COMPOSITION AND PHYSICOCHEMICAL PROPERTIES OF OAT STARCHES

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BY

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ABSTRACT

Starches from two new oat varieties namely AC Stewart (*Avena sativa L.*) and NO 753-2 (*Avena nuda L.*) were isolated and some of their characteristics determined. The total amount of starch lipids (TSL) extracted by acid hydrolysis was 1.60% (w/w) in both starches. The free lipid content (extracted by chloroform-methanol (CM) 2:1 v/v at 25°C) was 0.36 and 0.30% (w/w) in NO 753-2 and AC Stewart starches, respectively. The bound lipid content (extracted by hot n-propanol-water 3:1 v/v from the residue left after CM extraction) was 1.27 and 1.37% (w/w) in NO 753-2 and AC Stewart starches, respectively. The total amylose contents were nearly the same (~23%, w/w) in both starches, of which 14.1 (NO 753-2) and 15.3% (AC Stewart) were complexed by native lipids. The starches differed widely in their degree of swelling, gelatinization transition temperatures and enthalpy, susceptibility towards acid and enzyme hydrolysis, gel strength, paste viscosity, thermal stability and retrogradation characteristics. The results suggest that the extent of interaction of starch chains within the amorphous and crystalline regions of the granule was stronger in NO 753-2 than in AC Stewart.

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LIST OF ABBREVIATIONS

BU	-Brabender units
CL	-Chain length
CTAB	-Cetyltrimethylammonium bromide
CM	-Chloroform-methanol
DP	-Degree of polymerization
DSC	-Differential scanning calorimetry
DMSO	-Dimethylsulfoxide
ECL	-Exterior chain length
ESR	-Electron spin resonance
GMP	-Monopalmitin
GME	-Monoelaidin
GMO	-Monoolein
GML	-Monolinolein
GMS	-Monostearin
GMM	-Monomyristin
ICL	-Interior chain length
PW	-n-Propanol-water
SSL	-Sodium stearoyl-2-lactylate
SDS	-Sodium dodecyl sulfate
SEM	-Scanning electron microscopy
SEL	-Solvent extracted lipids
TSL	-Total starch lipids

DEDICATED TO MY LOVING PARENTS

CHAPTER 1

INTRODUCTION

Oats have been grown for thousands of years as a forage crop and a feed grain rather than for use as human food. Unlike other world cereal crops such as wheat, corn, barley, and rice, oats have received little attention as a subject of major scientific or industrial study.

Oats are unique in their uses and attributes in comparison to most other cereal grains. Most oats used as food are consumed as whole grain products so that the nutritional quality characteristics of the oat are reflected in the food product. The major food uses of oats are in hot and cold cereals, bread products, cookies, and infant foods. Oat products are often used in bakery items to produce desired texture or to increase moisture retention (Paton, 1986). A specially fine-ground oat flour was marketed for antioxidant purposes and found to be very effective to some extent in milk and milk powder, butter, ice-cream, cereals and other products that are sensitive to lipid oxidation during storage (Paton, 1979).

Due to increased awareness regarding the nutritional value of oats, considerable attention has been paid to this cereal grain in recent years (Paton, 1986). Nonetheless, it is difficult to obtain any commercial oat starch as a food ingredient since the starch industry utilizes primarily wheat, potato, maize and rice as raw material.

Many researchers have investigated physicochemical properties of oat starch including pasting, swelling, gelatinization, X-ray diffraction and granular characteristics (Paton, 1977, 1979, 1987; MacArthur and D'Appolonia, 1979; Doublier *et al.*, 1987a,b). Considerable difference has been observed between the physicochemical properties of oat starch and that from other cereal grains and also between oat starch cultivars (Paton, 1977,1987; Gudmundsson and Eliasson, 1989; Hoover and Vasanthan, 1992; Sowa and White, 1992). Oat starch has been shown to differ from other cereal starches in several ways: 1) Starch gels are less susceptible to retrogradation during storage than other cereal starch gels, e.g. maize and barley (Gudmundsson and Eliasson, 1989). 2) During heating of oat starch suspensions, both amylose and amylopectin are coleached from the granules (Doublier *et al.*, 1987a,b). 3) Starch granules have a marked tendency to aggregate into clusters (Hoover and Vasanthan, 1992). However, there is a dearth of information on the variation in crystallinity, digestibility and retrogradation properties between oat starches isolated from different cultivars.

The objectives of this study were to compare the thermal, digestibility, rheological and retrogradation properties of oat starches extracted from two new varieties, NO 753-2 (hull-free oat) and AC Stewart (hulled oat). These properties were investigated by differential scanning calorimetry (DSC), X-ray diffractometry, rheology, scanning electron microscopy (SEM), freeze-thaw stability and paste clarity.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch-General considerations

Starch is a potential carbohydrate polymer with widespread use in foods as well as in technical applications. It is not only available at relatively low cost, but is also convertible to versatile products through biological or physicochemical modifications of the molecular or granular structure. Besides its technical uses, mainly in paper, textile, and chemical industries, starch is of importance in food applications for improving quality of food because of its unique physicochemical properties.

2.1.1 Occurrence

Starch is the major energy reserve carbohydrate in the plant kingdom (Manners, 1985c). It is synthesized in the form of granules irrespective of whether energy storage is transient (e.g. in leaves) or longterm (e.g. in seeds). The storage of starch in the form of granules is a convenient method since starch is an insoluble source of energy that can be gradually made available through the action of enzymes.

Starch can be found in all organs of most higher plants and their modifications (Imberty *et al.*, 1991). It is metabolically easily accessible, in contrast to cellulose, and therefore, is an excellent source of energy. In all cases, it is produced in plastids. In fact,

the ability to produce starch is a characteristic feature of the plastids of higher plants (Badenhuizen, 1965).

Starches can be deposited in tubers, bulbs, seeds, rhizomes, stems, and unripe fruits. The most important starches of commerce are from seeds of cereal grains (maize, rice, wheat, etc.) and from underground storage organs (potato, sweet potato, cassava) (French, 1975).

2.1.2 Characteristics of the starch granule

The starch granule is a densely packed, semi crystalline material, and it is not soluble in cold water. The size, morphology and chemical composition of the starch granules are plant specific, and are also influenced by the growing conditions of the plant (Shannon and Garwood, 1984). In the cereal or legume seed endosperm, potato tuber, and other plant reserve organs, starch is deposited in the form of granules that range in size between 1 and 100 μm and have a variety of shapes depending on their botanical source. Potato starch granules, for example, are large (perhaps 100 μm in diameter) and oval, whereas rice starch granules are smaller (at most 10 μm in diameter) and much more angular (Jenkins *et al.*, 1993). In each case, an examination of these granules under optical or electron microscopy reveals pronounced concentric rings (French, 1984). These rings are alternately of semi-crystalline and amorphous composition as shown by their differing

susceptibility to α-amylase attack. These rings are referred to as "growth rings", and are generally observed to be between 1200 and 4000 Å in size (Manners, 1985c; Jenkins *et al.*, 1993).

At a higher level of organisation, the semi-crystalline rings are composed of stacks of alternating crystalline and amorphous lamellae. The combined repeat distance of crystalline and amorphous lamellae accounts for the peak observed in small angle X-ray and neutron scattering experiments. The crystallinity is associated with the branched amylopectin component of the starch granule. The currently accepted crystalline structure consists of a radial arrangement of clusters of amylopectin (Jenkins *et al.*, 1993). Each cluster contains a region high in branch points (the amorphous lamella) and a region where short chain segments of amylopectin have formed double helix structures (the crystalline lamella) (Fig. 2.1a and b). Lineback (1984) proposed a typical and plausible model for the overall distribution of amylose and amylopectin within the granule (Fig. 2.2).

Cameron and Donald (1992) have developed a model which allows quantification of the various parameters needed to describe this complex structure. The starch granule structure is modelled as a finite number of lamellae of alternating electron density (crystalline regions C with high electron density, and amorphous regions A with low electron density) embedded in a background region (B) of a third electron density, assumed to correspond to the amorphous growth rings (Fig. 2.1c).

2.2. Components of starch

2.2.1 Carbohydrate components

Starch contains two major high molecular weight carbohydrate components. Amylose has traditionally been considered to be a linear polymer composed of glucopyranose units linked through α-D-(1→4) glycosidic linkages (Fig. 2.3A). Although there is now evidence that amylose is not completely linear (Hizukuri *et al.*, 1981), its behaviour is that of a linear polymer. Amylopectin is a branched polymer (Fig. 2.3B) containing short (degree of polymerization (DP)= 20-25 glucopyranose residues) chains linked to the C-6 hydroxymethyl position of certain glucose moieties via α-D-(1→6) linkages. In addition to the fine structure, amylose and amylopectin differ in many respects, as shown in Table 2.1 (Biliaderis, 1991).

2.2.1.1. Amylose

Amylose is found with molecular weights ranging from 10^5 - 10^6 and with the number of glucose residues per molecule, DP, ranging from 500 to 5000. Although considered to be an essentially linear α-(1→4)-glucan, amylose extracted from starches is not completely hydrolysed by the α-(1→4)-specific, α-amylase, unless an α-(1→6) glucan hydrolase (e.g. pullulanase) is also added (Manners, 1985c). That a certain

Fig. 2.1 Schematic representation of the arrangement of amylopectin molecules within a growth ring of a starch granule. (a) A single amylopectin cluster with double helix formation. (b) Schematic representation of the arrangement of amylopectin molecules within a semi-crystalline growth ring. (c) The structure may be modelled in terms of a stack of lamellae alternating in electron density (crystalline regions C and amorphous regions A), embedded in a background region (B) of a different electron density. Adopted from Jenkins *et al.* (1993).

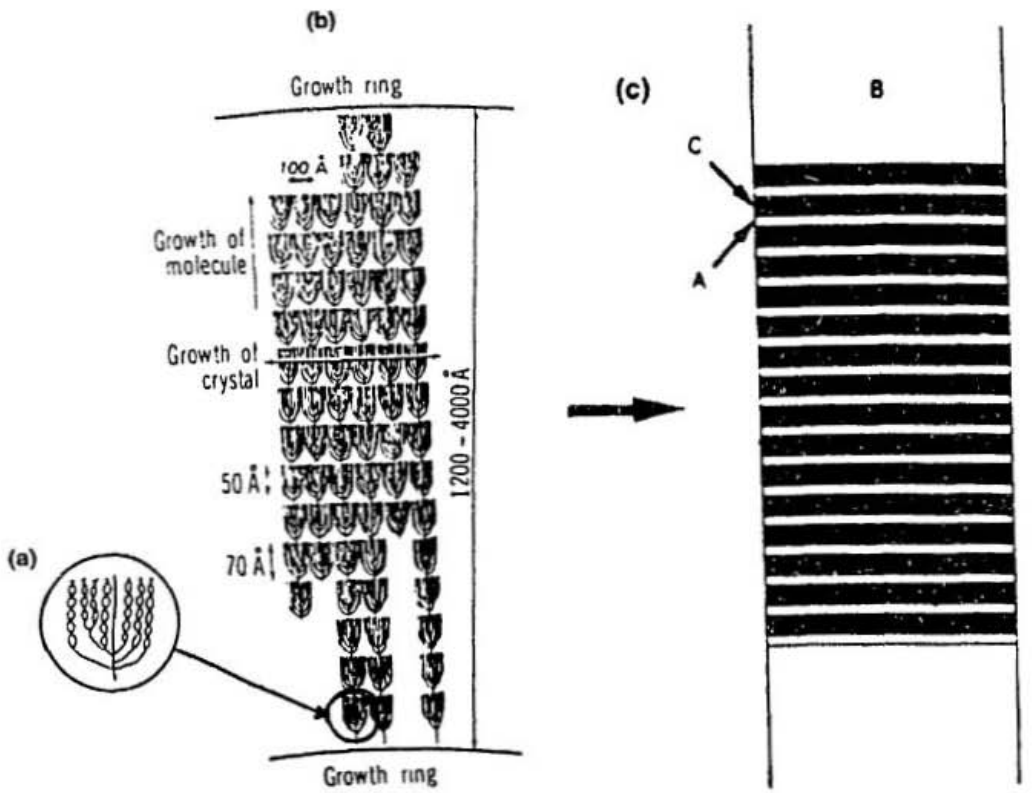

(b)
Growth ring
100 Å
Growth of molecule
Growth of crystal
50 Å
70 Å
1200 - 4000 Å
(a)
Growth ring
(c)
B
C
A

Fig. 2.2 Schematic of the organization (structure) of a starch granule. Adopted from Lineback (1984).

