato	*Bacillus subtilis*	55	14.9	Fuwa et al., (1997)
	Pancreatic		43.3	Fuwa et al., (1997)
	Porcine pancreatic		48.8-63.4	Zhang and Oates, (1994)
Cassava	*Bacillus subtilis*	24	44.0	Valetudie et al., (1993)
	Porcine pancreatic		52.9	Valetudie et al., (1993)
True yam (*Dioscorea alata*)	*Bacillus subtilis*	24	3.5	Valetudie et al., (1993)
	Porcine pancreatic		4.7	Valetudie et al., (1993)
	Bacillus subtilis		15.3	Valetudie et al., (1993)
New cocoyam	pancreatic porcine	24	15.6	Valetudie et al., (1993)
2. Cereals				
Oat	Pancreatic porcine	72	32	Hoover and Vasanthan, (1994)
Wheat	Pancreatic porcine	72	63	Hoover and Vasanthan, (1994)
3.Legume				
lentil	Pancreatic porcine	72	65	Hoover and Vasanthan, (1994)
Field pea	Pancreatic porcine	24	22.2	Ratnayake et al., (2001)

structure is superior to that of 'A' type starches, and hence more resistant to α-amylolysis. The *in vitro* digestibility of starches is presented in (**Table 2-7**). Among tuber and root starches, potato shows the highest resistance to α-amylase. A meaningful comparison can not be made with regards to variations in the extent of hydrolysis due to differences in α-amylase source and reaction time.

2.6 Heat-moisture treatment (HMT)

Heat-moisture treatment of starches is defined as a physical modification that involves incubation of starch granules at low moisture level (< 35% water [w/w] during a certain period of time, at a temperature above the glass transition temperature but below the gelatinization temperature (**Fig. 2-15**). The conditions used for heat-moisture treatment of starches from various botanical origins are listed in (**Table 2-8**).

2.6.1. Influence of heat-moisture treatment on granule morphology

The granule morphology of maize, wheat, potato, yam, and lentil starches has been shown to remain unchanged after HMT. (Kulp and Lorenz, 1981; Stute, 1992; Hoover and Vasanthan, 1994; Franco et al., 1995; Hoover and Manuel, 1996). However, Kawabata et al. (1994) observed cracks on the surface of heat-moisture treated potato and maize starches.

Fig. 2-15 Schematic representation of the temperature and moisture differences in gelatinization, annealing, and heat-moisture treatment (Adapted from Manuel, (1996)

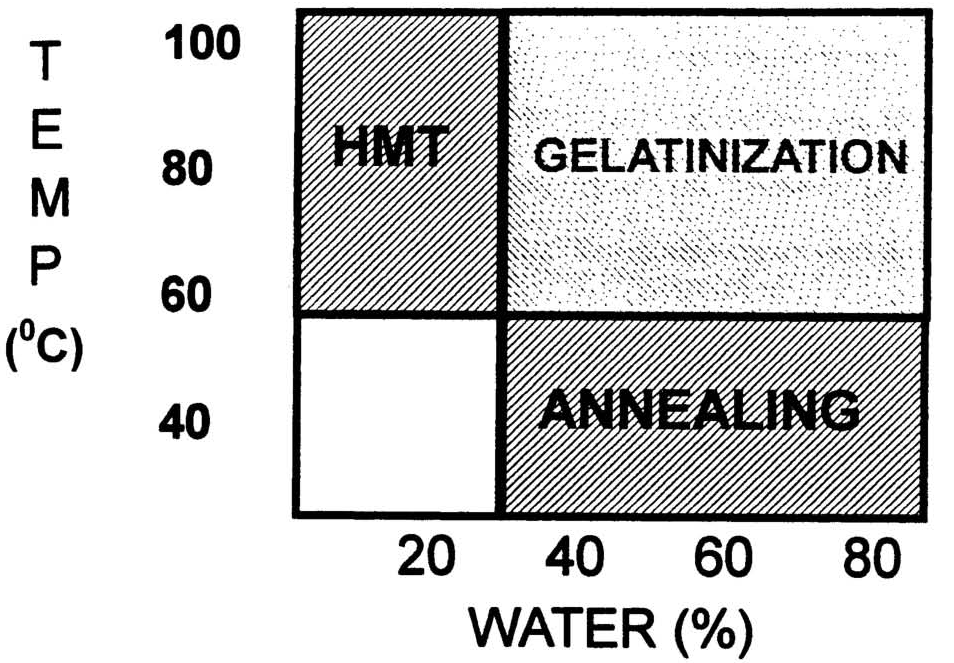

TEMP (°C)
100
80
60
40
HMT
GELATINIZATION
ANNEALING
20
40
60
80
WATER (%)

Table 2-8 Literature available for heat-moisture treatment conditions of different starches (Adapted from Jacobs and Delcour, 1998)

Starch	Time (^{0}C)	Time	Water content	Reference
1.Tuber and roots				
Potato	95-100	16h	18-27	Sair, (1967)
	100	16h	18-27	Lorenz and Kulp, (1981); Kulp and Lorenze, (1981); Donovan et al., (1983)
	80-120	15-60min	5-7	Kuge nad Kitamura, (1985)
	110-120	140/240min	20	Stute, (1992)
	100	16h	10-30	Hoover and Vasanthan, (1994a,c); Hoover et al., (1994)
	110	30min	16.5	Kawabata et al., (1994)
	84-105	16h	20-35	Eerlingen et al., (1996,1997)
Cassava	100	16h	18-27	Lorenz and Kulp, (1982)
	110	3-16	18-24	Abraham, (1993)
Arrow root	100	16h	18-27	Lorenz and Kulp, (1982)
Yam	100	16h	10-30	Hoover and Vasanthan, (1994)
2. Cereals				
Barely	100	16h	18-27	Lorenz and Kulp, (1982)
Maize (normal, waxy, high amylose)	95-110	16h	18-27	Sair, (1967)
	120	30/180min	25	Fukui and Nikuni, (1969)
	125	5/20	14	Kawabata et al., (1994)
	100	4h	25	Schierbaum and Kettlitz, (1994)
	100	16h	18-27	Franco et al., (1995)
	100	16h	30	Hoover and Manuel, (1996)
Wheat	120	30/180min	25	Fukui and Nikuni, (1969)
	100	16h	18-27	Lorenz and Kulp, (1981); Kulp and Lorenze, (1981)
	100	16h	10-30	Hoover and Vasanthan, (1994),Hoover et al., (1994)
	100	4h	25	Schierbaum and Kettlitz, (1994)
Rice	120	30-180	25	Fukui and Nikuni, (1969)
Rye	100	4h	22/25	Radosta et al., (1992); Schierbaum and Kettlitz, (1994)
Triticale	100	16h	18-27	Lorenz and Kulp, (1982)
Oat	100	16h	10-30	Hoover and Vasanthan, (1994); Hoover et al., (1994)
3.Legume				
Lentil	100	16h	10-30	Hoover and Vasanthan, (1994); Hoover et al., (1994)
Pea	100	16h	30	Hoover et al., (1993)

2.6.2 Influence of heat-moisture treatment on X-ray pattern and X-ray intensities

Heat-moisture treatment has been shown to change the wide angle X-ray pattern from the B to A- (or A+B) type for potato starch (Sair, 1967; Donovan et al., 1983; Kuge and Kitamura, 1985; Stute, 1992; Hoover and Vasanthan, 1994; Kawabata et al., 1994; Sekune et al., 2000) and also for yam starch (Hoover and Vasanthan, 1994).The transition from 'B' to 'A' type X-ray pattern was confirmed by ^{13}CCP/MAS NMR based on variations in C-1 multiplicity (Gidley and Bociek), 1985). Lorenz and Kulp, (1982) observed a shift from 'C' to 'A' type on heat-moisture treatment of cassava and arrowroot starches. However, several authors have shown (Sair, 1967; Fukui and Nikuni, 1969; Donovan et al., 1983; Radosta et al., 1992; Hoover and Vasanthan, 1994; Franco et al., 1995; Hoover and Manuel, 1996) that the A' type X-ray pattern of cereal starches remains unchanged after heat-moisture treatment. Decreased X-ray intensities have been reported after heat-moisture treatment of potato (Hoover and Vasanthan, 1994), barley (Lorenz and Kulp, 1982), and cassava (Abraham, 1993). However, cereal starches generally exhibit either increased or unchanged intensities after heat-moisture treatment (Sair, 1967; Fukui and Nikuni, 1969; Donovan et al., 1983; Radosta et al., 1992; Hoover and Vasanthan, 1995; Hoover and Manuel, 1996). Several theories have been put forward to explain changes in X-ray patterns and intensities on heat-moisture treatment. These are listed below:

1) Destruction of crystallites (decrease X-ray intensities) [Hoover and Vasanthan, 1994].

2) Growth of new crystallites (increase X-ray intensities) [Hoover and Vasanthan, 1994].
3) Reorientation of the already existing crystallites (may increase or decrease X-ray intensities) [Lorenze and Kulp, 1982 Hoover and Vasanthan, 1994; Hoover and Manuel, 1995;]
4) Changes in the packing arrangement (B to A type crystallinity) of the double helices (**Fig. 2-16**), which results in a change in the X-ray pattern. [Stute, 1992; Hoover and Vasanthan, 1994; Hoover and Manuel, 1996]
5) Interaction between amylose-amylose, amylose-amylopectin and amylopectin -amylopectin chains(increase X-ray intensities due to formation of new crystallites) [Hoover and Vasanthan, 1994; Hoover and Manuel, 1996]
6) Formation of crystalline amylose-lipid complexes (increase X-ray intensities) [Kawabata et al., 1994; Lorenz and Kulp, 1984; Fukui and Nikuni, 1969; Hoover and Manuel, 1996]

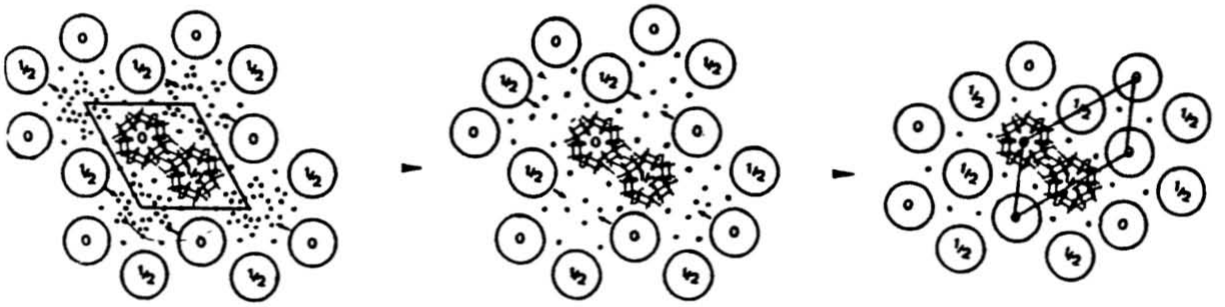
2.6.3 Influence of heat-moisture treatment on amylose-lipid complexes

Lorenz and Kulp, (1984), Hoover and Vasanthan, (1994), and Hoover and Manuel, (1996) observed a decreased apparent amylose content on heat-moisture treatment of wheat and potato starches, indicating additional interaction between native starch lipid and amylose chains.

Fig 2-16 Model of the polymorphic transition from B to the A starch, in the solid state (Imberty et al., 1991-with permission), parallel double helices =0 and 1/2, water molecules (.) dots

A type

B type



2.6.4 Influence of heat-moisture treatment on gelatinization parameters

Heat-moisture treatment increases the gelatinization transition temperatures and broadens the gelatinization temperature range (Sair, 1967; Kulp and Lorenz, 1981; Donovan et al., 1983; Radosta et al., 1992; Stute, 1992; Kobayashi, 1993; Hoover et al., 1993, 1994; Hoover and Vasanthan, 1994; Erlingen et al., 1996; Hoover and Manuel, 1996). The gelatinization enthalpy were decreased (Donovan et al., 1983; Kuge and Kitamura, 1985; Radosta et al., 1992; Stute, 1992; Kobayashi, 1993; Hoover et al., 1994; Hoover and Vasanthan, 1994; Erlingen et al., 1996) or unchanged after heat-moisture treatment. The increase in gelatinization temperature on heat-moisture treatment has been attributed to interaction between amylose chains (within the bulk amorphous region) and /or between amylose chain and the branched segment (within the intercrystalline region) of amylopectin. This in turn decreases the destabilization effect of the amorphous region on the melting of starch crystallites during gelatinization (Hoover and Vasanthan, 1994). Increase in gelatinization temperature after heat-moisture treatment suggest that double helices present in the native granule are disrupted during the polymorphic transformation (B to A+B) that occurs on heat-moisture treatment of tuber starches (Hoover and Vasanthan, 1994).

2.6.5 Influence of heat-moisture treatment on swelling power and amylose leaching

Swelling power and amylose leaching were generally found to decrease on heat-moisture treatment. This has been attributed to an interplay of three factors:

1) changes in the packing arrangement of the starch crystallites, 2) interaction between or among starch chains in the amorphous regions of the granule and 3) amylose-lipid interaction.

Decrease in amylose leaching was attributed to interplay of factors 2 and 3 (Kulp and Lorenz, 1981; Hoover and Vasanthan, 1994; Hoover and Manuel, 1996; Collado and Corke, 1999).

2.6.6 Influence of heat-moisture treatment on the susceptibility of starch to acid hydrolysis

The susceptibility to acid hydrolysis decreased after heat-moisture treatment of maize, (Hoover and Manuel, 1996), pea (Hoover et al., 1993), potato (Hoover and Vasanthan, 1994), and wheat, lentil, oat, and yam (Hoover and Vasanthan, 1994) starches. The decrease was attributed to starch chain interactions within the amorphous and crystalline domains during heat-moisture treatment, which renders these regions less susceptible to H_3O^+.

2.6.7 Influence of heat-moisture treatment on the susceptibility of starch to α-amylase hydrolysis

Depending on botanical origin and treatment conditions, increased or decreased susceptibility to α-amylase hydrolysis were observed as a result of heat-moisture treatment (Kulp and Lorenz, 1981; Lorenz and Kulp, 1982; Kuge and Kitamura, 1985; Hoover et al., 1993; Kobayashi, 1993; Hoover and Vasanthan, 1994; Franco et al., 1995; Hoover and Manuel, 1996). the reasons for the different

behaviors are likely to be ascribed to variations in the magnitude of interaction between starch chains during heat-moisture treatment.

3. Materials and methods

3.1 Materials

Dioscorea alata (true yam), Alocassia *indica* (taro), *Manihot esculenta* (cassava), *Solanum tuberosum* (potato) and *Xanthosoma sagitifolium* (new coco yam) were grown on experimental plots (under identical environmental conditions). Crystalline porcine pancreatic α-amylase (EC 3.2. 1.1, type 1A), was purchased from Sigma Chemical Co., (St. Louis, MO, USA). All other chemicals and solvents were of ACS certified grade. Solvents were distilled from glass distilled before use.

3.2 Methods

3.2.1 Starch isolation

All tubers and roots were divided into two lots representing the whole sample. Each lot was subdivided into two lots. Each sub lot was further subdivided into two sub lots. Starch was extracted and purified using the procedure of Hoover and Hadziyev, (1981). The tubers were peeled, washed, diced, dipped in ice-cold water containing 100 ppm $NaHSO_3$ and homogenized at low speed in a Waring blender. The slurry was squeezed through a 100-mesh polyester sieve cloth and the filtrate centrifuged at 700 x g for 15 min. The supernatant and the amber-brown layer of protein atop the starch layer was removed. Further purification was achieved by repeated suspension in water, centrifugation and removal of contaminating proteins and cells. The purified starch was dried overnight at 30^0C in a vacuum oven to a moisture content of ~10%.

3.2.2 Granule morphology

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

3.2.3 Proximate analysis

Quantitative estimation of moisture, ash, nitrogen, and starch were performed by the standard AACC methods (1984). Starch lipids were determined by the procedure outlined by Vasanthan and Hoover, (1992). Total phosphorus content was determined by the method of Morrison (1964).

3.2.3.1 Moisture content

Preweighed (3-5g, db) samples of starch were dried in a forced air oven (Fisher scientific, Isotemp 614G, USA) at 130^{O}C for 1 hr. The samples were then removed and cooled in a desiccator. The moisture content was calculated as the percentage weight loss of the sample.

3.2.3.2 Ash content

Preweighed samples (3-5g, db) were transferred into clean, dry porcelain crucibles, charred using a flame and then placed in a pre-heated (550^{O}C) muffle

furnace (Lab Heat, Blue M, USA) and left overnight and weighed. The ash content was calculated as percentage weight of the remaining material.

3.2.3.3 Nitrogen content

The nitrogen content was determined according to Micro Kjeldahl method. The samples (0.3g, db) were weighed on nitrogen-free paper and placed in the digestion tubes of a Buchi 430 (Buchi Laboratorimus-techink AG, Flawill/Schweiz) digester. The catalyst (2 Keltabs M pellets) and 20mL of concentrated H_2SO_4 acid were added and the samples were digested in the Buchi 430 digester until a clear yellow solution was obtained. The digested samples were then cooled, diluted with 50mL of distilled water, 100mL of 40% (W/W) NaOH was added, and the released ammonia was steam distilled into 50 ml of 4% H_3BO_3 containing 12 drops of end point indicator (N-point indicator, EM Science, NJ, USA) using a Buchi 321 distillation unit until 150mL of distillate was collected. The amount of ammonia in the distillate was determined by titrating it against 0.05N/H_2SO_4. Percentage nitrogen was calculated as follows:

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

3.2.3.4 Lipid content

Surface lipids were extracted at room temperature (25-27^{o}C) by mixing starch (5g, db) with 100ml of 2:1 (v/v) chloroform - methanol under vigorous agitation in a wrist action shaker for 1hr. The solution was then filtered (Whatman No. 4 filter paper) into a round bottom flask and the residue was washed thoroughly with small amounts of chloroform-methanol solution. The solution was then evaporated to dryness using a rotary evaporator (Rotavapor - R110, Buchi Laboratorimus - Technik AG, Flawill/Schweiz, Switzerland). The crude lipid extracts were purified by the method of Bligh and Dyer (1959) before quantification. Bound lipids were extracted using the residue left from surface lipid extraction. The residue was refluxed with 3:1 n-propanol-water (v/v) in a soxhlet apparatus at 90-100^{o}C for 7hr. The solvent was evaporated using a rotary evaporator. Crude lipid extract was purified by the Bligh and Dyer (1959) method before quantification. Total starch lipid was determined by hydrolyzing starch (2g, db) with 25mL of 24% HCl at 70 - 80^{o}C for 30min and extracting the hydrolysate three times with 1-hexane (Vasanthan and Hoover, 1992b). The mixture was evaporated to dryness using the same rotary evaporator used for surface lipid and bound lipid extractions.

3.2.3.4.1 Lipid purification (Bligh and Dyer [1959] method)

The crude lipid extracts were purified by extraction with chloroform-methanol - water (1:2: 0.8 v/v/v) and forming a biphasic system (Chloroform-methanol - water, 1:1:0.9, v/v/v) by addition of chloroform and water at room temperature in

a separatory funnel. The chloroform layer was then diluted with benzene and brought to dryness on a rotary evaporator.

3.2.3.5 Total phosphorous

Total starch phosphorous was determined according to the method of (Morrison, 1964). Dry starch sample (6mg) was placed into a hard glass test tube. Concentrated sulfuric acid (5mL) was then added, and the tube gently heated untill charring was complete. Hydrogen peroxide (30% w/v H_2O_2) was then added dropwise to completely clarify the solution, and the tube well shaken. The tube was then gently boiled for (2min) and allowed to cool to room temperature. The contents were diluted with water (4mL),using the water to wash down the walls of the tube. Sulfite solution (0.1mL) [33% (w/v) Na_2SO_3. $7H_2O$] was then added with stirring, followed by addition of 2% w/v ammonium paramolybdate (0.1mL) [$(NH_4)_6Mo_7O_{24}.4H_2O$] directly into the solution. Finally, ascorbic acid (0.01g) was added, and the solution was heated in boiling water bath for 10min. After cooling to room temperature, the contents were diluted to 10mL, and the absorbance at 822nm was determined using a UV-visible spectrophotometer (LKB Novospec Model 4049). A standard curve was produced using known amounts of KH_2PO_4 (**Fig.I -1** in Appendix I).

3.2.3.6 Amylose content

Apparent and total amylose content was determined by a modification (Hoover and Ratnayake, 2001 of the method of McGrance et al., (1998).

3.2.3.6.1 Apparent amylose content

Starch (20mg, db) was dissolved in 90% dimethylsulfoxide (8mL) [DMSO] in screw-cap reaction vials. The contents of the vials were vigorously mixed for 20 min and then heated in a water bath ,with intermittent shaking at 85°C for 15min. The vials were then cooled to ambient temperature and the contents were diluted with water to 25 mL in a volumetric flask. 1.0 mL of diluted solution was mixed with water (40 mL) and 5 ml 1_2/KI solution (0.0025M KI) and then adjusted to a final volume of 50mL. The contents were allowed to stand for 15min at ambient temperature, before absorbance measurements at 600nm (Hoover and Ratnayake, 2000).

3.2.3.6.2 Total amylose content

The total amylose content of starch samples were determined by the above procedure, but with prior defatting with hot n-propanol-water (3:1 v/v) for 7h. In order to correct for over estimation of apparent and total amylose content (due to complex formation between iodine and the long outer branches of amylopectin), amylose content was calculated from a standard curve prepared using mixtures of pure potato amylose and amylopectin (over the range 0 -100% amylose and amylopectin 100 - 0 %).

3.2.4 Estimation of starch damage

The starch damage was estimated following the AACC (1984) procedure. Starch samples (1g, db) were digested with fungal α-amylase from *Aspergillus oryzae* (12500 sigma units) having specific activity of 50 -100 units/mg, in a water bath at 30^0C for 15min. The enzyme action was terminated by adding 3mL of 3.68N H_2SO_4 and 2mL 12% $Na_2WO_4.2.H_2O$ (2ml), . The mixtures were allowed to stand for 2min and then filtered through Whatman No 4 filter paper. The amount of reducing sugars in the filtrate were determined using the method of Bruner (1964) [section 2.4.3]. The percentage starch damage was calculated as follows:

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

Where M = mg maltose equivalents in the digest; W = mg starch (db); 1.05 = molecular weight conversion of starch to maltose and 1.64 = the reciprocal of the mean percentage maltose yield from gelatinized starch. The latter is an empirical factor, which assumes that under the conditions of the experiment, the maximum hydrolysis is 61%.

3.2.5 Starch fractionation

Amylose and amylopectin were extracted from the tuber and root starches utilizing the aqueous leaching procedure described by Montgomery and Senti (1958).

3.2.5.1 Amylose

Starch (40g, db) was extracted at 2% concentration by adding slurry to water at 98^0C and maintaining this temperature for 15min with stirring the solution. Phosphate buffer consisting of a mixture of 0.2M NaH_2PO_4, (45mL) and 0.2M Na_2HPO_4 (55mL), diluted to 200mL with distilled water, was used to maintain the pH of the solution between 6.0-6.3. The solution was cooled rapidly to room temperature and then centrifuged (IEC-centra MP-4, International Equipment Co., Needham, MA, USA) at 10000rpm for 20min in order to separate the supernatant and the gel-like material which settled to the bottom of the centrifuge tube. The supernatant was saved and the gel was re-extracted with hot distilled water and phosphate buffer. The supernatant obtained after centrifugation was combined with the first supernatant and the gel was re-extracted a third time. The supernatant from the third extraction was discarded and the gel was saved for the isolation of amylopectin. Amylose was isolated from the supernatant obtained after centrifugation. To the supernatant, 1-butanol (250mL) was added and stirred for 5h. The supernatant was decanted after centrifugation and the butanol-amylose complex was collected and recrystallized by adding it to 1L of boiling distilled water containing 100mL of 1-butanol. Stirring was continued until the solution became clear. The hot solution was filtered with suction and cooled slowly with stirring. An additional 150mL of 1-butanol was added, and stirring continued for 16h. The complextion process was repeated three times. The complex was then mixed with acetone in a blender (Waring Commercial blender, Dynamics Corporation of America, New Hartford, CT, USA) and filtered

(Whatman No. 4). The filtered complex was resuspended in the blender with 95% ethanol, recovered by filtration, treated with 99% ethanol in the blender, recovered by filtration and washed with diethylether. The recovered amylose was allowed to air-dry for 24h and then vacuum-dried at 40^0C overnight (Montgomery and Senti, 1958).

3.2.5.2 Amylopectin

Amylopectin was precipitated from the gel after mixing with methanol (1000ml) in a Waring blender for 45sec. The resulting white precipitate was allowed to settle,the methanol was decanted and fresh methanol (100mL) was added, followed by further blending (45sec.). The mixture was filtered under suction, and the precipitate was collected, again mixed with methanol in the blender, and recovered by filtration. The precipitated amylopectin was air-dried for 24h and vacuum-dried at 40^0C overnight (Montgomery and Senti, 1958).

3.2.6 Determination of the purity of isolated amylose by gel permeation chromatography (GPC)

Gel permeation chromatography on isolated tuber and root amylose was done by the McPherson and Jane, (1999) method with modifications. 5ML of the sample solution containing 15mg of isolated tuber and root amylose and 0.5mg of glucose (as marker) was injected into an Econo-Column (1.5×100cm, i.d × l) (Bio-Rad Laboratories, Richmond, CA, USA) packed with Sepharose CL-2B (Sigma Chemical Co., St. Louis, MO, USA). 0.02% NaCl was used to elute the

sample at 30mL/h flow rate. Fractions of 4.8mL were collected and analyzed for iodine affinity (0.0025M I_2/0.0065M KI solution) and total carbohydrate (Dubois et al., 1956).