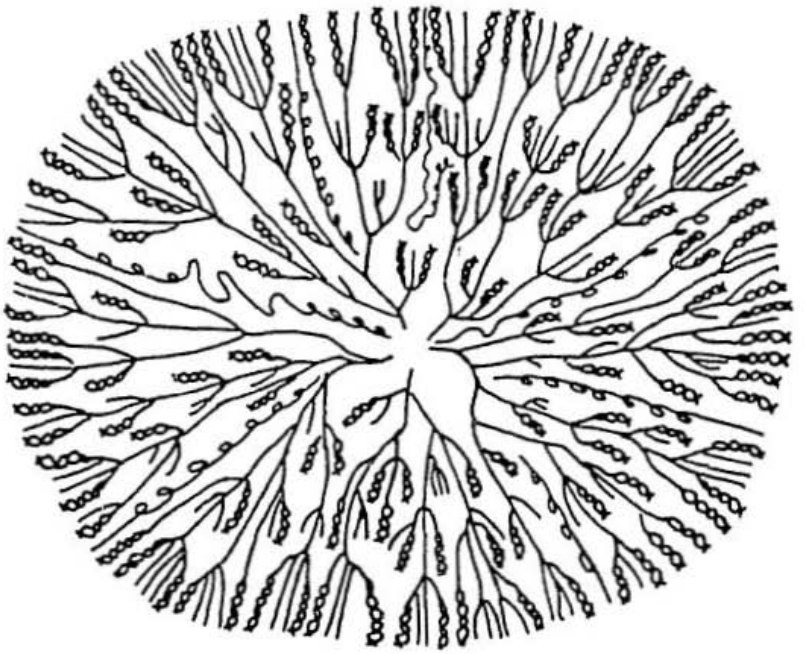


Fig. 2.3 Structure of the amylose and amylopectin components of starch. A, diagram of a portion of an amylose molecule; B, enlarged view of the shaded section of A, showing the chemical formula; C, diagram of a portion of an amylopectin molecule; D, enlarged view of the shaded section of C, showing the chemical formula. Adopted from Pazur (1965).

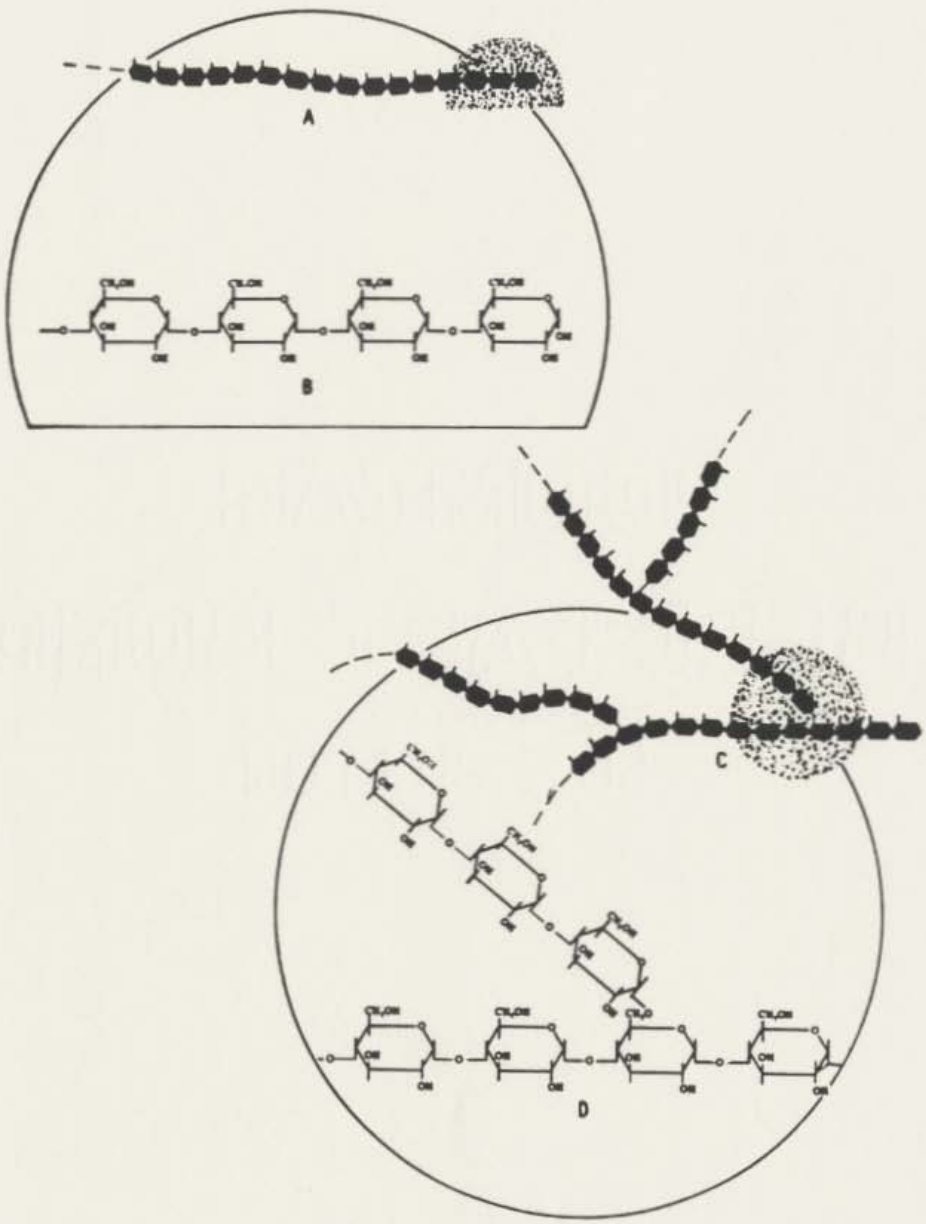

A
B
C
D

Table 2.1 Structure and physicochemical properties of starch polymers .

Property	Amylose	Amylopectin
Molecular structure	Essentially linear, α-(1→4)	Branched, α-(1→4) and α-(1→6)
Degree of polymerization	~10^3	~10^4-10^5
Molecular weight	1.5 X 10^5-10^6	(50-500) X 10^6
Average chain length	~10^3	~20-25
Iodine complex, λ_{max} (color)	600-640 nm (blue)	530-550 nm (purple)
Digestibility (%)		
β-Amylase	~70	~55
β-Amylase and debranching enzyme	~100	~100
Stability of dilute aqueous solutions	Unstable (retrogrades)	Stable
Gel texture	Stiff, irreversible (T_m >100°C)	Soft (thermally reversible at < 100°C)
Film properties	Strong, coherent	Brittle

Adopted from Biliaderis (1991).

degree of branching is present in amylose has been confirmed recently by Hizukuri *et al.* (1981), who have demonstrated that amylose from different sources contains, on average, 2-8 branch points per molecule, the side-chains ranging in chain-length from 4 to ≥100 glucose units. In a recent study it was found that 1.6% of the glucose in amylose was in fact found in an $\alpha(1\rightarrow6)$ branching point (Curà and Krisman, 1990).

The solution behaviour of amylose has been interpreted as being that of a random coil, with no marked helical content in aqueous solution in the absence of complexing agents (Ring *et al.*, 1985). Small-angle X-ray scattering data suggest that the amylose chain is highly disordered but that short-range helical structures might exist (Braga *et al.*, 1985). These helical structures are both irregular and labile. Although amylose is slightly branched, as described above, the branching does not influence its behaviour in solution; amylose behaves as a linear polymer.

There are two features of amylose in solution that are of special interest in relation to baking. The first is the great tendency to form intramolecular hydrogen bonds, which means a strong tendency toward crystallization (also referred to as retrogradation). An amylose solution is therefore, not very stable. For a 2.4% amylose solution, turbidity is observed only a few minutes after cooling to 32°C (Miles *et al.*, 1985b). The development of crystallinity can be followed by the X-ray powder technique, and it is found that crystallinity develops at a slower rate than turbidity. Moreover, the crystallinity develops

in the polymer-rich phase. The second feature of interest concerning amylose in solution is its ability to form helical inclusion complexes. When the proper ligand is present, amylose forms a helix with the ligand in the cavity. The formation of a complex between amylose and iodine causes the typical blue colour, as this complex has an absorption maximum at $\lambda = 640$ nm (Williams *et al.*, 1970).

2.2.1.2 Amylopectin

Amylopectin is an α-(1→4)-D-glucan containing 4-5% α-(1→6)-D-glycosidic linkages (branch points) (Imberty *et al.*, 1991). This highly branched polymer has one of the highest molecular weights (greater than 10^8) known among naturally occurring polymers. Because of the short length of the exterior chains, amylopectin does not form strong complexes with iodine.

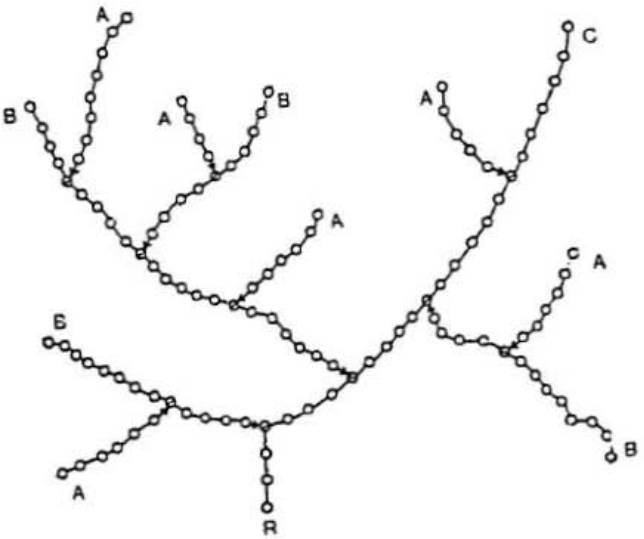
A number of terms are now frequently used to describe the structural parameters of amylopectin-type molecules. Exterior chains are those parts of a chain between the non-reducing end-group and the outermost branch point, whilst interior chains represent parts of a chain between branch points in the interior of the molecule (Fig. 2.4). A-Chains are linked to the molecule only by the potential reducing group, whilst B-chains are similarly linked, but also carry one or more A-chains. The C-chain carries the sole reducing group in the molecule, although for some purposes, the possible presence of a

reducing group is not significant, and the C-chain can be regarded as a B-chain (Meyer and Bernfeld, 1940).

It must be emphasised that analytical methods for measurements of the chain length (CL), exterior chain length (ECL) and interior chain length (ICL) give average values, and that individual chains can vary considerably in length. From the above definitions and Fig. 2.4, it can be seen that an amylopectin molecule contains roughly equal numbers of exterior and interior chains. If the molecule has an A:B chain ratio of 1:1, then each A-chain is also an exterior chain, and each B-chain, on the average, consists of one exterior and two interior chains (Robin *et al.*, 1975). In fact, many B-chains are known to consist of one exterior and only one interior chain. Other B-chains contain one exterior and three or more interior chains. The proportion of very long B-chains will decrease as their CL increases. Nevertheless there is now evidence for a small proportion of B-chains containing perhaps 50 or more glucose residues which play a key role in the overall molecular structure.