3.2.6.1 Iodine affinity

An aliquot of 0.250mL from each fraction was mixed with 5mL of I_2/KI (0.0025M I_2 in 0.0065M KI solution). The reaction mixture was allowed to stand for 15min at 25^0C for color development, and the absorbance was then measured using a LKB Novaspec-4049 spectrophotometer (LKB Biochrom Ltd., Cambridge, England) at 600nm.

3.2.6.2. Determination of total carbohydrate (Dubois et al., 1956)

Fractions of 0.125mL were diluted to 2mLusing distilled water and the amount of total carbohydrate of each diluted fraction was analyzed as follows. 1mL of 5% (w/v) phenol solution was added to 2mL of the sample solution in a test tube, 5mL of conc. H_2SO_4 was added directly onto the sample liquid surface and the mixture was allowed to stand for 10min. The test tubes were transferred into a 30^0C water bath after mixing. After 15min, the absorbance was taken against a reagent blank at 490nm. A standard series was prepared with known amounts of maltose (Fisher Scientific, Fair Lawn, NJ, USA) (**Fig. I-2** in Appendix I).

3.2.7 Determination of amylose structure

3.2.7.1 Degree of polymerization (DP) of amyloses

Isolated amylose (0.01g) was completely dissolved in 10mL of DMSO by heating at 60^0C in a water bath. The resulting solution was divided into two equal volumes and the degree of polymerization (DP) was calculated (Jane and Robyt, 1984) using the equation shown below:

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

Total carbohydrate and total reducing power were calculated according to the procedures outlined by Dubois et al., (1956) and Bruner, (1964), respectively.

3.2.7.2 Determination of total reducing sugar (Bruner, 1964)

3.2.7.2.1 Preparation of 3,5 dinitrosalicylic acid (DNS)

20g of DNS was dissolved in 700mL NaOH. The mixture was stirred well to dissolve DNS and then diluted to 1L with distilled water and filtered through a medium porosity fitted glass-filter. The reagent was storde in a dark bottle under refrigeration until used.

In the determination of total reducing sugars, 1mL of the sugar solution was taken into a screw-capped tube and the volume was adjusted up to 2mL using distilled water. Then, 2mL of 3,5 dinitrosalicylic acid was added. The mixture was heated in a boiling water-bath for 5min for color development. The tubes were then cooled in an ice-bath for 10min, and then distilled water was added to make the volume up to 12mL. The absorbance at 540nm was read at 25^0C using a UV-

visible spectrophotometer (LKB Novaspec-4049 spectrophotometer (LKB Biochrom Ltd., Cambridge, England) against a reagent blank. The standard curve was produced using known amounts of maltose (Fisher Scientific, Fair Lawn, NJ, USA) (**Fig. I-3** in appendix I).

3.2.8 Determination of amylopectin structure

3.2.8.1 β amylolysis limit

The β amylolysis limit of isolated amylopectin was determined by the method of Nilsson, (1999). Amylopectin (5mg) was gelatinized in 1M NaOH (8mL) for 1h at 45^0C, followed by the addition of 1M citric acid to adjust the pH to 6.0. The final sample volume was adjusted to 10mL. One fraction (5mL) of the sample was withdrawn and used as a blank. The remaining 5mL incubated with β amylase (5u) for 2h at 37^OC with continuous stirring. Complete hydrolysis was confirmed by addition of another 5u of β amylase and prolonged hydrolysis time in a parallel sample. The β amylolysis was calculated as shown below:

$$\% \beta \text{ amylolysis} = \frac{\text{Reducing capasity (as maltose)}}{\text{Total carbohydrate (as maltose)}} \times 100$$

3.2.8.2 Number average chain length ($\bar{C}$ln) of amylopectins

Debranching was carried out by a modification of the method of Shi and Seib, (1992). Amylopectin (5.5mg db) was dissolved in 3.0mL of 0.05M sodium acetate buffer (pH 3.5) by boiling for 5min. After cooling to 25^0C, 2ml of isoamylase

solution (85 units/mL of 0.05M sodium acetate buffer pH 3.5) was added and the sample kept at 37^{0}C in a shaking water bath for 48h. The number average chain length was calculated as shown:

$$\bar{C}ln = \frac{\text{weight of carbohydrate } (\mu g)}{\text{Reducing sugar } (\mu g \text{ maltose})} \times 2$$

3.2.8.3 Exterior and interior chain length of amylopectin

The average length of the exterior chain (ECL), *i.e*, those chains located outside the branching points and the interior chain length (ICL)was calculated according to equations 1 and 2 (Manners, 1989).

$$ECL = CL \times (\% \beta \text{ Limit } /100) + 2 \rightarrow \text{Eq (1)}$$

$$ICL = CL - ECL - 1 \rightarrow \text{Eq (2)}$$

The addition of 2 is made in equation (1) since the ECL in β limit dextrin in on average two glucose units. The actual branch point residue is regarded as neither exterior nor interior unit. Therefore the subtraction of 1 in equation (2).

3.2.9 X-ray diffraction

X-ray diffractograms of the starches were obtained with a Rigaku RU X-ray diffractometer (Rigaku-Denki Co., Tokyo, Japan) with the following operating conditions as: target voltage 40kV, current-100mA, aging time-5min, scanning range-3-350, scan speed-2.000°/min, step time-4.5sec, divergence slit width-1.00, scatter slit width-1.00 and receiving slit width-0.60.

3.2.10 Determination of relative crystallinity

Relative crystallinity of the starches was calculated using the method of Nara et al., (1978) [**Fig. II-1** in Appendix II], using the peak-fitting software Origin-version 6.0 (Microcal Inc., Nothampton, MA, USA). Amorphous starch was prepared by heating a 10% starch solution at 95^0C for 30min with continuous agitation and then drying at 100^0C for 24h. The dried sample was ground into a free flowing powder using a RP Pulaerit comminucator (Geoscience Instruments Corp., New York, NY, USA) with denatured alcohol as the solvent. The ground sample was air dried for 24h and passed through a 250μm sieve.

3.2.11 Swelling factor (SF)

The SF of the starches when heated to 50-9^0C in excess water was determined according to the method of Tester and Morrison, (1990). The SF was reported as the ratio of the volume of swollen starch granules to the volume of dry starch. This method measures only intragranular water and hence the true SF at a given temperature. Starch samples (50mg db) were weighed into screw cap tubes, and 5mL of water added. Tubes were then heated in a shaking water bath at the appropriate temperature for 30 min. Tubes were cooled to 20^0C and 0.5mL of blue dextran (5mg-mL^{-1}) was added. Contents were mixed by inverting the tubes. The tubes were then centrifuged at 1500 x g for 5 min and the absobance of the supernatant and a starch-free reference was measured at 620nm using a

spectrophotometer (Novospec Model 4049). The absorbance of the reference which contains no starch was also measured at 620 nm.

Calculation of SF was based on starch weight corrected to 10% moisture, assuming a density of 1.4 g-ml^{-1}.

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

$FW = 5.5 (A_R / A_S) - 0.5$

A_R and A_S represent the absorbance of the reference and sample respectively.

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

$V_0 = W / 1400$

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

$V_1 = 5.0 - FW$

Hence the volume of the swollen starch granules (V_z) is

$V_z = V_0 + V_1$ and

$SF = V_z / V_0$

This can also be expressed by the single equation

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

3.2.12 Amylose leaching (AML)

Starch (20mg, db) in water (10mL) was heated (50-90^{0}C) in volume calibrated sealed tubes for 30min. The tubes were then cooled at ambient temperature and centrifuged at 2000 g for 10min. The supernatant liquid (1mL) was withdrawn and its amylose content was determined as described by Hoover and Ratnayake, (2001).

3.2.13 Gelatinization parameters

Gelatinization parameters were measured using a Seiko DSC (Seiko Instrument Inc., Chiba, Japan) differential scanning calorimeter equipped with a thermal analysis data station and data recording software. Water (11μL) was added with a microsyringe to starch (3.0mg) in the DSC pan, which was then sealed, reweighed and allowed to stand for 2h at room temperature in order to attain an even distribution of water. The scanning temperature range and the heating rates were 20-120°C and 10°C/min^{-1}, respectively. In all measurements, the thermogram was recorded with an empty aluminum pan as the reference. The transition temperatures reported are the onset (T_o), peak (T_p), and conclusion (T_c). The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram (**Fig. II-2** in appendix II) and a base line under the peak and was expressed in terms of joules per unit weight of dry starch (J/g).

3.2.14 Differential scanning calorimetry of retrograded starch

Water (3μL) was added with a microsyringe to starch (3.0mg) in DSC pans, which were then sealed, reweighed and allowed to stand for 2h at room temperature for moisture equilibration. The sealed pans were then heated to 120°C at 10°C/min to gelatinize the starch. The gelatinized samples were stored at 4°C for 24 h to increase nucleation, and then at 40°C for 7 days to increase propagation. Subsequently, the samples were equilibrated at room temperature

for 2h, and then rescanned in the calorimeter from 20 to 120°C at 10°C/min to measure retrogradation transition temperatures and enthalpy.

3.2.15 Acid hydrolysis

The starches were hydrolyzed with 2.2N HCl at 35°C (1.0g starch/40mL acid) for 12days. The starch slurries were shaken by hand daily to resuspend the deposited granules. At 24h intervals, aliquots of the reaction mixtures were neutralized and recentrifuged (2000g) and the supernatant liquid was assayed for total carbohydrate (Dubois et al, 1956). The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial starch.

3.2.16 Enzymatic digestibility

Enzymatic digestibility (0-72h) studies on tuber and root starches were done using a crystalline suspension of porcine pancreatic α-amylase (Sigma Chemical Co., St. Louis, MO, USA) in 2.9M saturated sodium chloride containing 3mM calcium chloride, in which the concentration of α-amylase was 30mg-mL^{-1}, and the specific activity was 790 units per milligram of protein. One unit was defined as the α-amylase activity which liberates 1mg maltose in 3min at 20°C at pH 6.9. Hydrolysis was carried out for 6h, 12h, 24h and 72h. The procedure was essentially that of Knuston et al. (1982). Starch (100 mg, db) was suspended in distilled water (25mL) and 5mL aliquots were placed in a constant temperature water bath at 37°C. Then 4.0mL of 0.1 M phosphate buffer (pH 6.9) containing 0.006 M NaCl were added to the slurry and the mixture was gently stirred before

adding α-amylase suspension (12 units / mg starch). The reaction mixtures were shaken by hand every 6h to resuspend the deposited granules. Aliquots (1mL) were removed at specific time intervals, and .ethanol (0.2 ml) of 90% pipetted in to the reaction mixtures. Followed by centrifugation at 3000g. Aliquots of the supernatant were analyzed for soluble carbohydrate (Bruner, 1964). Percentage hydrolysis was calculated as the amount (mg) of maltose released per 100mg of dry starch. Controls without enzyme, but subjected to the above experimental conditions, were run concurrently to eliminate spontaneous hydrolysis of starch (Hoover and Vasanthan, 1994).

3.2.17 Heat-moisture treatment

Starch (15g, db) was weighed into glass containers and the moisture content was brought to 30%. The sealed samples (in glass jars) were heated in a forced air oven (Fisher Scientific, Isotemp 615g), USA) at 100°C for 10h. After cooling, the jars were opened and starch samples were air-dried to a moisture content of ~10%. The experimental conditions used for heat-moisture treatment were based on the findings of (Hoover and Vasanthan, 1994; Hoover et al.,1993).

3.2.18 Statistical analysis

All determinations were replicated three times and mean values and standard deviations reported. Analysis of variance (ANOVA) were performed and the mean separations were done by Tukey's HSD test ($p<0.05$) using Sigmastat Version 2.0 (Jandel Scientific/SPSS Science, Chicago,IL, USA).

4 Results and discussion

4.1 Morphological granular characteristics

The starch granules ranged from oval to round to spherical to polygonal in shape with characteristic dimensions in the range 3-110μm (**Table 4-1**). On the basis of starch granule size, new cocoyam had a very large surface area per unit weight compared to that of the other starches. The granule surface of all starches appeared to be smooth and showed no evidence of pin holes under the scanning electron microscope. Heat-moisture treatment did not alter the size or shape of the starch granules. Similar observations have been made on heat-moisture treated maize (Hoover and Manuel, 1996) and wheat (Hoover and Vasanthan, 1994) starches.

4.2 Chemical composition

The data on chemical composition are presented in **(Table 4-1**). The purity of the starches was judged on the basis of composition (low nitrogen and low ash level) and microscopic observation (absence of any adhering protein). The low nitrogen content was in the range 0.01-0.09% and indicated the absence of non-starch lipids (lipids associated with endosperm protein). Therefore, the total lipids (0.03-0.4%) (**Table 4-1**) obtained by acid hydrolysis mainly represent free and bound starch lipids (Vasanthan and Hoover, 1992). The total lipid content was in the range reported for most tuber and root starches (0.1-1.14%) (Hoover, 2001). The free lipid obtained by extraction with chloroform-methanol (2:1 v/v at 25°C) was in the range 0.02-0.09% of the total weight.

The bound lipid content obtained by extraction of the chloroform-methanol residue with n-propanol-water (3:1v/v for 7h) was in the range 0.01-0.3% (**Table 4-1**). The apparent amylose content (determined by I_2 before removal of bound lipid) was in the range 19.8-26.1% (**Table 4-1**). The total amylose content (determined by I_2 binding after removal of bound lipids by n-propanol water was in the range 22.4-29.3% (**Table 4-1**). A comparison of the apparent and total amylose content (**Table 4-1**) showed that the percentage of total amylose complexed by native starch lipids ranged from 10.1-15.5% (**Table 4-1**). New cocoyam differed from the other starches in exhibiting a significantly higher content of total lipid and a much higher proportion of lipid complexed amylose chains (**Table 4-1**). The phosphorus content ranged from 0.01-0.10% (**Table 4-1**). Potato contained more phosphorus (0.10%) than any of the other starches (0.01-0.03%) used. The extent of starch damage (0.25-1.50%) was low in all starches (**Table 4-1**).

4.3 Molecular structure

The average degree of polymerization (DP_n) of isolated amylose followed the order: potato > new cocoyam > cassava > taro > true yam. The DP_n of potato (4850) and cassava (2500) was close to the values reported by Takeda et al, (1984). The DP_n of the other starches have not been reported previously, and therefore, no comparisons are possible. The average chain length ($\bar{C}l_n$) of isolated amylopectins followed the order: true yam > potato > taro > cassava > new cocoyam.The $\bar{C}l_n$ of potato (28) and cassava (24) were close to reported

Table 4-1 Chemical composition (%) and some properties of tuber and root starches

Characteristic	Composition(%)[a]				
	True yam	Taro	New cocoyam	Cassava	Potato
Moisture	10.2 ± 0.20^{p}	$11.2 \pm 0.10^{q,r}$	11.3 ± 0.03^{r}	13.5 ± 0.24^{s}	$13.1 \pm 0.15^{t,s}$
Ash	$0.12 \pm 0.01^{p,q}$	0.14 ± 0.01^{q}	$0.15 \pm 0.02^{r,p,q}$	$0.11 \pm 0.02^{s,q,p}$	0.25 ± 0.01^{t}
Nitrogen	$0.05 \pm 0.01^{p,t,r}$	$0.03 \pm 0.01^{q,p}$	0.08 ± 0.03^{r}	$0.02 \pm 0.01^{s,p,q}$	$0.09 \pm 0.02^{t,r}$
Lipid					
Solvent extracted					
Chloroform-methanol[b]	$0.02 \pm 0.01^{p,r,t,s}$	$0.08 \pm 0.02^{q,r}$	0.09 ± 0.04^{r}	$0.06 \pm 0.02^{s,r,q,t}$	$0.08 \pm 0.01^{t,r,q}$
n-Propanol-water[c]	$0.01 \pm 0.00^{p,s,q}$	$0.04 \pm 0.01^{q,s}$	0.30 ± 0.05^{r}	$0.08 \pm 0.03^{s,t}$	0.12 ± 0.02^{t}
Acid hydrolyzed[d]	0.03 ± 0.01^{p}	$0.15 \pm 0.01^{q,t,s}$	0.40 ± 0.03^{r}	0.12 ± 0.02^{s}	0.20 ± 0.04^{t}
Amylose content					
Apparent[e]	24.6 ± 0.30^{p}	26.1 ± 0.21^{q}	22.3 ± 0.12^{r}	19.8 ± 0.20^{s}	25.2 ± 0.22^{t}
Total	$28.5 \pm 0.70^{p,q}$	29.3 ± 0.62^{q}	26.4 ± 0.20^{r}	22.4 ± 0.13^{s}	$28.1 \pm 0.50^{t,p,q}$
Amylose complexed with lipid[f]	$10.1 \pm 0.40^{p,q,t}$	$10.9 \pm 0.30^{q,s}$	15.5 ± 0.60^{r}	11.6 ± 0.32^{s}	$10.4 \pm 0.30^{t,q}$
Phosphorus	0.03 ± 0.01^{p}	$0.02 \pm 0.01^{q,p}$	$0.02 \pm 0.00^{r,p,q}$	$0.01 \pm 0.00^{s,p,q,r}$	0.1 ± 0.05^{t}
Starch damage	$1.23 \pm 0.20^{p,t}$	$0.85 \pm 0.30^{q,p,r}$	$1.20 \pm 0.02^{r,p}$	0.25 ± 0.01^{s}	$1.5 \pm 0.04^{t,r}$
Granule shape	oblong to oval	round to variable	polygonal to variable	round to variable	oval to spherical
Granule size (diameter) [μm]	12-100	10-50	3.0-10	5.0-45	10-110

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

[b]Lipids extracted from chloroform-methanol 2:1 (v/v) at 25°C (mainly unbound lipid)

[c]Lipids extracted by hot n-propanol-water 3:1 (v/v) from the residue left after chloroform-methanol extraction (mainly bound lipid)

[d]Lipids obtained by acid hydrolysis (24%) HCl of the native starch (total lipid)

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

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

values (Suzuki et al., 1985; Hizukuri, 1985). The $\bar{C}l_n$ of the other starches have not been reported previously, and therefore, no comparisons are possible. The average exterior chain length (ECL) of amylopectin followed the order: potato > true yam > taro > cassava > new cocoyam. The corresponding order for the average interior chain length (ICL) was: true yam > potato > taro > new cocoyam > cassava (**Table 4-2**).

4.4 X-ray pattern and crystallinity

Potato and true yam showed the typical "B" type X-ray pattern (Zobel, 1988b) with reflection intensities at 5.5, 17, and 22-24^{0}2θ angle (**Fig. 1**). However, the other three starches showed a "A" type X-ray pattern (**Fig. 4-1**). Both "A" and "B" type starches are based on parallel standard double helices, in which the helices are more closely packed in the "A" type starch. Furthermore, they also differ in the content of intra-helical water ("B">"A") [Imberty, 1988; Imberty et al., 1988]. The type of crystalline polymorph has been shown (Hizukuri et al., 1981) to be influenced by the chain length (CL) of amylopectin ("A" type CL< 19.7, B type CL ≥ 21.6), growth temperature (Hizukuri et al., 1961) and fatty acids (Hizukuri, 1996). The relative crystallinity (**Table 4-3**) of potato (30%) and cassava (31%) were comparable to the values reported by Zobel, (1988b) for the above two starches. However, the relative crystallinity of new cocoyam (45%) was much larger than the value reported (24%) by Takeda et al., (1983). The differences in relative crystallinity (**Table 4-3**) among the starches probably represent differences in: 1) crystallite size, 2) orientation of double helices within the

Table 4-2 Structure of amylose and amylopectins of tuber and root starches

Starch source	Amylose[1]	Amylopectin			
	DP_n[2]	CL_n[3]	ECL[4]	ICL[5]	β amylolysis(%)
True yam	1,800 ± 45[a]	29.0	18.5	9.5	57 ± 4[a]
Taro	2,200 ± 60[b]	26.1	16.6	8.4	56 ± 2[a]
New cocoyam	2,775 ± 59[c]	24.2	15.8	7.4	57 ± 2[a]
Cassava	2,500 ± 62[d]	24.5	16.2	7.3	58 ± 2[a]
Potato	4,850 ± 75[e]	28.1	18.6	8.5	59 ± 3[a]

[1]Amylose from all starches was free of amylopectin (confirmed by gel permeation chromatography [GPC]). The yield of amylose (by GPC) was 26.6,28.8,26.0, and 26.7%, respectively in true yam, taro, new cocoyam, cassava, and potato

[2]Number average degree of polymerization.

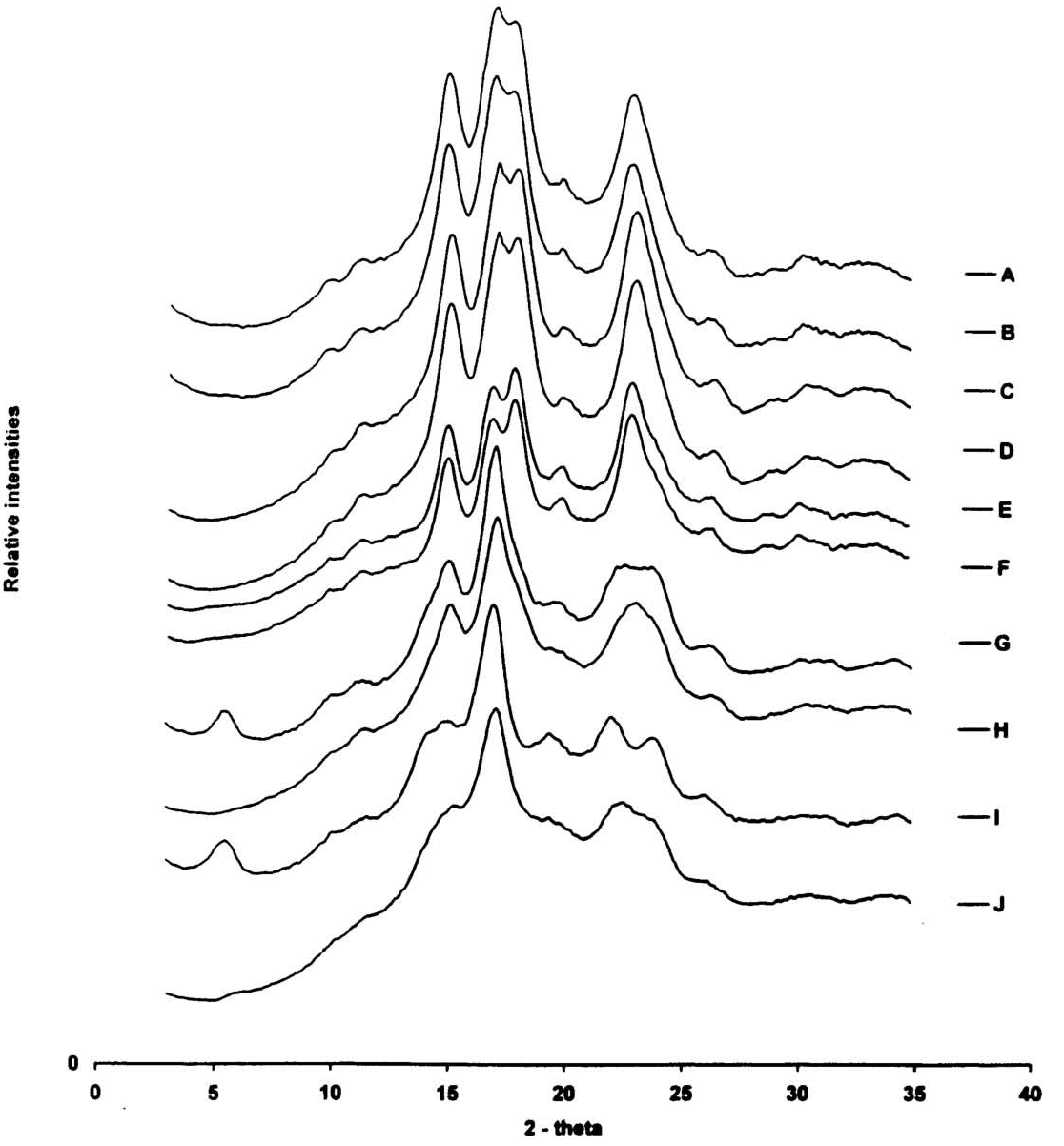
[3]Average chain length

[4]External chain length = $\frac{\overline{Cl}_n \times \beta \text{ amylolysis limit (\%)}}{100} + 2$

[5]Internal chain length = $(Cl_n - ECL) - 1$

Means within a column with different superscripts are significantly different ($p < 0.05$)

Fig 4-1 X-ray diffraction patterns of native and heat-moisture treated (HMT) tuber and root starches (10h, 30% moisture, 100^0C). (A) native cassava, (B) HMT cassava, (C) native taro, (D) HMT taro, (E) native new cocoyam, (F) HMT new cocoyam, (G) native true yam, (H) HMT true yam, (I) native potato, (J) HMT potato


Relative intensities
A
B
C
D
E
F
G
H
I
J
0
0
5
10
15
20
25
30
35
40
2 - theta

crystallite, 3) average chain length of amylopectin (Cl_n), and 4) mole percentage of the short chain fraction of amylopectin (DP 10-13).