Although the main structural features of amylopectin have been known for some 50 years, details of the fine structure are still lacking. Indeed, there is still some uncertainty about the detailed arrangements of the constituent linear chains of (1 ➛4)-linked α-D-glucose residues, and during the period 1970-81, three different molecular structures for amylopectin were proposed (French, 1972; Robin *et al.*, 1974; Manners

Fig. 2.4 Segment of a hypothetical branched (1→4)-α-D-glucan. In amylopectins, the exterior chains may contain 12-16 glucose residues, but in phytoglycogen and animal glycogens, they are about half this length. ∘-, a (1→4)-linked α-D-glucose residue; ∘←, a (1→6)-linked α-D-glucose residue; R, a free reducing group; A,B and C, types of chain. Adopted from Manners (1989).


A
B
A
B
A
C
A
A
C
A
B
A
B
R

and Matheson, 1981). One of these molecular structures, the so called cluster model, has emerged as the most probable structure, although there are some variations of the cluster model, and it is not yet clear whether it applies to all amylopectins, irrespective of the botanical source of the starch (Manners, 1989).

The fine structure of amylopectin represents something of a paradox (Manners, 1989). In purely chemical terms, it could scarcely be simpler. It is composed of only one monosaccharide residue, the great majority of which are joined together by (1→4)-α-D-glycosidic linkages to form chains of varying lengths. Amylopectin also contains about 4-5% of (1→6)-α-D-glycosidic linkages which interlink the chains to form a three dimensional macromolecule. The problems arise from the very high molecular weight of amylopectin (~10^7) giving a DP of ~10^5. Since the average length is usually 20-25 glucose residues, it follows that each macromolecule contains several thousand individual chains (Manners, 1985c).

From a chemical point of view, three types of glucose residues can be described. There are 4-5% of non-reducing end-groups, which lead to tetra-o-methyl-D-glucose on methylation analysis. There are an equal number of branch point residues which are triply linked by two (1→4)- and one (1→6)-α-D-glycosidic linkages to adjacent residues. These give rise to di-o-methyl-D-glucose on methylation analysis. However, some 90% of the glucose residues are indistinguishable from each other by chemical-analytical techniques

(Manners, 1989).

Substantial progress in investigating the fine structure of amylopectin has become possible by the use of highly purified debranching enzymes. These enzymes specifically hydrolyse the (1→6)-α-D-glycosidic inter-chain linkages in amylopectin and derived dextrins, but have no action on the major (1→4)-α-D-glycosidic linkages (Manners, 1974). Their specificity is therefore opposite to that of the conventional α- and β-amylases.

Four classes of enzymes have been widely used in studies of the fine structure of amylopectin. α-Amylases hydrolyse non-terminal (1→4)-α-D-glycosidic linkages by a partly random action to give eventually a mixture of maltose, branched oligosaccharides (α-dextrins) and either maltotriose or glucose depending on the relative concentrations of enzyme and substrate (Manners, 1989). The α-dextrins contain the original (1→6) interchain linkages and two or more adjacent (1→4)-α-D-glycosidic linkages. The α-dextrins are a heterogeneous mixture with DPs ranging from 4 to more than 10, and individual α-dextrins may contain one, two or even three (1→6) inter-chain linkages, depending on their proximity to each other in the original macromolecule, and whether the enzyme can hydrolyse (1→4)-α-D-glycosidic linkages in very short interior chains containing perhaps only two, three or four glucose residues. There is some evidence that α-amylases require a linear segment of at least five glucose residues for rapid hydrolysis.

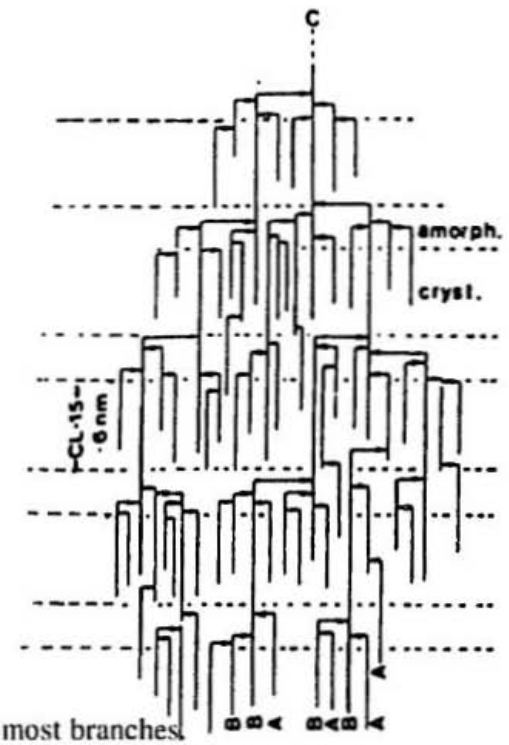
β-Amylases hydrolyse, in stepwise fashion, alternate linkages in their substrates, so that linear molecules are completely converted into maltose (French, 1975; Manners, 1985c). It should be noted that chains containing an odd number of residues will give one molecule of maltotriose or glucose, depending on the concentration of enzyme, along with the many molecules of maltose.

With branched substrates, enzyme action is confined to the exterior chains, since β-amylase cannot hydrolyse or bypass (1→6) inter-chain linkages. Enzyme action ceases leaving 'stubs' containing an average of two glucose residues per chain. With A-chains, the stubs actually contain either two or three residues per chain, whilst with B-chains, either one or two residues are present in the stub (French, 1975).

Amylopectins are also degraded by plant and animal phosphorylases (Manners, 1985c). In the presence of inorganic phosphate, enzyme action results in a stepwise cleavage of adjacent linkages in the exterior chains to give about 40% of α-D-glucose 1-phosphate. Like β-amylase, the phosphorylases cannot degrade or by-pass the inter-chain linkages, and enzyme action ceases giving 'stubs' of about four glucose residues.

The structure of amylopectin has been investigated extensively, and a number of molecular models have been proposed (Manners, 1989). The general view that has emerged during the last 15 years or so is that amylopectin has a cluster type structure (see Fig. 2.5). In this structure, only a small portion of an amylopectin molecule is shown to demonstrate the concept of "clusters" of interchain branching points (French, 1972;

Fig. 2.5 Cluster model of amylopectin. Adopted from Robin *et al.* (1974).



A - Outer most branches.
C - Only chain which carries the reducing group.
CL - Chain Length.

Robin *et al.*, 1974; Manners and Matheson, 1981).

2.2.2 Noncarbohydrate components

A number of minor noncarbohydrate constituents, particularly lipids, proteins, phospholipids and plant cell-wall materials may also be present in starch (Gracza, 1965). These occur in such small amounts that is not clear whether they are trace components of the starch granule or are contaminants that are not completely removed during isolation of the starch. Although they are present in small amounts, they can, nevertheless, affect the functional properties and behaviour of the starch (Lineback and Rasper, 1988).

The minor components of starches may be considered in three categories, according to their location: (1) Particulate material- fragments of non-starch substances (e.g. insoluble proteins and cell-wall material) that separate with starch (2) Surface components- material associated with the surface of granules (e.g. residues of amyloplast membranes, water soluble proteins and inorganic material) and that may be removed by extraction procedures which do not cause disruption of the granule internal structure (3) Internal components- material buried within the granule matrix (e.g. lipids that appear to be associated with the amylose fraction, nitrogenous phospholipids and internal proteins) and inaccessible to extraction without granule disruption (Galliard and Bowler, 1987).

2.2.2.1. Proteins

Gracza (1965) reported the presence of 0.24% protein (N X 6.25, dry basis) in oat starch. The nitrogen is generally considered to be present as protein, but it may also be part of lipids (e.g. lysophosphatidylcholine in wheat starch). In general, as the purity of isolated starch increases, the amount of protein present decreases. The protein present is of different types, depending on, among other things, the starch preparation procedure.

Other proteins present might be enzymes, either remaining from the starch synthesis or those necessary for the hydrolysis of starch when the seed germinates. Two types of amylases are present: α-amylases and β-amylases. α-Amylase is an endoenzyme, meaning that it breaks α(1→4) bonds randomly (more or less). The size of the starch molecules will quickly be reduced owing to the action of α-amylase, and consequently the viscosity of a starch suspension rapidly decreases when α-amylase is present (Robyt and Whelan, 1968a). β-Amylase is an exoenzyme, meaning that the α(1→4) glycosidic bonds are hydrolyzed from the nonreducing end during the release of maltose. However, when the enzyme meets an α(1→6) linkage, its action is stopped, and the β-amylase leaves β-limit dextrins as a result of its action (Robyt and Whelan, 1968b; Manners, 1974).

2.2.2.2. Lipids

Lipids are among the minor components of starch granules that have received most attention, particularly those from within the granule matrix. These are distinguished clearly from other lipids associated with starch granules on the basis of selective extraction methods. Morrison *et al.* (1975) have used cold and hot solutions of water-saturated butanol to separate 'non-starch' lipids (removed by cold solvent from ungelatinized granules) from the 'starch' lipids that are released only slowly with cold solvents and that require gelatinization of starch for efficient extraction.

The way in which lipids are distributed at the surface of starch granules is not known. The surface lipid content of well-washed starch granules of wheat is sufficient to form a bimolecular lipid layer on the surface of granules of average diameter of 10 μm (Galliard and Bowler, 1987). However, there is no evidence of an intact 'membrane' around granules of isolated starch. It is more likely that lipid material is distributed unevenly at the granule surface. Whether an uneven distribution of lipid and other materials on the surface of granules contributes to the observed resistance of parts of the granule surface to attack by α-amylase remains to be determined.

Lipids associated with native cereal starches have been differentiated as non-starch, surface and internal lipids the last being true starch lipids inside the granules. Non-starch lipids can reside either in a free state or they are bound together with proteins to the

surface of granules. This fraction of lipids is composed of triacylglycerols, diacylglycerols, diacylglucolipids, phospholipids, tocopherols, sterols and carotenoids constituting aleurone layer and germ (Gibinski *et al.*, 1993). Free fatty acids as well as monoacylglycerols resulting from lypolysis on isolation and storage of starch (Liukkonen and Laakso, 1992) are also present. Monoacyl lipids, penetrated into interior of granules on isolation of starch, are considered as surface lipids (Liukkonen and Laakso, 1992). Internal lipids of cereal starch are composed exclusively of monoacylglycerols, free fatty acids, and lysophospholipids. Such lipids reside either inside the cavity of the amylose helix or in the spaces between amylose and amylopectin. Therefore, rigorous conditions are needed to swell the starch granules sufficiently to permit complete lipid extraction.

2.3 The amylose-lipid complex

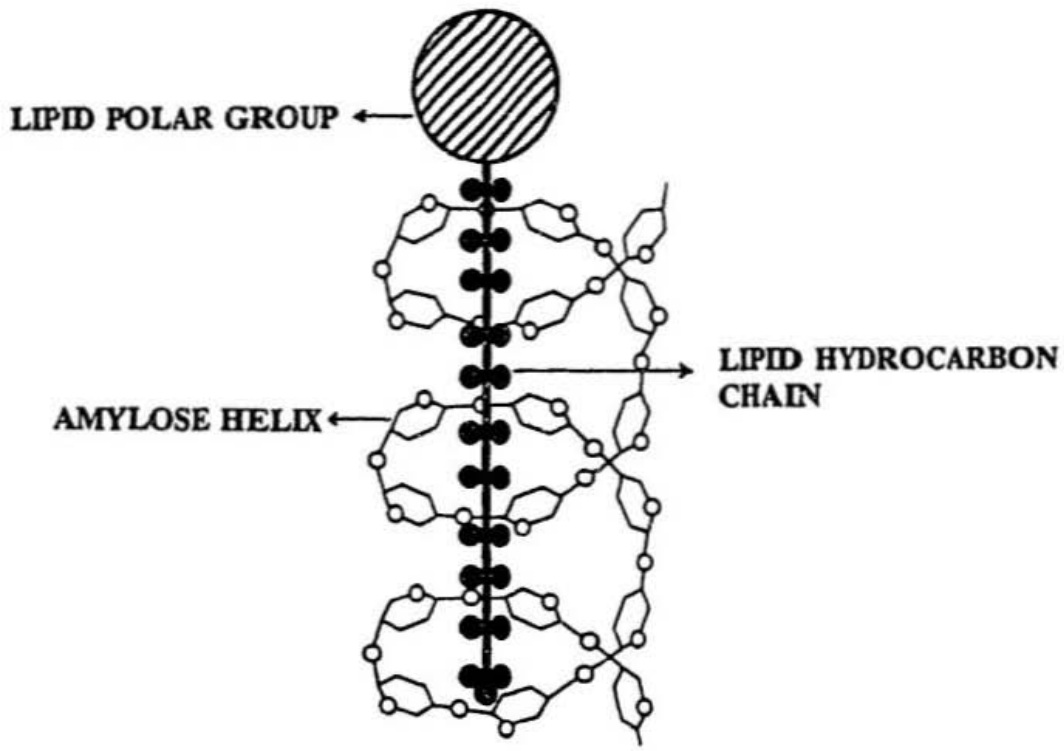
Amylose forms crystalline complexes with a variety of polar and non-polar organic compounds. These complexes play an essential role in every process associated with the utilization of starch-containing materials. Thus, texture and structural stability of cereals and starch-based products are greatly influenced by complex formation of linear starch components with various ligands (Krog and Lauridsen, 1976; Lund, 1984).

2.3.1 Structure

There is now ample evidence from a range of physical and chemical studies about the nature and structure of amylose-lipid complexes, as represented in Fig. 2.6. The amylose molecule forms a single helix and its hydrophobic cavity is occupied by a ligand. For a polar lipid such as a monoacylglycerol, each turn of the helix consists of six glucose residues, and on the average there are two to three turns for each monoacylglycerol molecule. On the average, about two-thirds of the hydrocarbon chain is involved in the complex (Carlson *et al.*, 1979). Each amylose molecule may contain several helices with ligands. When the ligand is more bulky than a fatty acid chain, the turn in the helix can consist of seven or eight glucose residues (Yamishita *et al.*, 1973; French and Murphy, 1977). The list of ligands that complex with amylose is long and includes fatty acids, monoacylglycerols, surfactants such as sodium dodecyl sulphate (SDS), cetyltrimethylammonium bromide (CTAB), lecithin, alcohols, carboxylic acids, and even cyclic compounds such as cyclohexanol (Mikus *et al.*, 1946; Osman *et al.*, 1961; Lagendijk and Pennings, 1970; Eberstein *et al.*, 1980; Gough *et al.*, 1985; Eliasson and Krog, 1985; Kowblansky, 1985; Eliasson, 1986b; Evans, 1986; Eliasson, 1988).

One of the strongest pieces of evidence for the structure of amylose-lipid complexes is the characteristic 'V' pattern in X-ray diffraction studies (Osman *et al.*, 1961; Zobel *et al.*, 1967). The 'V' type pattern is readily observed in cereal starches. The complex is not

Fig. 2.6 Schematic illustration of monostearin-amylose helical complex with the whole chain inside the helical space. Adopted from Carlson *et al.* (1979).


LIPID POLAR GROUP
LIPID HYDROCARBON CHAIN
AMYLOSE HELIX

formed only by monoacyl lipids; lecithin also gives the 'V' pattern (Osman *et al.*, 1961). The unit cell of the complex is orthorhombic with the dimensions a= 13.0 Å, b= 23.0 Å, and c= 8.05 Å (Mikus *et al.*, 1946). The packing of the helices is the same; the ligand is either a fatty acyl or iodine, although some variations in d-spacing due to the ligand in the complex have been reported (Osman *et al.*, 1961). Moreover, the d-spacings observed were also related to the treatment of the complex- to whether it was extracted with carbon tetrachloride or methanol. The amylose chains in the crystals are folded and form a lamellar structure (Manley, 1964).

The circumstance that native starch does not show the 'V' pattern (except in a few special cases (Zobel, 1988a)) has been taken as evidence that the amylose complex does not exist in the native starch but is formed during starch gelatinization . However, the absence of the 'V' pattern does not prove the absence of the amylose-lipid complex, it merely proves that the complex is not arranged in crystalline domains that we can detect by the X-ray diffraction technique.

2.3.2 Formation of the complex

To achieve the best conditions for the formation of a complex between amylose and monoacyl lipids, both should be in solution. If a solution of monoacyl lipids is added to a solution of amylose, the complex forms as a white precipitate, which can be collected

by centrifugation for further studies. The presence of uncomplexed lipid can be checked by DSC if the chain melting temperature is suitable. It has been noted that the existence of uncomplexed lipid is common when dimethylsulphoxide (DMSO) is used as solvent, perhaps because the monoacyl lipids and DMSO compete for the same site in the helical cavity (Raphaelides and Karkalas, 1988).

Complex-forming ability differs among the monoacylglycerols (Osman *et al.*, 1961; Lagendijk and Pennings, 1970; Krog, 1971; Hoover and Hadziyev, 1981). Monoacyl lipids with a large polar group, or diacyl lipids such as lecithin, were shown to have poor amylose-complexing ability (Osman *et al.*, 1961; Krog, 1971). To form a precipitate, a higher amount is required for a diacylglycerol than for a monoacylglycerol (Osman *et al.*, 1961).

When the complex-forming abilities of monopalmitin (GMP), monoelaidin (GME), monoolein (GMO), and monolinolein (GML) were compared, GME was found to be the best when the monoacylglycerols were added as aqueous dispersions at 30°C (Riisom *et al.*, 1984). At these conditions GMP forms β-crystals, the cis-unsaturated monoacylglycerols form a cubic phase, and GME is in the border region between the β-crystalline form and the lamellar phase. If all monoacylglycerols were added as liposomes (liposomes prepared with the aid of sodium cholate), their complexing ability was increased.

2.3.3 Thermal behaviour of the complex

Differential scanning calorimetric studies of Kugimiya *et al.* (1980) have shown that the presence of an endothermal transition typical of cereal starches near 100°C was due to the melting of amylose-lipid complexes. Formation of these complexes can be observed as an exothermal transition at a temperature range of 60 to 80°C when starch free of lipid is gelatinized with a water-soluble lipid. However, the melting temperatures depend on the water content (Biliaderis *et al.*, 1985; Eliasson, 1986b). The size of the melting endotherm of the amylose-lipid complex can be used to calculate the amylose content of starch (Roos, 1992).

2.4 The amylopectin-lipid complex

It has been assumed that amylopectin molecules do not form complex with polar lipids. When emulsifiers are added to an amylopectin solution, no precipitate is formed, as it would with amylose (Krog, 1971). However, a slight decrease in iodine absorption is observed. On the other hand, when amylopectin and monoacylglycerols were suspended in water at 60°C for several hours, a precipitate was formed (Batres and White, 1986). The least amount of complex was formed with monostearin (GMS), whereas GMP gave the highest amount and monomyristin (GMM) produced a moderate amount. The iodine affinity of these complexes was lower than that of amylopectin.

DSC studies have shown that retrogradation of starch decreases when monoacyl lipids are added to the starch (Russell, 1983). This effect has been explained by the formation of amylose-lipid complex. It should be remembered, however, that these DSC measurements show the retrogradation of amylopectin. It is not evident how the amylose-lipid complex can affect the retrogradation of amylopectin. The simplest explanation may be that the amylose-lipid complex does not affect the retrogradation of amylopectin and that it is the polar lipid that directly affects the amylopectin. This explanation is supported by the fact that it is possible to decrease the retrogradation of waxy maize starch or amylopectin by the addition of monoacyl lipids (Eliasson and Ljunger, 1988).

Several indirect measurements have indicated the existance of an amylopectin-lipid complex. As described above, the retrogradation of waxy maize starch and amylopectin is affected by monoacyl lipids but not by triacylglycerols (Eliasson and Ljunger, 1988). Equilibrium dialysis indicates that stearic acid is bound to waxy maize starch; 0.76 g stearic acid/g amylopectin was bound (Hahn and Hood, 1987). The corresponding value for amylose was 5.25 g stearic acid/g amylose.

2.5 The crystalline nature of starch

Classic work of Katz and his collaborators (Katz and Van Itallie, 1930) has shown that starch is a semi-crystalline material. Using X-ray diffraction techniques these authors

have distinguished three types of crystalline structure in intact starch granules, giving diffraction patterns which were designated as A-, B-, and C-patterns (Fig. 2.7). The structural type depends on the botanical source of the starch: the A-type pattern is given by most starches of cereal origin (rice, wheat, and corn); the B-type pattern is shown by tuber, fruit, and high amylose corn (>40%) starches as well as by retrograded starch; and C-type pattern, which is intermediate between A and B-type, is observed for legume seed starches (pea and bean). The reasons for these differences are not properly understood. Slight differences in the chain length and chain profile of amylopectin molecules may be responsible for existing differences in X-ray patterns (Hizukuri, 1985).

Recent studies with low molecular weight monodisperse oligosaccharides have suggested that the A-type crystal is the most thermodynamically stable form, while the B-type is the kinetically favoured polymorph (Gidley, 1987; Gidley and Bulpin, 1987). All other factors being equal, the A-type structure is preferred over the B-type under conditions of (a) higher crystallization temperature, (b) higher polymer concentration, and (c) shorter chain lengths. The work of Hizukuri (Hizukuri, 1986; Hizukuri *et al.*, 1983) on the structure of amylopectins from a variety of starch sources also concurs with the view that the average chain length of the branched starch molecules is the major determinant of crystalline polymorphism observed among native starches; amylopectins of B-type starches have longer chain lengths than those of the A-type (Hizukuri *et al.*,

1983).

According to the modelling studies of Wu and Sarko (1978a,b), using diffraction powder data of oriented amylose A- and B-type fibers, crystallinity in starch is due to packing of double helices. The arrangement of duplexes into the crystal lattice is nearly the same in both structures, except that the unit cell of B amylose contains 36 water molecules in a channel formed by the hexagonally packed double helices; in A amylose this channel is occupied by another double helix and the water molecules (eight / unit cell) are distributed in interstitial spaces between strands (Fig. 2.8). Recent studies by Imberty *et al.* (1987) have established that the A-type polymorph is characterized by packing of left-handed parallel-stranded duplexes. This arrangement is consistent with the overall architecture of the amylopectin molecule and chain polarity requirements arising from biosynthetic considerations (French, 1972). There still exists a controversy whether the C-type is a distinct structure of simply a mixture of A- and B-type crystallites (Sarko and Wu, 1978; Zobel, 1988a).

Only a small portion of the starch granule is believed to be crystalline and the concept of the partially crystalline nature of starch is now widely accepted (Biliaderis, 1991). Kainuma and French (1971) have visualized the granule as a gel matrix in which the crystalline regions are embedded. These crystallites exhibit the X-ray pattern and optical birefringence and are relatively resistant to chemical and enzymic attack. In

Fig. 2.7 Typical diffractometer patterns for starches giving A-, B-, and C-type X-ray spectra. Adopted from Banks and Greenwood (1975).