Heat-moisture treatment changed the X-ray pattern ("B" → "A+B") of both potato and true yam starches (**Fig. 4-1**). A shift from "B" to "A" crystal type as a result of heat-moisture treatment was confirmed by ^{13}CCP/MAS NMR based on variations in C-1 multiplicity (Gidley and Bociek, 1985). The X-ray pattern of taro, cassava and new cocoyam , however, remained unchanged on heat-moisture treatment **(Fig. 4-1)**. The relative crystallinity of potato and true yam decreased by 9% and 8%, respectively after heat-moisture treatment (**Table 4-3**). However, the relative crystallinity of the other starches remained practically unchanged (**Table 4-3**).

Imberty et al. (1988) and Imberty and Perez, (1989) have shown that double helices of "A" and "B" type starches are packed in a pseudohexagonal array. The lattices of "B" type starches have a large void (channel) in which 36 water molecules can be accommodated. However, in "A" type starches, the lattices contain a helix in the center rather than a column of water. In both "A" and "B" type starches, there is a spacing of double helices that corresponds to 1.1nm distance between the axes of the two double helices. The change in X-ray pattern ("B'→"A+B") on heat-moisture treatment can be attributed to: 1) dehydration (vaporization of the 36 water molecules in the central channel of the B-unit cell), and 2) movement of a pair of double helices into the central channel, (that was originally occupied by the vaporized water molecules). Double helical movement during heat-moisture treatment could disrupt starch crystallites and /or

Table 4-3 X-ray pattern and relative crystallinity (%) of native and heat-moisture treated tuber and root starches

Starch source[a] & treatment	X-ray pattern	Relative crystallinity[b] (%)
True yam		
Native	B	32.0 ± 0.2^{p}
HMT[c]	A+B	23.0 ± 0.8^{q}
Taro		
Native	A	31.0 ± 0.5^{p}
HMT[c]	A	30.0 ± 0.1^{p}
New cocoyam		
Native	A	45.0 ± 0.3^{p}
HMT[c]	A	43.5 ± 0.2^{p}
Cassava		
Native	A	37.0 ± 0.5^{p}
HMT[c]	A	36.0 ± 0.1^{p}
Potato		
Native	B	30.0 ± 0.7^{p}
HMT[c]	A+B	22.0 ± 0.4^{q}

[a]Moisture content ~ 16%(w/w)

[b]Relative crystallinity $= \Sigma |Is\text{-}Ia| / \Sigma |Ic\text{-}Ia| \times 100$, where Is-Ia = difference between the sample and morphous intensities and Ic-Ia + difference between the crystallinity (quartz) and amorphous intensities

[c]Heat-moisture treated (100°C, 30% moisture, 10h)

Means within a column with different superscripts(for native starch and its heat-moisture treated counterpart) are significantly different ($p < 0.05$)

change crystallite orientation. This would then explain the observed changes in crystallinity on heat-moisture treatment of B-type starches (**Table 4-3**).

4.5 Swelling factor (SF) and amylose leaching (AML)

The SF and AML at different temperatures are presented in (**Tables 4-4 and 4-5**), respectively. SF and AML of native and heat-moisture treated starches increased with increase in temperature. The extent of this increase was more pronounced at temperatures beyond 70°C. The, SF of native starches followed the order: potato > cassava > taro > true yam > new cocoyam. Whereas, AML followed the order: potato ~ taro > true yam > cassava > new cocoyam. The interplay between the extent of interaction between starch chains (in the amorphous and crystalline domains), phosphate content (**Table 4-1**), and the amount of lipid complexed amylose chains (**Table 4-1**) may have been responsible for the observed differences in SF (**Table 4-1**) and AML (**Tables 4-5**). The decrease in AML on heat-moisture treatment (**Table 4-5**) suggests that additional interactions may have occurred between amylose-amylose (AM-AM) and amylose-amylopectin (AM-AMP) chains during heat-moisture treatment. This type of mechanism may also be partly responsible for the observed decrease in SF on heat-moisture treatment (**Table 4-4**). Tester and Morrison, (1990) have shown by comparative studies, on non-waxy and waxy maize starches, that swelling is primarily a property of amylopectin and that amylose is a diluent. Furthermore, Cooke and Gidley, (1992) have suggested that the forces holding the granule together are mainly at the double helical level and that the starch

Table 4-4 Swelling factor[a] of native and heat-moisture treated tuber and root starches

Starch source	Treatment	Temperature (^{0}C)				
		50	60	70	80	90
True yam	Native	0.0	0.0^p	5.3 ± 0.3^p	26 ± 0.2^p	33 ± 0.1^p
	HMT[a]	0.0	1.2 ± 0.2^q	3.3 ± 0.2^q	7.9 ± 0.4^q	19.5 ± 0.6^q
Taro	Native	0.0	2.5 ± 0.1^p	8.5 ± 0.2^p	$36 \pm .0.1^p$	34.2 ± 0.0^p
	HMT[a]	0.0	0.0^q	6.0 ± 0.5^q	13.4 ± 0.2^q	23.3 ± 0.4^q
New cocoyam	Native	0.0	5.0 ± 0.2^p	10.6 ± 0.3^p	18.0 ± 0.6^p	22 ± 0.62^p
	HMT[a]	0.0	4.0 ± 0.5^q	9.2 ± 0.4^q	12.6 ± 0.1^q	13.1 ± 0.2^q
Cassava	Native	0.0	4.6 ± 0.2^p	31.0 ± 0.7^p	43.0 ± 0.4^p	36.5 ± 0.2^p
	HMT[a]	0.0	2.1 ± 0.1^q	16.4 ± 0.3^q	24.5 ± 0.2^q	23.5 ± 0.5^q
Potato	Native	0.0	37.6 ± 0.4^p	57.4 ± 0.5^p	60.0 ± 0.5^p	54.0 ± 0.3^p
	HMT[a]	0.0	10.2 ± 0.2^q	17.2 ± 0.6^q	18.5 ± 0.8^q	16.4 ± 0.4^q

[a]Starches (50mg) were heated with water (5ml) at the specified temperatures for 30min

[b]Heat-moisture treated (100°C, 30% moisture,10h)

Means within the same column with different superscripts (for native starch and its heat-moisture treated counterpart)are significantly different ($P < 0.05$)

Table 4-5 Amylose leaching (%) [a] of native and heat-moisture treated tuber and root starches

Starch source		Temperature (°C) 50	60	70	80	90
True yam	Native	0.0	0.0	1.1 ± 0.5^{p}	13.0 ± 0.3^{p}	20.1 ± 0.3^{p}
	HMT[a]	0.0	0.0	0.1 ± 0.0^{q}	2.0 ± 0.5^{q}	5.5 ± 0.1^{q}
Taro	Native	0.0	0.3 ± 0.3^{p}	2.3 ± 0.2^{p}	22.1 ± 0.2^{p}	23.0 ± 0.6^{p}
	HMT[a]	0.0	0.0^{q}	0.2 ± 0.0^{q}	7.3 ± 0.4^{q}	12.2 ± 0.1^{q}
New cocoyam	Native	0.0	0.1 ± 0.1^{p}	0.6 ± 0.1^{p}	2.9 ± 0.6^{p}	5.4 ± 0.4^{p}
	HMT[a]	0.0	0.0^{q}	0.4 ± 0.1^{q}	2.0 ± 0.2^{q}	2.2 ± 0.5^{q}
Cassava	Native	0.0	7.0 ± 0.2^{p}	15 ± 0.3^{p}	16.6 ± 0.5^{p}	17.2 ± 0.1^{p}
	HMT[a]	0.0	6.3 ± 0.2^{q}	11.5 ± 0.4^{q}	15.3 ± 0.1^{q}	16.1 ± 0.1^{q}
Potato	Native	0.0	4.5 ± 0.5^{p}	18.1 ± 0.1^{p}	22 ± 0.3^{p}	22.2 ± 0.2^{p}
	HMT[a]	0.0	1.9 ± 0.1^{q}	6.7 ± 0.2^{q}	8.7 ± 0.6^{q}	9.2 ± 0.3^{q}

[a]Starches (20mg) were heated with water (10ml) at the specified temperatures

[a]Heat-moisture treated (100°C, 10h, 30% moisture

Means within the same column with different superscripts (for native starch and its heat-moisture treated counterpart) are significantly different ($P < 0.05$)

"crystallinity" functions as a means of achieving dense packing rather than as a primary provider of structure. This implies that the decrease in SF on heat-moisture treatment (**Table 4-4**) could also be due to a decrease in granular stability, resulting from unraveling of double helices that may have been present in a crystalline array in the native granule. Disruption of crystallites on heat-moisture treatment (**Table 4-3**) could have contributed to the large decrease in SF observed for true yam and potato starches (**Table 4-4**).

4.6 Gelatinization parameters

The gelatinization temperatures, onset [(T_o), mid point (T_p), and conclusion (T_c)] and gelatinization enthalpy (ΔH) are presented in (**Table 4-6**). The gelatinization temperatures of native starches followed the order: taro > true yam > new cocoyam > cassava > potato. Whereas, the gelatinization temperature range (T_c-T_o) followed the order: cassava > taro > true yam > potato. The ΔH followed the order: true yam > potato > taro > new cocoyam > cassava.

The differences in gelatinization temperatures among the starches can be attributed to the interplay of three factors: 1) molecular structure of amylopectin (unit chain length, extent of branching 2). starch composition (amylose to amylopectin ratio, amount of lipid complexed, amylose chains, phosphorous content), and 3) granular architecture (crystalline to amorphous ratio). Difference in T_c-T_o (**Table 4-6**) suggests that the degree of heterogeneity of crystallites within the granules of the five starches are different. The ΔH values have been shown to represent the number of double helices that unravel and melt during

Table 4- 6 DSC characteristics of native, and heat moisture treated tuber and root starches[a]

		Transition temperatures				
Starch source	Treatment	T_0 [b] (^{0}C)	T_p [b] (^{0}C)	T_c [b] (C)	T_c-T_0 [b] (^{0}C)	ΔH [c] (J/g)
True yam	Native	75 .0± 0.3^p	80.0 ± 0.5^p	91.2 ± 0.3 ^{p}	16.5± 0.^{p}	17.8 ± 0.1^p
	HMT[d]	79.8 ± 0.2^q	88.2 ± 0.6^q	98.5 ± 0.5^q	18.9 ±0.3^q	15.5 ± 0.1^q
Taro	Native	76.8 ± 0.2^p	83.0 ± 0.6^p	95.2 ± 0.7^p	18.4 ± 0.2^p	14.5 ± 0.3^p
	HMT[d]	79.2 ± 0.3^q	89.5 ± 0.1^q	98.5 ± 0.2^q	19.5 ± 0.2^q	12.2 ± 0.2^q
New cocoyam	Native	71.5 ± 0.6^p	77.2 ± 0.3^p	85.4 ± 0.9^p	13.6 ±0.3^p	13.1 ± 0.4^p
	HMT[d]	75.4 ± 0.6^q	83.1 ± 0.2^q	90.3 ± 0.8^q	14.7 ± 0.5^q	11.5 ± 0.4^q
Cassava	Native	63.0 ± 0.5^p	71.5 ± 0.2^p	81.5 ± 0.2^p	18.7 ± 0.6^p	12.3 ± 0.2^p
	HMT[d]	66.4 ± 0.5^q	79.1 ± 0.2^q	87.0 ± 0.3^q	20.6 ± 0.3^q	11.7 ± 0.3^q
Potato	Native	59.6 ± 0.4^p	66.3 ± 0.4 ^{p}	76.0 ± 0.5^p	16.5 ± 0.4^p	16.3 ± 0.1^p
	HMT[d]	61.2 ± 0.3^q	75.6 ± 0.5^q	86.5 ± 0.4^q	25.3 ± 0.2^q	11.5 ± 0.2^q

[a]Starch: water ratio is, 1:3 (w/w) dry basis)
[b]T_0,T_p, T_c, T_c-T_0 represent the onset, mid-point, end of gelatinization, and the gelatinization temperature range, respectively
[c]Enthalpy of gelatinization
[d]Heat-moisture treated (100°C, 30% moisture, 10h)
Means within the same column with different superscripts (for native starch and its heat-moisture treated counterpart) are significantly different ($P< 0.05$

gelatinization (Cooke and Gidley, 1992). Thus the higher ΔH values for true yam and potato starches (**Table 4-6**) could be attributed to the presence of a higher number of double helices (due to their longer amylopectin chain length [**Table 4-2**]) and /or weaker interaction between adjacent amylopectin double helices within the crystalline domains of the native granule (this postulate seems plausible, since crystallite disruption on heat-moisture treatment occurs only in potato and true yam [**Table 4-3**]).