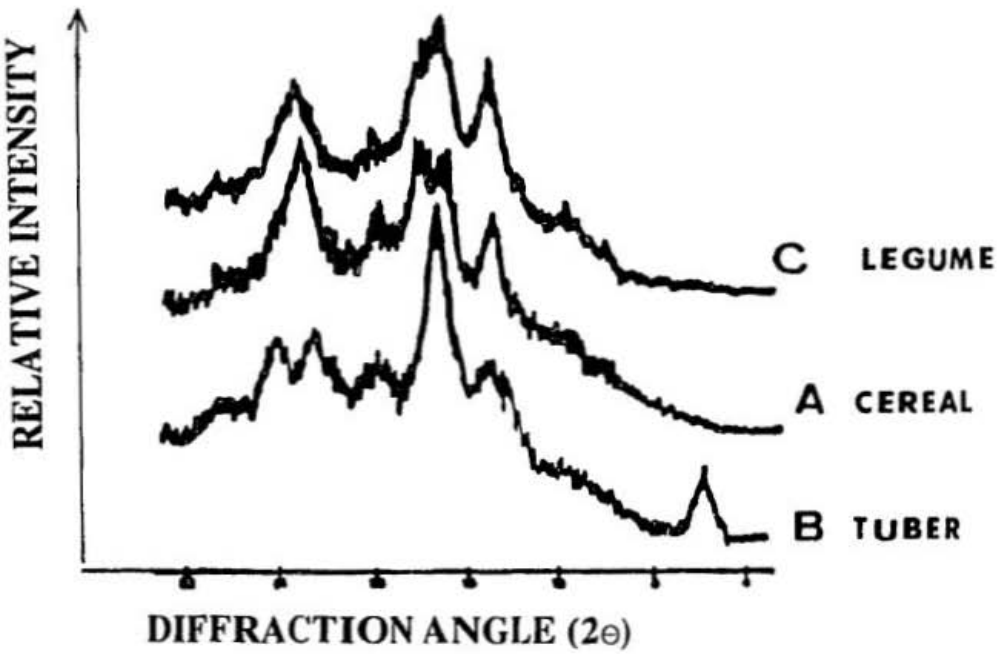
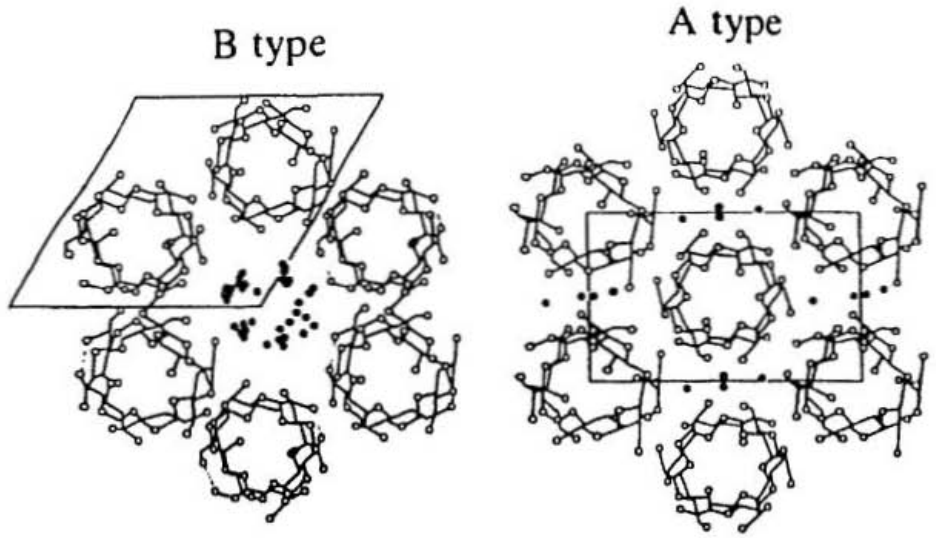

RELATIVE INTENSITY
C LEGUME
A CEREAL
B TUBER
DIFFRACTION ANGLE (2θ)

Fig. 2.8 Double-helix packing arrangements in A- and B-type unit cell structures of crystalline amylose. Adopted from Wu and Sarko (1978a,b).


B type
A type

contrast, the gel phase (amorphous regions) is less dense and more susceptible to chemical and enzymatic modifications and absorbs water more readily at temperatures below the gelatinization temperature. Diffusion of small molecules within the starch granule and swelling upon hydration are properties also associated with the gel or amorphous phase (French, 1972). It is of interest to note that as a result of the fine structure of amylopectin, there is no sharp demarcation between crystallites and amorphous regions in starch. Instead, a range of structures is expected between well-developed crystallites and fully disordered regions. In this type of supermolecular structure, the amorphous and crystalline phases are interdependent. Levels of crystallinity, determined by integration of the areas under the crystalline diffraction peaks, range between 15 and 45%, depending on the source of starch (Zobel, 1988b). Another intriguing question regarding starch structure is the degree to which amylose and amylopectin are separated or mixed within the granule. Zobel (1988b) presented several arguments to support the notion that amylose is more closely associated with amylopectin in potato than in cereal starches. Viewed under polarized light, starch granules also show birefringence (the well-known Maltese cross), which indicate chain orientation of some sort (Biliaderis, 1991).

Another crystallographically distinct structure, typically observed when linear starch molecules form complexes with various ligands, is the V- form. The V-pattern is evident when lipid-containing cereal starches are subjected to heat-moisture treatments

at temperatures below the gelatinization temperature or in their freshly cooked gels. The V-polymorph is believed to consist of closely packed single helices of amylose in which the complexing ligand molecules reside within the helix (Zobel *et al.*, 1967). Native cereal starches do not exhibit the V-pattern in X-ray analysis. Nevertheless, there is circumstantial evidence that internal granular monoacyl lipids (lysophospholipids, free fatty acids) are associated somehow with the glucan molecules *in situ* (Galliard and Bowler, 1987). It is likely that some form of complexing between lipids and starch chain exists in the granule but of insufficient perfection and/or limited extent of long-range ordering to demonstrate a well-defined V-diffraction pattern (Biliaderis, 1991).

2.6 Swelling and solubility of starch

Starches show different patterns of swelling and solubility when heated at different temperatures (Hashim *et al*, 1992). Cereal starches show a two stage swelling and solubility pattern (Blanshard, 1978; Doublier *et al.*, 1987a,b; Hoover and Vasanthan, 1994). In the region of the gelatinization temperature, there is limited swelling and only a small amount of carbohydrate is solubilised. At around 90°C there is a large increase in the extent of swelling and a more substantial loss of starch from the granule. Normally, amylose leaves the granule first and it is only at the higher temperature stage of the pasting process that the amylopectin is lost (Doublier, 1981). However, for oat starch both

amylose and amylopectin are leached out together when held in the 90 to 100°C temperature range (Launay *et al.*, 1986).

The swelling power is defined as the swollen sediment weight (g) per g of dry starch while the solubility is expressed as the percentage (by weight) of the starch sample that is dissolved after heating in water at 95°C (Soni *et al.*, 1993). The swelling power test is one of a number of closely related tests (also known as water-holding capacity or water-retention capacity tests) which measure the uptake of water during gelatinization of starch (Leach *et al.*, 1959; Medcalf and Gilles, 1965; Freeman and Verr, 1972; Sollars, 1973; Kulp and Lorenz, 1981; Konik *et al.*, 1993). The swelling power test has several advantages over the conventional viscograph procedure. It utilises only one gram of starch, is relatively easy to carry out with simple laboratory equipment and can be performed on large numbers of samples simultaneously (Konik *et al.*, 1993).

Tester and Morrison (1990) developed a method for measuring the volume of water absorbed by starch granules heated in excess water, based on the observation that blue dextran dye (molecular weight 2×10^6) will dissolve in supernatant and interestitial water but not in the intragranular water. This method measures only intragranular water and hence the true swelling factor at a given temperature. The swelling factor is reported as the ratio of the volume of swollen starch granules to the volume of the dry starch.

2.7 Enzyme digestibility of starch

Digestibility of carbohydrates is very important in diabetics and in hyperlipidaemia. The rate and extent of starch degradation in the small intestine affect glucose absorption and the corresponding insulin response (Wolever, 1991). In ruminants, ingestion of large amounts of highly degradable carbohydrates may result in rumen acidosis and depression of feed intake. Knowledge of starch degradability is also important for beer brewing and bread baking technology.

Degradation of starch by ruminants can be determined *in vitro* using rumen fluid from fistulated cows. Starch degradability can also be determined *in vitro* using α-amylase. However, it is not possible to predict accurately the degradation of starch in feedstuffs by ruminal fluid with α-amylase (Wolters and Cone, 1992).

The digestibility of starch by α-amylases has been the subject of numerous investigations (Greenwood and Milne, 1968a; Thoma *et al.*, 1971; Leegwater *et al.*, 1971; Wootton and Chaudhry, 1979; Preiss and Levi, 1980; Robyt, 1984; Dreher *et al.*, 1984; Wolters and Cone, 1992; Tsuge *et al.*, 1992; Sreenath, 1992; Gorinstein, 1993; Wootton and Mahdar, 1993; Camire and Camire, 1994; Coma *et al.*, 1995; Lee *et al.*, 1995). A wide variation in digestibility of native, gelatinized, and chemically modified starches under *in vivo* and *in vitro* conditions, depending on the source of starch and food processing and storage conditions, has been reported (Björck *et al.*, 1989).

It is generally accepted that intact granular (raw) starches are less digestible than their gelatinized counterparts (McBurney, 1991; Sreenath, 1992) and that cereal starches (A-type) are more easily digested than root or tuber starches (B-type) (Dreher *et al.*, 1984). Cooking greatly improves the digestibility of poorly digested starches, presumably owing to granular disorganization and changes in crystallinity of starch materials. Similarly, glucose and insulin responses are significantly higher after intake of cooked versus raw starches (Vaaler *et al.*, 1984). The *in vitro* digestibility of legume starches and seeds is generally lower than those of cereals (Hoover and Sosulski, 1985). With increasing degree of gelatinization, there is a concomitant improvement in the *in vitro* digestibility of baked products (Wootton and Chaudhry, 1980). Incompletely gelatinized products (as assessed by calorimetry) made from whole wheat by various industrial processes also exhibited reduced rates of α-amylosis (Holm *et al.*, 1988).

2.7.1 Mode of action of α-amylase

Amylose is often used as a substrate for studies of α-amylase action patterns because the linear dextrins formed can be identified and quantitated more easily than the complex mixture of linear and branched dextrins formed during hydrolysis of amylopectin or solubilized starch (Manners, 1985b). α-Amylosis of amylose can be

considered to take place in two stages (Myrbäck and Neumuller, 1950). Initially, rapid breakdown of macromolecules to short-chain dextrins is accompanied by a large decrease in viscosity, loss in iodine staining power, and limited formation of reducing sugars. Three mechanisms can be postulated to describe dextrinization, this initial phase of amylose hydrolysis (Robyt, 1984). In the single-chain mechanism, the enzyme forms an active complex with the substrate and hydrolyzes it completely in one direction in a "zipper"-type manner to form large amounts of small dextrins throughout hydrolysis. This mechanism is not found with cereal α-amylases. In the multiple (or repetitive) attack mechanism, the enzyme catalyzes a number of bond scissions per encounter with the substrate before diffusing away and forming an active complex with another substrate molecule. This type of attack would produce less dextrin than the single-chain mechanism during early stages of hydrolysis. There is also no evidence that cereal α-amylases react in this way (Banks *et al.*, 1970), although it is difficult to eliminate the possibility of a small amount occurring. Strong evidence shows, however, that α-amylases from other sources may utilize this mechanism (Robyt and French, 1967; Thoma, 1976). Completely random hydrolysis of internal bonds of amylose is achieved in the multichain process, in which the enzyme hydrolyzes one bond per encounter with a substrate molecule. This mechanism best describes the behaviour of cereal α-amylases.

A large amylose molecule (approximately 500,000 molecular weight) has about 3,000 α-(1→4) bonds, of which six (0.2% of the total) are more resistant than the others to hydrolysis by cereal α-amylases because of their position at the ends of the molecule. There is little impediment, therefore, to rapid hydrolysis by the enzyme, but the proportion of less susceptible bonds increases surprisingly rapidly during hydrolysis. After hydrolysis of only 5% of the total amylose bonds, the less susceptible bonds represent 30% of the remainder, and this doubles to 60% after 10% of the amylose bonds have been hydrolyzed. Obviously, enzyme hydrolysis slows down markedly after only limited amylose degradation (Hill and MacGregor, 1987).

Amylopectin is a highly branched molecule in which relatively short unit chains of α-(1→4)-linked glucose residues (average chain length, 20-26) are joined by α-(1→6) bonds (Manners, 1985a). These α-(1→6) bonds are resistant to hydrolysis by α-amylase, and they also inhibit hydrolysis of α-(1→4) bonds in their vicinity (Manners, 1962). Therefore, amylopectin has a higher proportion of α-amylase-resistant bonds than amylose.

2.8 Starch gelatinization

Starch gelatinization is one of the most important phase transitions in foods. A group of starch scientists and technologists recently defined starch gelatinization

(Atwell *et al.*, 1988) as " the collapse (disruption) of molecular orders within the starch granule manifested by irreversible changes in properties such as granular swelling, crystallite melting, loss of birefringence, viscosity development, and starch solubilization. The point of the initial gelatinization and the range over which it occurs is governed by starch concentration, methods of observation and granule type, and heterogeneties within the granule population under observation."

Starch granules are insoluble in cold water but swell when heated in an aqueous medium. Initially, the swelling is reversible and the optical properties of the granule are retained(e.g., birefringence). But when a certain temperature is reached, the swelling becomes irreversible and the structure of the granule is altered significantly. The process is called "gelatinization" and the temperature at which gelatinization occurs is called the "gelatinization temperature". At this temperature the granule looses its birefringence and material from the granule diffuses into the water (Lund, 1984).

Aside from the swelling during gelatinization, the viscosity of the medium also increases (Bornet, 1993). Both the molecular and granular structures contribute to the increase in viscosity. Initially, gelatinization occurs in the more accessible an amorphous regions. As the temperature is raised above that for initiation of gelatinization, intermolecular hydrogen bonds which maintain the structural integrity of the granule continue to be disrupted. Water molecules solvate the liberated hydroxyl

groups and the granule continues to swell. As a consequence of severe disruption of hydrogen bonds, the granule will be fully hydrated and finally the micellar network separates and diffuses into the aqueous medium. After disruption of the granules, the viscosity decreases. The increase and decrease in viscosity during gelatinization can be followed by a Brabender Amylograph. The increase in viscosity in the early heating stages is due mainly to the release of amylose while, in later stages, the continued viscosity increase is due to interaction of extragranular material and swelling of the granules.

Based on changes in characteristics of starch granules (using a Brabender Amylograph) during and after heating in aqueous medium, Olkku and Rha (1978) summarized the steps of gelatinization: (1) granules hydrate and swell to several times their original size.(2) granules lose their birefringence. (3) clarity of the mixture increases. (4) marked, rapid increase in consistency occurs and reaches a maximum. (5) linear molecules dissolve and diffuse from ruptured granules. (6) upon cooling, uniformly dispersed matrix forms a gel or paste-like mass.

Marchant and Blanshard (1978) postulated three constituent processes for starch gelatinization based on nonequilibrium thermodynamics:(1) diffusion of water into the starch granules, (2) a hydration-facilitated helix-coil transition which is a melting process, and (3) swelling as a result of crystallite melting.

The detection of a glass transition endotherm just prior to the gelatinization endotherm led Slade and Levine (1988) to postulate that the process of starch gelatinization is inherently non-equilibrium in character, in that it occurs when starch granules are subjected to heat in the presence of plasticizing water in which crystallite melting is indirectly controlled by the kinetically constrained continuous amorphous environment, which was in a glassy state prior to gelatinization.

Because gelatinization is of great importance to many food processing operations, several analytical techniques have been employed to probe this phenomenon and determine quantitatively the amount of gelatinized starch in processed foods. These include light microscopy, scanning electron microscopy, light transmission, viscometry, swelling and solubility measurements, X-ray diffraction, nuclear magnetic resonance, and enzymic methods (Zobel, 1984). Because gelatinization is an endothermic process, thermal analysis methods, and differential scanning calorimetry (DSC) in particular, have attracted most interest during the last decade in studies of phase transitions of aqueous starch systems (Biliaderis *et al*, 1986). DSC can provide the characteristic temperatures and enthalpies of the various transitions as well as allowing measurements over a wide range of starch concentrations.

2.8.1 Factors affecting gelatinization

2.8.1.1 Water

The effect of water on starch gelatinization has been extensively studied (Collison and Chilton, 1974; Wootton and Bamunuarachchi, 1979; Donovan, 1979; Eberstein *et al.*, 1980; Burt and Russell, 1983; Maurice *et al.*, 1985; Biliaderis *et al.*, 1986; Eliasson, 1986a; Huang *et al.*, 1994). In DSC studies of wheat starch suspensions with water contents ranging from 32 to 67%, a minimum moisture content of 32% was necessary for gelatinization (Wootton and Bamunuarachchi, 1979). This finding is in good agreement with the results for mixtures of wheat starch and water heated in an oven, in which the minimum moisture content for gelatinization was 30% (Collison and Chilton, 1974). Thus, these authors concluded that gelatinization did not occur when the water content was less than 30%. Eberstein *et al.* (1980) used DSC to study gelatinization in systems with low water content and found that an enthalpy change was no longer observed at about 20% water content, indicating that gelatinization could no longer occur in such a system. In systems with higher water contents, the enthalpy change was independent of starch concentration, but in systems with a water content of about 60% or less, it decreased strongly. This result indicated that only a portion of the gelatinization process, namely swelling up to the absorption of 1.5 times the amount of water, is calorimetrically detected. However, later work with rice starches showed that melting does occur in

systems with less than 30% water content, but at higher temperatures (Maurice *et al.*, 1985; Biliaderis *et al.*, 1986). In a normal rice starch at 10% water content, the onset temperature appears to be above 140°C. In systems with water contents below 30%, water was reported to act as a plasticizer, decreasing the glass transition temperature of the amorphous parts of the granule. This behaviour assists the melting or reorganization of starch crystallites and amylose-lipid complexes to occur at lower temperatures. A three-phase model, proposed to explain the thermal behaviour of mixtures of granular starch and water, included two distinct types of amorphous material and the crystallites formed by the clusters of amylopectin.

2.8.1.2 Lipids

Effect of the lipid on starch gelatinization is related to hydrocarbon chain length. While short chain polar lipids may actually accelerate the rate of gelatinization, medium and long chain compounds inhibit the swelling of granules and uptake of water (Camire *et al.*, 1990). Polar lipids have long been known to affect the behaviour of starch pastes and are thought to interact with linear amylose chains to inhibit swelling and hydration (Gray and Schoch, 1962).

Monoacyl lipids present during gelatinization of starch affect the swelling power, the solubility, and the size and shape of the starch granules (Eliasson, 1986b; Tester

and Morrison, 1990). Most monoacyl lipids cause a decrease or delay in swelling and solubility. The sediment volume of wheat starch heated to 70°C is lower when monoacylglycerols are present. However, if the monoacylglycerols are added after the heating they do not affect the sediment volume (Van Lonkhuysen and Blankestijn, 1976). Monoacylglycerols decrease the solubility more than, for example, sodium stearoyl-2-lactylate (SSL) (Ghiasi *et al.*, 1982a). The swelling power is also lower in the presence of monoacylglycerols than with SSL. The morphological changes that occur during gelatinization of wheat starch are the same in the presence of monoacyl lipids as without such additives. However, the changes in the size and shape of the granules occur at a somewhat higher temperature in the presence of added lipids.

2.8.1.3 Protein

Gelatinization of starch from some sources may be influenced by the presence of protein. Chandrashekar and Kirleis (1988) found that water absorption of sorghum at 70 and 80°C was reduced, and gelatinization was decreased when the kafirin fraction was increased from 46 to 70% of the total protein content. Kafirin is an alcohol-soluble prolamine that forms a capsule around sorghum starch granules, preventing their rapid gelatinization under ordinary cooking conditions.

2.8.1.4 Sugars

Effects of sugars on the gelatinization of starches have been extensively studied (D'Appolonia, 1972; Derby *et al.*, 1975; Hoseney *et al.*, 1977; Savage and Osman, 1978; Bean and Yamazaki, 1978a,b; Koepsel and Hoseney, 1980; Wootton and Bamunuarachchi, 1980; Spies and Hoseney, 1982; Chungcharoen and Lund, 1987; Slade and Levine, 1987; Hansen *et al.*, 1989; Paredes-Lopez and Hernàndez-Lopez, 1991; Tomasik *et al.*, 1995). It has been reported that sugars increase the onset temperature of gelatinization of starches, and disaccharides retard the start of gelatinization process more than monosaccharides.

The precise mechanism, by which saccharides delay starch gelatinization, is still unclear. Hansen *et al.* (1989) classified the mechanism into three types; (a) competition between the saccharides and starch for available water and associated changes in free volume (Derby *et al.*, 1975; Hoseney *et al.*, 1977); (b) the ability of saccharides in water system to retard gelatinization by inhibiting the swelling of the starch granules (Savage and Osman, 1978; Wootton and Bamunuarachchi, 1980); and (c) the ability of saccharides to penetrate the starch granule and interact in the amorphous areas, thus stabilizing this region and increasing onset temperature of gelatinization (Oosten, 1984; Johnson *et al.*, 1990b).

2.9 Starch retrogradation

Retrogradation is an important property of starch gels. It has been defined as "a process which occurs when the molecules comprising gelatinized starch begin to reassociate in an ordered structure" (Atwell *et al.*, 1988).

After gelatinization or pasting of starch, the amylose and amylopectin molecules may be considered to be "dissolved". The entangled amylose molecules have a strong tendency to associate through the formation of hydrogen bonds with adjacent amylose chains when the solution (sol) is cooled or upon standing for long periods of time (Fig. 2.9). This phenomenon is known as retrogradation and manifests itself through the formation of precipitates or gels. If the original starch solution is diluted and cooled slowly, the amylose molecules have sufficient time to align themselves in such a way that several hydrogen bonds can be formed between adjacent parallel chains. The resulting bundles of parallel chains are insoluble aggregates, which precipitate. If the starch paste is relatively concentrated, a gel is produced almost immediately upon cooling. This gelation is interpreted as being due to an inability of the amylose chains to form insoluble aggregates. Hydrogen bonds are formed where possible between the entangled chains, resulting in junction zones (points) and yielding the elastic network of a gel. As the starch gel stands, additional hydrogen bonds form slowly, causing the gel to shrink and some of the water to separate (the process of syneresis). Areas where chains or portions of them

are associated through hydrogen bonding are crystalline and are called micelles. Thus the process of retrogradation is accompanied by an increase in crystallinity, although it is not the crystallinity of the original granule.

A considerable amount of experimental data is available (differential scanning calorimetry and X-ray diffraction) to suggest that the main underlying cause of bread staling is retrogradation (Kulp and Ponte, 1981). Although numerous investigations have been undertaken on this phenomenon, the exact mechanism of retrogradation, particularly at a molecular level, is not clear (Biliaderis, 1991).