Heat-moisture treatment increased T_o, T_p, T_c and T_c-T_o (potato > true yam > cassava > taro > new cocoyam) [**Table 4-6**], but decreased ΔH (potato > true yam> cassava > taro > new cocoyam) [**Table 4-6**] .

The melting temperatures (T_o, T_p, T_c) of the starch crystallites are controlled indirectly by the surrounding amorphous region. The reduction in granular swelling on heat-moisture treatment (**Table 4-4**) would reduce the destabilization effect of the amorphous region on crystallite melting. Consequently, a higher temperature would be required to melt crystallites of heat-moisture treated starches. This would then explain the increase in T_o, T_p, and T_c on heat-moisture treatment (**Table 4-6**). The decrease in ΔH (**Table 4-6**) on heat-moisture treatment suggests that some of the double helices present in crystalline and in non-crystalline regions of the granule may have disrupted under the conditions prevailing during heat-moisture treatment. Thus, fewer double helices would unravel and melt during gelatinization of heat-moisture treated starches.

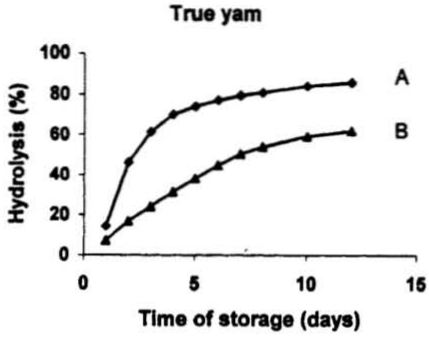
In B-type starches, the packing of helices is less compact than in A-type starches, (Gidley, 1987). Furthermore, there are 36 water molecules per B-type

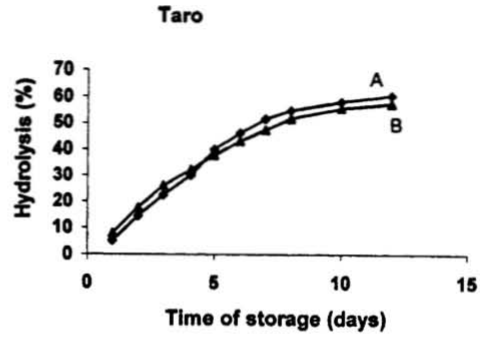
unit cell, whereas only 4 water molecules are present within the A-type unit cell (Sarko and Wu, 1978). Consequently, on heat-moisture treatment, the double helical chains forming the crystallites of B-type starches would be more mobile, and hence more prone to disruption than those of A-type starches. This would then explain, the large difference in ΔH between native and heat-moisture treated B-type starches (**Table 4-6)**. The changes in gelatinization parameters on heat-moisture treatment are more pronounced in potato starch due to its higher phosphate monoester content (**Table 4-1**). Phosphate groups are mainly located on C_2, C_3, and C_6 of the glucose unit of potato starch (Hizukuri et al., 1970). Repulsion between negatively charged phosphate groups on adjacent amylopectin chains would hinder strong interaction between double helices. Consequently, crystallites of potato starch would be very susceptible to disruption on heat-moisture treatment. This would then explain the large decrease in T_o,T_p, T_c and ΔH on heat-moisture treatment of potato starch.

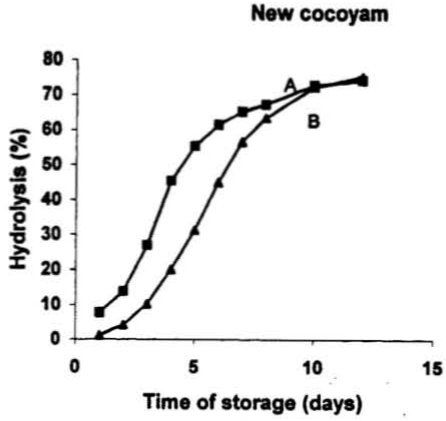
4.7 Acid hydrolysis

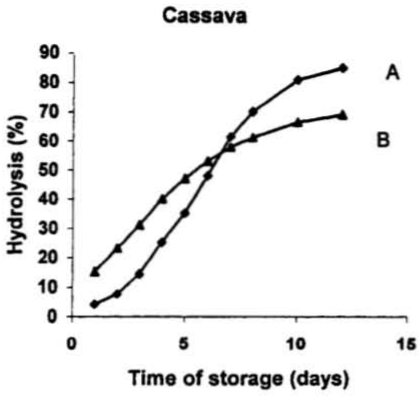
Acid hydrolysis of native and heat-moisture treated starches are presented in (**Fig. 4-2**). The extent of hydrolysis of native starches during the first few days (corresponding mainly to the degradation of amorphous regions) followed the order: true yam > potato > taro > cassava > new cocoyam. Thereafter, the extent of hydrolysis (corresponding mainly to the degradation of crystallites) followed the order: true yam > cassava > new cocoyam > potato > taro. Differences in the extent of acid hydrolysis between native starches has been attributed to granule

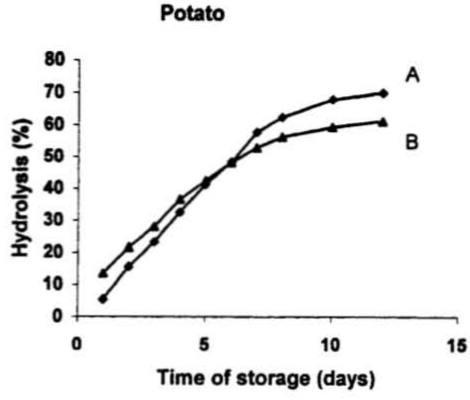
Fig. 4-2 Acid hydrolysis of native and heat-moisture treated (10h, 30% moisture, 100^0C) tuber and root starches, A= native, B= heat-moisture treated


True yam
Hydrolysis (%)
100
80
60
40
20
0
0
5
10
15
Time of storage (days)
A
B


Taro
Hydrolysis (%)
70
60
50
40
30
20
10
0
0
5
10
15
Time of storage (days)
A
B


New cocoyam
Hydrolysis (%)
80
70
60
50
40
30
20
10
0
0
5
10
15
Time of storage (days)
A
B


Cassava
Hydrolysis (%)
90
80
70
60
50
40
30
20
10
0
0
5
10
15
Time of storage (days)
A
B


Potato
Hydrolysis (%)
80
70
60
50
40
30
20
10
0
0
5
10
15
Time of storage (days)
A
B

hydrolysis by reducing chain flexibility, and thereby hindering the conformational change (chair →half chair) required for efficient protonation of glycosidic oxygens; and 3) disruption of double helices in the amorphous region (increases hydrolysis by making glycosidic oxygens more accessible to protonation). The influence of heat-moisture treatment on acid hydrolysis varied among the starch sources. For instance, throughout the time course of hydrolysis, heat-moisture treated true yam starch was hydrolyzed to a much lesser extent than that of its native counterpart (**Fig. 4-2**). However, heat-moisture treated taro, cassava and potato showed increased hydrolysis until the 4^{th}, 6^{th}, and 5^{th} day respectively. After which , hydrolysis was less than their native counterpart (**Fig. 4-2**). The decreased susceptibility of heat-moisture treated true yam starch towards acid hydrolysis (**Fig. 4-2**) suggests that extensive amylose chain interactions on heat-moisture treatment (**Table 4-5**) probably negates the influence of crystallite disruption on acid hydrolysis. In potato and true yam starches, the extent of crystallite disruption (**Table 4-2**) and amylose - amylose interactions (**Table 4-5**) during heat-moisture treatment are similar. Therefore, the different hydrolysis patterns shown by heat-moisture treated true yam and potato starches (**Fig. 4-2**), suggests that double helical structures that may have been present in the amorphous regions of potato starch are probably disrupted on heat-moisture treatment, and are thus rendered more accessible to attack by H_30^+. This would then explain the increase in hydrolysis shown by heat-moisture treated potato starch during the first 5 days of hydrolysis (**Fig. 4-2**). The results suggest that double helical structures are either absent and/or are present only in trace

amounts in the amorphous regions of true yam [due to its shorter amylose chain length (**Table 4-2**)]. The increased susceptibility of heat-moisture treated new cocoyam starch towards acid hydrolysis (during the first 10days) can be attributed to the interplay of the following factors: 1) action of H_30^+ on disrupted double helices in the amorphous region; 2) weak interaction between amylose chains during heat-moisture treatment (**Table 4-5**); and 3) action of H_30^+ on free amylose chains (that were originally complexed with lipids in the native granule). The increase in acid hydrolysis on heat-moisture treatment is more pronounced in cassava than in taro due to weaker amylose - amylose interactions (**Table 4-5**) in the former.

4.8 Enzyme hydrolysis

The susceptibility of tuber and root starches towards hydrolysis by porcine pancreatic α-amylase followed the order: New cocoyam > cassava > taro > potato ~ true yam (**Table 4-7**). Differences *in vitro* digestibility of native starches by α-amylase has been attributed to the interplay of many factors such as: granular size, surface area, type of unit cell (A, B or A+B), amylose to amylopectin ratio, amount of lipid complexed amylose chains, crystallinity and extent of distribution of α (1→6) branch points between the amorphous and crystalline regions of amylopectin (Jane et al.,1997; Planchot et al., 1997; Holm et al., 1983; Hoover and Sosulski, 1991). The results indicate that differences in granule size (potato > true yam > taro > cassava > new cocoyam) and the presence of α (1→6) branch points (the location of α (1→6) branch points in the

Table 4- 7 Enzyme hydrolysis (%) of native and heat-moisture treated tuber and root starches by porcine pancreatic ∝-amylase

Starch source	Treatment	Number of hours			
		6	12	24	72
True yam	Native	1.2 ± 0.2^{p}	1.5 ± 0.2^{p}	3.1 ± 0.2^{p}	4.9 ± 0.3^{p}
	HMT[a]	4.2 ± 0.3^{q}	8.5 ± 0.3^{q}	11.3 ± 0.5^{q}	17.2 ± 0.5^{q}
Taro	Native	8.2 ± 0.4^{p}	15.3 ± 0.1^{p}	22.1 ± 0.2^{p}	38.0 ± 0.2^{p}
	HMT[a]	12.3 ± 0.5^{q}	19.2 ± 0.4^{q}	28.1 ± 0.3^{q}	45.4 ± 0.1^{q}
New cocoyam	Native	18.8 ± 0.6^{p}	24.2 ± 0.1^{p}	36.1 ± 0.4^{p}	62.5 ± 0.1^{p}
	HMT[a]	20.2 ± 0.7^{q}	29.1 ± 0.6^{q}	40.2 ± 0.1^{q}	67.3 ± 0.3^{q}
Cassava	Native	15.3 ± 0.4^{p}	22.1 ± 0.2^{p}	34.2 ± 0.6^{p}	56.2 ± 0.4^{p}
	HMT[a]	19.4 ± 0.5^{q}	38.4 ± 0.3^{q}	48.1 ± 0.5^{q}	69.5 ± 0.1^{q}
Potato	Native	1.8 ± 0.6^{p}	2.1 ± 0.3^{p}	3.8 ± 0.1^{p}	5.9 ± 0.5^{p}
	HMT[a]	8.3 ± 0.5^{q}	14.7 ± 0.6^{q}	$23.1 \pm$	32.8 ± 0.1^{q}

[a]Heat-moisture treated (100°C, 30%miosture, 10h)

Means (for the different stages of hydrolysis) within the same column (for native starch and its heat-moisture treated counterpart) with different superscripts are significantly different ($p<0.05$)

crystallite regions would weaken the crystalline structure thereby increasing the accessibility of α-amylase into the granule interior) in the crystalline regions of A-type starches (new cocoyam, taro, cassava) are the factors that influence hydrolysis of native starches. This postulate is based on the fact that, differences among starches with respect to the level of amylose content (**Table 4-1**), amylose lipid complexes (**Table 4-1**) and crystallinity (**Table 4-2**) are too small to account for the observed differences on enzyme hydrolysis.

In all starches, enzyme susceptibility increased on heat-moisture treatment (potato > true yam > cassava > taro > new cocoyam). Gallant, (1974) has shown that one of the limiting factors in α-amylolysis could be the nature of the granule surface with respect to crystallinity. Furthermore, Planchot et al., (1997) have postulated, that the fraction of total crystalline material is an important factor defining the rate and extent of α-amylase hydrolysis. The initial step of α-amylolysis corresponds to adsorption of α-amylase on the granule surface. Thus, crystallite disruption near the granule surface on heat-moisture treatment of true yam and potato (**Table 4-3**) starches, could facilitate the rapid entry of α-amylase into the granule interior. This would then explain the more pronounced increase in enzyme hydrolysis observed on heat-moisture treatment of the above starches (**Table 4-7**). The extent of crystallinity disruption during heat-moisture treatment was nearly the same in both true yam and potato starches (**Table 4-3**). Therefore, the more pronounced increase in hydrolysis after heat-moisture treatment of potato starch (**Table 4-7**) could be attributed to interactions (during heat-moisture treatment) involving amylose chains (**Table 4-5**) being of a lower

order of magnitude than in true yam [Interaction between amylose chains (within the amorphous region) would decrease the accessibility of α-amylase towards the α- (1→4) glycosidic linkages]. In A-type starches, crystallites are not disrupted on heat-moisture treatment (**Table 4-3**). Therefore, the extent of increase in hydrolysis on heat-moisture treatment (cassava > taro > new cocoyam) mainly reflects the interplay between: 1) the number of double helices that may have disrupted in the amorphous regions during heat-moisture treatment (disrupted double helices would increase accessibility of the unraveled chains to the binding sites of α-amylase), and 2) the extent of interaction that occurs between amylose chains (taro > new cocoyam > cassava) during heat-moisture treatment (**Table 4-5**).

4.9 Retrogradation

The melting enthalpies (ΔH_R) of amylopectin recrystallization are presented in (**Table 4-8**). ΔH_R reflects the extent of retrogradation during the storage period (7days at 40°C). ΔH_R followed the order: True yam > potato > taro > new cocoyam > cassava. This result confirms earlier reports that amylopectin from B-type starches retrogrades to a greater extent than amylopectins from A type(cereal) and A+B-type(legume) starches (Kalichevsky et al., 1990; Silverio et al., 1996). This was attributed to the shorter average amylopectin chain length of the A-type starches (Kalichevsky et al., 1990; Orford et al., 1987). Ward et al. (1994) postulated that differences in retrogradation between cereal amylopectins is influenced by: a) an increased molar proportion of unit chain with DP 14-24

Table 4-8 The enthalpy of retrogradation (ΔH_R) of native and heat-moisture treated tuber and root starches monitored by differential scanning calorimetry

Starch source	Treatment	Number of days of storage[a] 1	2	3	4	5	6	7
True yam	Native	7.4 ± 0.5^{p}	7.2 ± 0.9^{p}	7.6 ± 0.6^{p}	8.7 ± 0.3^{p}	9.2 ± 0.5^{p}	9.3 ± 0.8^{p}	9.8 ± 0.6^{p}
	HMT[b]	7.2 ± 0.8^{p}	7.1 ± 0.3^{p}	7.4 ± 0.5^{p}	8.1 ± 0.6^{p}	8.3 ± 0.6^{p}	8.4 ± 0.6^{p}	8.7 ± 0.1^{q}
Taro	Native	4.1 ± 0.6^{p}	5.2 ± 0.6^{p}	5.7 ± 0.8^{p}	6.3 ± 0.4^{p}	6.8 ± 0.8^{p}	7.1 ± 0.8^{p}	7.1 ± 0.8^{p}
	HMT[b]	3.9 ± 0.9^{p}	4.9 ± 0.2^{p}	5.7 ± 0.2^{p}	6.2 ± 0.9^{p}	6.9 ± 0.2^{p}	6.7 ± 0.1^{p}	6.9 ± 0.5^{p}
New cocoyam	Native	3.6 ± 0.7^{p}	3.4 ± 0.6^{p}	3.8 ± 0.6^{p}	4.1 ± 0.3^{p}	4.9 ± 0.3^{p}	5.2 ± 0.6^{p}	5.3 ± 0.7^{p}
	HMT[b]	3.5 ± 0.5^{p}	3.1 ± 0.2^{p}	3.8 ± 0.2^{p}	4.5 ± 0.4^{p}	4.8 ± 0.9^{p}	5.0 ± 0.8^{p}	5.4 ± 0.8^{p}
Cassava	Native	1.3 ± 0.5^{p}	1.4 ± 0.2^{p}	2.2 ± 0.3^{p}	2.3 ± 0.6^{p}	2.5 ± 0.8^{p}	3.4 ± 0.4^{p}	3.6 ± 0.3^{p}
	HMT[b]	1.5 ± 0.6^{p}	1.6 ± 0.2^{p}	2.5 ± 0.8^{p}	2.2 ± 0.3^{p}	2.7 ± 0.5^{p}	3.1 ± 0.5^{p}	3.2 ± 0.4^{p}
Potato	Native	4.9 ± 0.8^{p}	5.3 ± 0.4^{p}	5.9 ± 0.5^{p}	6.6 ± 0.3^{p}	7.2 ± 0.3^{p}	7.6 ± 0.3^{p}	8.2 ± 0.7^{p}
	HMT[b]	4.8 ± 0.3^{p}	5.6 ± 0.5^{p}	5.3 ± 0.6^{p}	5.5 ± 0.9^{p}	5.9 ± 0.8^{p}	$7.2 \pm 0.8p$	7.1 ± 0.5^{q}

[a] At 40°C

[b]Heat-moisture treated (100°C, 30%moisture, 10h)

Means (for the different days of storage) with different superscripts (for native starch and its heat-moisture treated counterpart) are significantly different ($p<0.05$)

(increases retrogradation, and (b) an increased molar proportion of short chains with DP 6-9 (inhibits retrogradation). A similar finding was also reported by Wursch and Gumy (1994).

In this study, differences in the extent of retrogradation between taro, new cocoyam and cassava (**Table 4-8**) can be explained on the basis of differences in their external chain length (**Table 4-2**). However, differences in retrogradation between true yam and potato are probably influenced to a greater extent by differences in their amylopectin chain length distribution (not determined in this study) rather than the external chain length. Heat-moisture treatment decreased retrogradation in B-type starches, but caused no significant changes to the retrogradation of A-type starches (**Table 4-8**). As discussed earlier, crystallites are disrupted in B-type starches, but remain unchanged in A-type starches on heat-moisture treatment (**Table 4-2**). Thus, after heat-moisture treatment, the degree of separation between the outer branches of adjacent amylopectin chains would be greater in the B-type starches, but would remain practically the same in A-type starches. Consequently, during gel storage, the formation and lateral association of double helices involving amylopectin chains, would be much slower, more difficult and less stronger for heat-moisture treated B-type starches. This would then explain the observed decrease in ΔH_R for true yam and potato, and the unchanged ΔH_R for new cocoyam, cassava and taro starches **(Table 4-8)**.

SUMMARY AND CONCLUSION

The results showed that starch chain interaction, crystalline disruption and dissociation of double helical structures (in the amorphous region) occur on heat moisture treatment. The extent of these structural changes and the accompanying changes to crystallinity, amylose leaching, granular swelling, acid and enzyme susceptibility, gelatinization and retrogradation were more pronounced in the B-type starches (potato and true yam).

Many tuber and root starches are not widely used in food applications due to their poor functional properties. Presently, chemical modification is widely used to tailor the properties of potato and cassava starches. This study has shown that heat-moisture treatment may be an alternative to chemical modification for altering the gelatinization and retrogradation properties of tuber and root starches. Many tuber and root crops are endemic to less developed countries. Thus, scientists in these countries need to tailor the properties of tuber and root starches by heat-moisture treatment (using different temperature / time combinations) to a level that is presently met by chemical modification. Such a study would help these countries to compete more effectively in the markets in both the food and non-food sectors.

DIRECTIONS FOR FUTURE RESEARCH

This study has shown that heat-moisture treatment can be used to modify the structure and properties of tuber and root starches. Further studies are needed to determine whether these starches could be modified to a level that is presently met by chemical modification. These studies are listed below:

(1) Determination of structure and property changes when tuber and root starches are subjected to heat-moisture treatment under different time/ temperature/ moisture combination regimes

(2) More detailed investigations (using different physical probes)on the influence of heat-moisture treatment on starch retrogradation

(3) The use of Atomic force microscopy to study the surface characteristics of native and heat-moisture treated starches. Changes in granular surface on heat-moisture treatment (not detectable by SEM) could influence susceptibility towards acid and enzyme hydrolysis

PUBLICATIONS

Gunaratne.A., and Hoover, R. (2001).Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches. *Carbohydr, Polym.*, (In press))

AWARDS RECEIVED FOR THIS STUDY

(1). George F. Stewart International Research Paper Competition, Institute of food technologists (IFT) Annual Conference (2001), New Orleans, Louisiana, USA, awarded third place for "Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches"

(2). Title of "Fellow of the School of Graduate Studies" (2000 -2001), in recognition of the continued academic excellence, Memorial University of Newfoundland, St. John's, Canada

(3). Barrowman Biochemistry Travel Award (2000-2001), in recognition of outstanding achievement and pursuit of excellence by a Memorial University student at the graduate level. Memorial University of Newfoundland, St.John's Canada

(4). Graduate Fellowship, Graduate School, Memorial University of Newfoundland, St. John's, Canada (31st, Aug, 2000 to 31st, Aug, 2002)

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Appendix 1

Fig.I-1 Standard curve for determination of total phosphorus (Morrison, 1964)

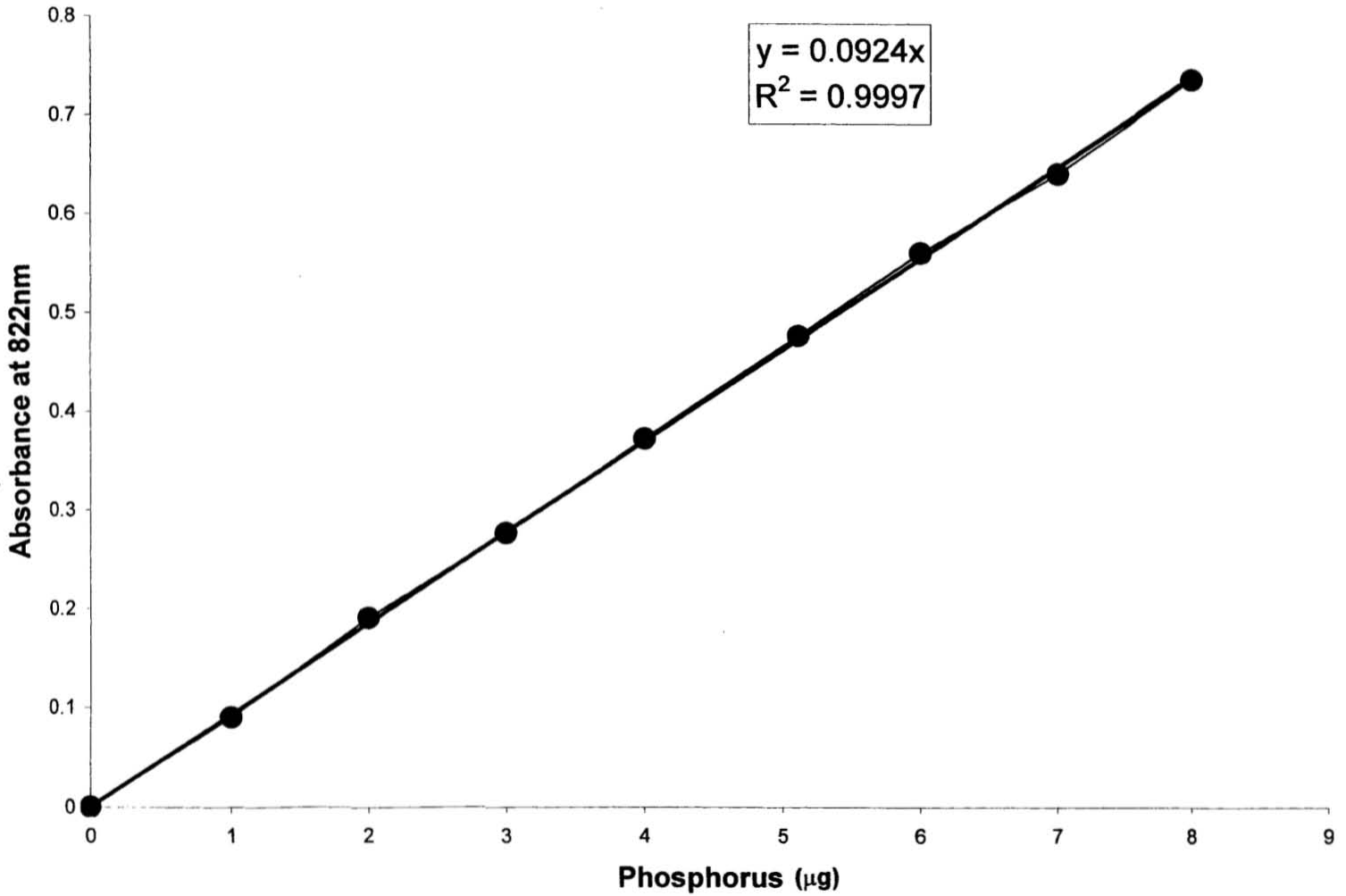

y = 0.0924x
R2 = 0.9997
Absorbance at 822nm
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
Phosphorus (µg)

Fig. I-2 Standard curve of total carbohydrate as maltose (Dubois et al., 1956)