The molecular structures and transformations that occur during gelation and retrogradation of starch and its components have been the subject of several investigations recently (Miles *et al.*, 1984, 1985ab; Ring, 1985; Ring *et al.*, 1987; Leloup, *et al.*, 1992; Sievert and Wursch, 1993; Svegmark and Hermansson, 1993; Cameron, *et al.*, 1994). As shown by Miles *et al.* (1985a,b), retrogradation consists of two separable processes: (1) gelation of amylose molecules exuded from the granules during gelatinization and (2) recrystallization of amylopectin. In order to isolate the roles played by amylose and amylopectin, it is convenient to discuss amylose gelation and amylopectin recrystallization separately.

2.9.1 Amylose gelation

Amylose, because of its linear nature, is considered primarily responsible for gelation of starch (Biliaderis, 1991). Amylose forms opaque, partially crystalline, thermo-irreversible gels. It is a flexible molecule that adopts a near spherical shape in aqueous solution (Miles *et al.*, 1985b). In dilute solution, the average intermolecular separation will be much greater than the molecular size. However, if the concentration is increased, a critical concentration (C^*) is reached where the intermolecular separation is similar to the molecular size and the molecules interpenetrate. The concentration C^* may be regarded as the so-called overlap or entanglement concentration. For amylose preparations, it was noted (Miles *et al.*, 1985b) that C^*~1.5%(w/w), which was very close to the critical concentration for gelation (C_g). Thus, the gelation process involves converting the weak, temporary network into a strong permanent network.

Characteristic features of gelation include the formation of a permanent elastic network and the development of opacity (Ring, 1987). Miles *et al.*(1985b) have shown that amylose solutions of low concentration (1.6-2.4%) become turbid during the early stages of amylose gelation. For normal polydisperse amylose, the increase in turbidity only slightly preceded network formation. In this case, gelation was envisaged as a separation of amylose into a coarse network of a polymer-rich phase interspersed with a solvent- rich phase (Miles *et al.*, 1985a). Subsequently, an increase in elasticity was

recorded followed by a slow increase in crystallinity in the polymer-rich phase (Hoover, 1995). The level of crystallinity within the gel was shown to be concentration dependent, but the rate of crystallization was concentration independent (Miles *et al.*, 1984). This suggests that the local polymer concentration is constant in the phase-separated network. Miles *et al.* (1985a) showed that stiffening of amylose gels is accompanied by the formation of B-type crystals.

The above discussion suggests that an amorphous amylose network containing crystallites provides permanency to the structure. Development of crystallinity can be measured by X-ray diffraction (Miles *et al.*, 1984). Unfortunately, the exposure times with conventional X-ray sources are too large to permit time-dependent studies of crystallization during the very early stages of network formation (Morris, 1990).

In hot aqueous solution, amylose molecules are flexible (Ellis and Ring, 1985), whereas within the amylose gel at least some regions of the amylose molecules are present as stiff double helices within the crystallite junction zones. This double helix formation may occur between the ends of molecules, favouring chain elongation (Fig. 2.10a). Once helix formation has occurred, lateral association may occur through crystallization (Fig. 2.10b). For high molecular weights, poor matching of chains during helix formation may initially favor fibrillar network formation, whereas low molecular weights may initially favor lateral association, due to better matching of chains upon

Fig. 2.9 A schematic representation of the processes and structures observed during heating and storage of aqueous suspensions of granular starch. Adopted from Biliaderis (1991).

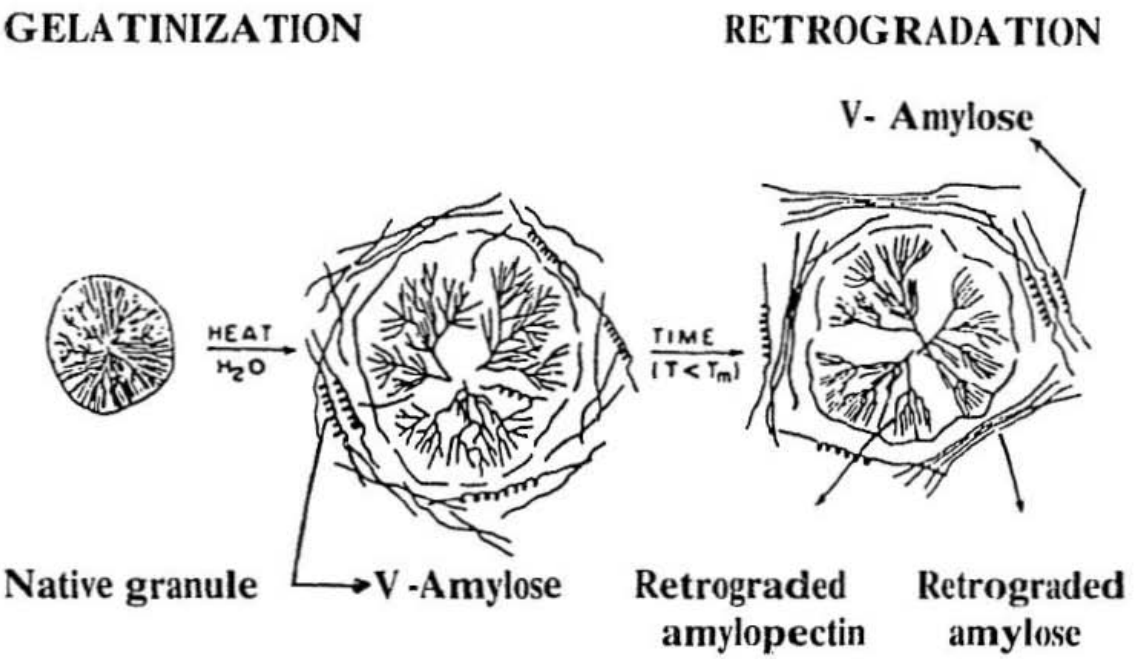
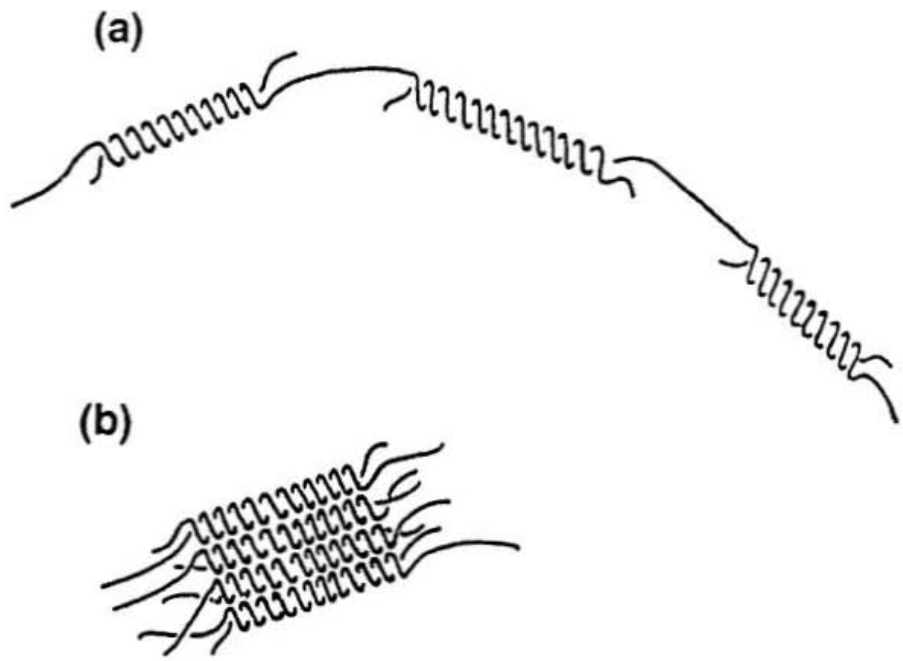

GELATINIZATION
RETROGRADATION
V- Amylose
HEAT
H_2O
TIME
$(T < T_m)$
Native granule
V -Amylose
Retrograded amylopectin
Retrograded amylose

Fig. 2.10 Double helix formation and association: (a) helix formation and chain elongation; (b) lateral association of helical regions. Adopted from Morris (1990).


(a)
(b)

helix formation, and the conversion of fibrils to thicker fibres (Morris, 1990).

2.9.2 Amylopectin crystallization

In contrast with amylose, amylopectin gelation is a much slower process and requires much higher polysaccharide concentrations (usually ≥ 15%), well above the C^* (≈0.9%) of this polymer (Biliaderis, 1991). In this respect, the gelation kinetics of amylopectin is different than that of amylose and is believed to reflect network formation via crystallization of the outer short chains of the molecule (Ring *et al.*, 1987). The slow crystallization rate of amylopectin corresponds kinetically with the staling events of ageing baked items (Kulp and Ponte, 1981).

Kalichevsky *et al.* (1990) studied the relationship between the fine structure of amylopectins from various botanical sources and their gelation behaviour. Amylopectins from pea, potato, and canna were found to exhibit higher rates of retrogradation (monitored by development of shear modulus) than those from wheat, barley and maize starches. However, there was no simple relationship between shear modulus and molecular structure of amylopectin.

2.9.3 Factors affecting starch retrogradation

Retrogradation affects the texture and acceptability of many starch- containing

foods. How this process is affected by interactions between starch and other food components needs to be understood to better control the keeping quality of starchy food products. Extent of retrogradation, and the nature of the crystallites formed, may be affected by: starch source (Orford *et al.*, 1987); concentration (Zeleznak and Hoseney, 1986; Orford *et al.*, 1987; Slade and Levine, 1987); storage temperature (Slade and Levine, 1987; Eliasson and Ljunger, 1988); acids (Muhrbeck and Eliasson, 1987; Russell and Oliver, 1989); salts (Ciacco and Fernandes, 1979); lipids and surfactants (Germani *et al.*, 1983; Russell, 1983; Evans, 1986; Eliasson and Ljunger, 1988; Krog *et al.*, 1989); and sugars (Maxwell and Zobel, 1978; Hase *et al.*, 1981; Germani *et al.*, 1983; Slade and Levine, 1987; l'anson *et al.*, 1990; Ward *et al.*, 1994; Eerlingen *et al.*, 1994; Bello-Pérez and Paredes-Lopez, 1995; Botlan and Desbois, 1995).

2.9.3.1 Source of starch

The retrogradation differs among starches from different sources (Rosario and Pontiveros, 1983; Gudmundsson and Eliasson, 1992; Ward *et al.*, 1994). Orford *et al.* (1987) investigated the extent of retrogradation of wheat, corn, potato and pea starches. Gels from all sources showed substantial increases in shear modulus over 7 days. Pea starch showed the highest increase, followed by potato, corn and wheat. Crystallinity (X-ray and DSC) paralleled the long term development of shear modulus, indicating

that this long term development was because of a crystallization process. All gels exhibited a B-type X-ray pattern. However, the above authors made no attempt to explain the differences in recrystallization between various starch sources. The G value of wheat was found to increase very slowly during storage compared to that of barley (Kalichevsky *et al.*, 1990). Gudmundsson and Eliasson (1989, 1991) have used DSC to investigate several cereal starches and found the following order in the extent of retrogradation: oat < rye < wheat.

2.9.3.2 Concentration

Starch concentration influences the extent of retrogradation. The most intense B-pattern is obtained for wheat starch gels with a water content of 47-50% (w/w) [Hellman *et al.*, 1954). For a starch gel with 63% (w/w) water stored 8 days at 24°C, the intensity of the B-pattern is only one-fifth the intensity observed for a starch gel with 50% water treated in the same way. Measurements by DSC have confirmed earlier X-ray results. The maximum ΔH value is obtained in a starch concentration of about 60% (w/w) (Longton and LeGrys, 1981; Eliasson, 1983; Zeleznak and Hoseney, 1986). Also the increase in storage modulus during storage is related to the starch concentration (Biliaderis and Zawistowski, 1990).