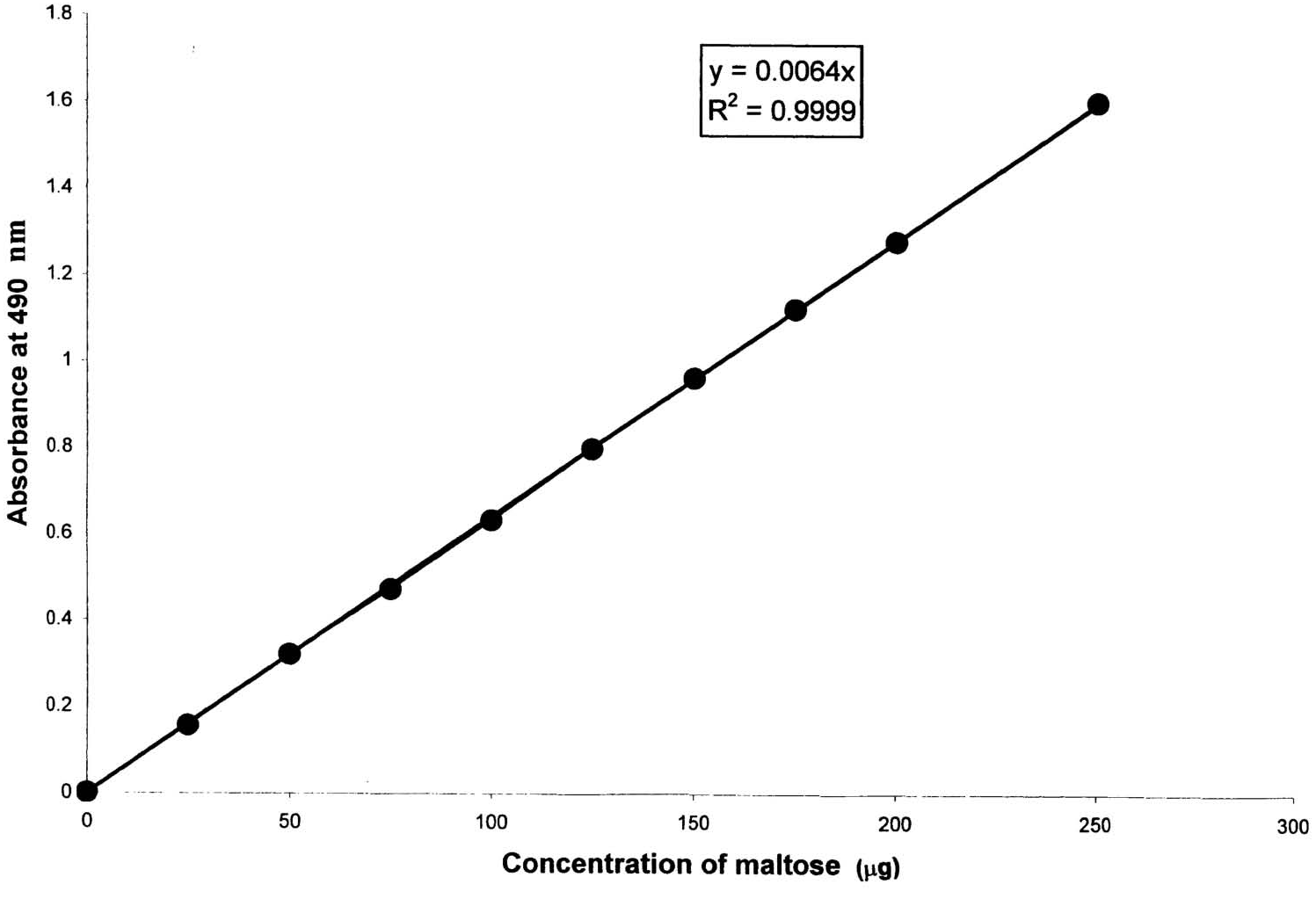
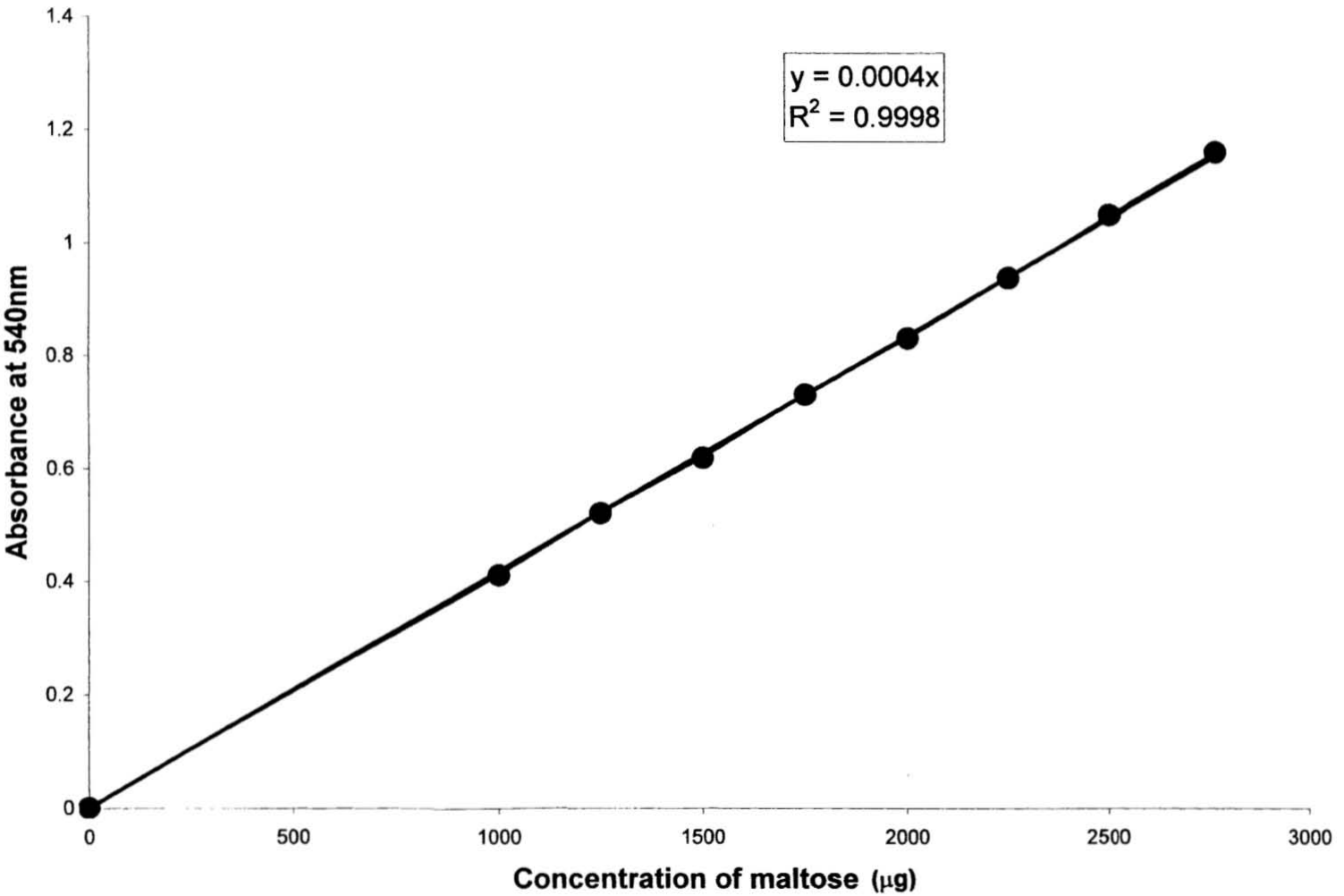

y = 0.0064x
R2 = 0.9999
Absorbance at 490 nm
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
300
Concentration of maltose (µg)

Fig.I-3 Standard curve for determination of reducing sugar as maltose (Bruner, 1964)


y = 0.0004x
R2 = 0.9998
Absorbance at 540nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
500
1000
1500
2000
2500
3000
Concentration of maltose (μg)

Appendix 2

Fig. II-1 Determination of relative crystallinity (Nara et al., 1978)

% Crystallinity = $\sum | I_s - I_a | / \sum | I_c - I_a | \times 100$, where $I_s - I_a$ = difference between the sample [I_s] and amorphous [I_a] intensities and $I_c - I_a$ = difference between the crystalline (quartz) [I_c] and amorphous [I_a] intensities.

The shaded area of the above figure represents $\sum | I_s - I_a |$, where, I_s = intensity of native starch (moisture content = 17%, w/w) and I_a = Intensity of amorphous starch.

According to the same method, the value of $\sum | I_c - I_a |$ can be calculated (not shown in the figure).

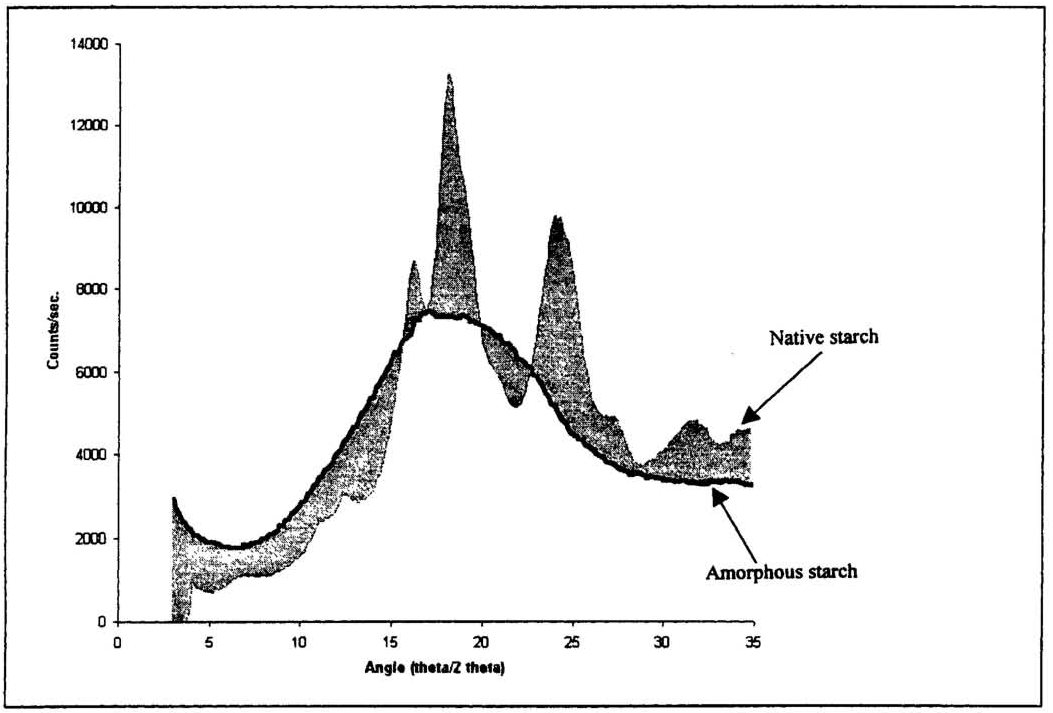
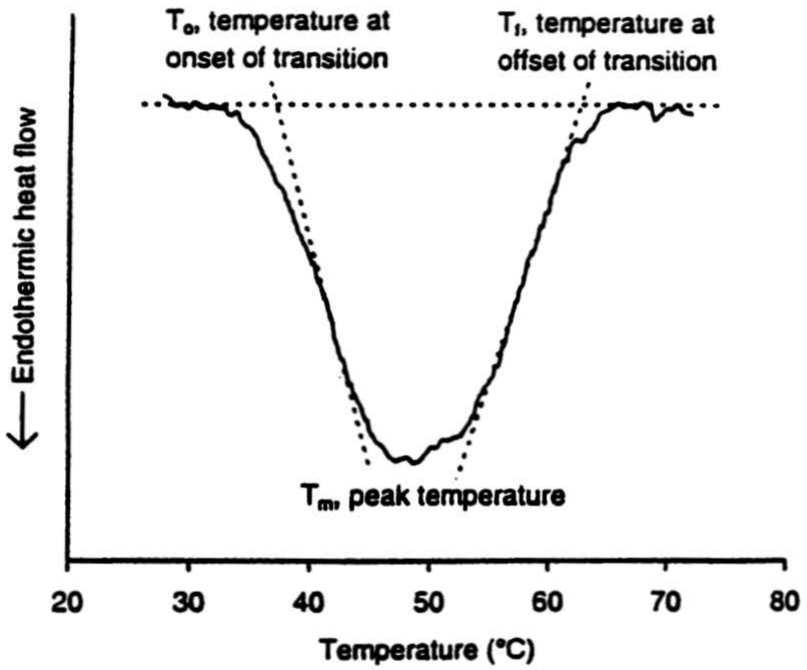

14000
12000
10000
8000
6000
4000
2000
0
Counts/sec.
0
5
10
15
20
25
30
35
Angle (theta/2 theta)
Native starch
Amorphous starch

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


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