2.9.3.3 Storage temperature

The retrogradation process is very sensitive to temperature (Colwell *et al.*, 1969; Eliasson, 1985; Biliaderis and Zawistowski, 1990). This is evident from using DSC and X-ray diffraction as well as rheological measurements. These results are easily understood from classic knowledge about crystallization. The higher the degree of supercooling, the more crystals will be formed, but the growth of the crystals is favored at a higher temperature. However, in the case of starch gel the supercooling cannot be brought too far; at some temperature, the glass transition temperature (T_g) of the starch as well as the freezing point of water will be passed, and during these conditions no crystallization will occur at all. The best conditions for crystallization of amylopectin therefore seem to be just above 0°C. The retrogradation process might then be avoided or delayed by storage at the correct temperature.

In order to bring about as much starch retrogradation as possible, the correct treatment would be to store the starch gel first at a low temperature (e.g. 4°C) to promote nucleation and then at a higher temperature (e.g. 40°C) to promote crystal growth. Such experiments have been performed, and after 24h at 4°C starch gels were stored for another 24h at 4, 12, 25, or 40°C. The highest values of ΔH_c were obtained after storage at 12 and 25°C (Zeleznak and Hoseney, 1987).

2.9.3.4 Lipids

Many researchers have demonstrated that lipids retard retrogradation. The effects of lipids have been reported by several investigators (Krog and Jenson, 1970; Kulp and Ponte, 1981). It is well known that amylose forms inclusion complexes with lipids (Krog, 1971; Birnbaum, 1977). Amylopectin also forms complexes with lipids (Gray and Schoch, 1962; Batres and White, 1986). It has been suggested that complex formation between lipids and the starch components could hinder crystallization during gel storage (Batres and white, 1986; Slade and Levine, 1987). However, it is also possible that reduced retrogradation in the presence of lipids could also correspond to a decrease in the mobility (due to water-water interaction and/or to an increase in the local viscosity of the starch suspension) of those starch chains which are involved in double helix formation and lateral associations during recrystallization (Hoover, 1995).

DSC studies have shown that lipids decrease crystallization of amylopectin and increase the amount of amylose-lipid complex (Eliasson, 1983; Russell, 1983). But how lipids or the amylose-lipid complexes retard recrystallization of amylopectin is still not clear.

2.9.3.5 Sugars

Results of several studies agree that sugars do affect melting temperatures of

retrograded starch as well as the extent to which retrogradation occurs. However, the literature is replete with conflicting information about the effect of individual sugars on retrogradation. Furthermore, the mechanism by which sugars influence retrogradation still remains unclear (Hoover, 1995).

Sugars are believed to penetrate the melted crystalline region of starch. Hence, "sugar-bridges" (a term coined by Spies and Hoseney, 1982) are formed in both crystalline and amorphous regions during gelatinization. When starch gels are cooled, the starch chains become less energetic and the hydrogen bonds become stronger. Retrogradation involves, among others, recrystallization of starch chain in the gels. In starch-water-sugar systems, sugars interfere with the hydrogen bonds between starch chains. Thus, stabilized starch gels retard retrogradation, i.e., the rate of retrogradation is reduced by sugars (Miura *et al.*, 1992).

Katsuta *et al.* (1992a) reported that sucrose stabilized the starch gel matrix and impeded retrogradation more than glucose and fructose. They suggested that the ability of saccharides to stabilize the starch-water system might be influenced by the conformation of saccharides, i.e. the number of equatorial OH (e-OH) groups in saccharide molecules might play an important role in maintaining the water structure surrounding the saccharides and starch molecules.

Katsuta *et al.* (1992b,c) studied the effects of mono-, di- and oligosaccharides

on stabilities of rice starch gels. In this study, they applied retrogradation kinetics on the basis of viscoelastic properties to starch-water-saccharide systems in order to investigate the effects of conformation of saccharides on stabilities of rice starch gels and water structure. The above authors showed that hexoses (with the exception of galactose) decreased the extent of retrogradation to a greater extent than pentose (ribose and xylose). While disaccharides, (maltose and sucrose) were more effective than monosaccharides in reducing retrogradation. Furthermore, among malto-oligosaccharides, the effectiveness in reducing retrogradation followed the order: maltotriose > maltotetraose > branched oligosaccharides. Similar findings were reported by Miura *et al.* (1992).

2.10 The oat grain

The oat kernel or caryopsis is usually longer and more slender than wheat or barley kernels, and most domestic cultivars are more extensively covered by hairs, or trichomes (Fig. 2.11), which often obscure other surface details (Fulcher, 1986). At maturity the caryopsis, usually called the groat, is tightly enclosed within the fibrous lemma and palea, which persist after threshing during harvesting operations and form the hull except in the case of hull-less oats. The caryopsis usually accounts for 65-75% and the hull 25-35% of the whole oat (McMullen, 1991).

The wall of the caryopsis consists of several cell layers derived from three different sources: the pericarp, from the ovary wall; the testa, from the inner integument; and the epidermis, from the nucellus (Bonne, 1961). The endosperm is located inside the wall layers of the caryopsis and is composed of the aleurone cells and the starchy endosperm (Fig. 2.11). The layer or two of cells which comprise the aleurone layer are more rectangular in shape and thicker walled than the starchy endosperm cells and contain aleurone grains. The aleurone cells secrete a variety of hydrolytic enzymes during germination which digest and mobilize the starchy endosperm reserves (McMullen, 1991). The wall and the aleurone layers from milling fraction referred to as the bran.

The starchy endosperm is composed of large thin-walled parenchyma cells filled with starch contributing approximately 55.8-68.3% of the weight of the mature kernel (Youngs, 1972) and comprises the commercial fraction called the starchy endosperm. The starchy endosperm is the primary source of nutrients for use by the embryo during germination.

The oat embryo, comprised of scutellum and the embryonic axis, is located on the anterior side, near the base of the caryopsis (Fig. 2.11). The epidermal layer of the scutellum or epithelium is in contact with the endosperm and secretes enzymes, which digest contents of the endosperm during germination (Bonne, 1961). The embryonic axis consists of the plumule and the radicle. The plumule

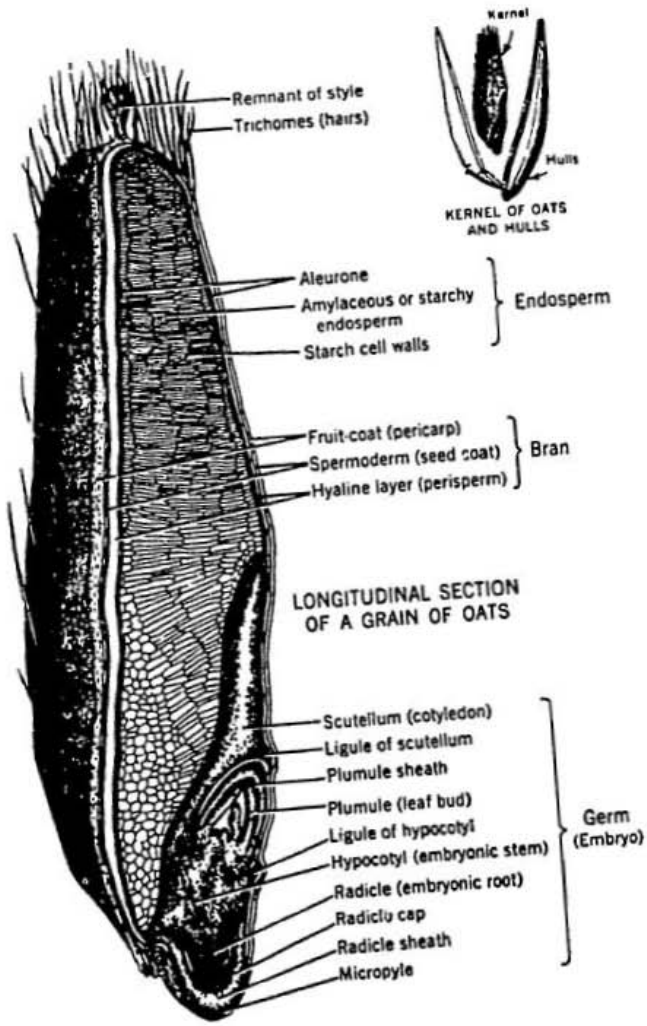
is made up of the coleoptile, which encloses the shoot apex and two leaf primordia. The root is comprised of the radicle and two or three adventitious roots, which produce the seminal root system. Youngs (1972) determined that the embryonic axis and the scutellum comprise 1.0-1.4% and 1.7-2.6%, respectively, of the groat.

2.11 Oat Starch

In most parts of the world, oats are grown and used almost exclusively on the farm as an animal feed; a very small percentage indeed finds its way into the human diet. Almost 80% of protein requirements for human is derived from cereals or cereal- based products. Although oats are well known to have both a higher protein content and better quality protein than other cereals, their utilization for human food is still limited to a few ethnic cultures. Perhaps the principal reason for this limitation is that by itself oat flour does not make a loaf bread. However, there has been an increased interest in North America in oats as a result of the overall search for protein concentrates and isolates (Wu *et al.*, 1973; Wu and Stringfellow, 1973; Cluskey *et al.*, 1973). High protein oat varieties have been developed and breeder activity in this field remains high in an effort to further improve the crop (Paton, 1979).

About 15-20 years ago Agriculture Canada's Research Branch developed a

Fig. 2.11 The oat caryopsis. Adopted from Leonard and Martin (1963).


Kernel
Hulls
KERNEL OF OATS AND HULLS
Remnant of style
Trichomes (hairs)
Aleurone
Amylaceous or starchy endosperm
Starch cell walls
Endosperm
Fruit-coat (pericarp)
Spermoderm (seed coat)
Hyaline layer (perisperm)
Bran
LONGITUDINAL SECTION OF A GRAIN OF OATS
Scutellum (cotyledon)
Ligule of scutellum
Plumule sheath
Plumule (leaf bud)
Ligule of hypocotyl
Hypocotyl (embryonic stem)
Radicle (embryonic root)
Radicle cap
Radicle sheath
Micropyle
Germ (Embryo)

considerable interest in oats as a potential source of food protein. Cultivars containing 18-22% protein in the dehulled seed (groat) are available, and even higher values may be obtained. Oat is a nutritious and inexpensive cereal but nevertheless is utilised mostly as feed, with 5% of the crop going to manufacture. There has been no identified specific functional property leading to an obvious market such as wheat has in bread, or barley in beer (Wood *et al.*, 1987).

Oat starch has been shown to differ from other cereal starches in several ways. Oat starch granules are characterized by angular or irregular shape, the granule size being mostly in the range of 3 μm to 10 μm (Paton, 1977; Mäkelä and Laakso, 1984; Gudmundsson and Eliasson, 1989; Hoover and Vasanthan, 1992). However, individual granules have a marked tendency to aggregate into bundles or clusters (MacArthur and D'Appolonia, 1979; Mäkelä and Laakso, 1984; Hoover and Vasanthan, 1992).

Oat starch is more like rice starch than wheat starch in both granule size and shape. Its amylose content (25-29%) is similar to wheat starch (MacMasters *et al.*, 1947), but oat amylose is more linear and oat amylopectin is more branched than that found in wheat (MacArthur and D'Appolonia, 1979).

Traditionally, oat starch has not generated widespread commercial interest due to its functional similarity to corn and wheat starch. It can be used in composite breads, but imparts a crumb-softening effect. Recent studies have identified some oat cultivars whose

starch pastes have unusual properties, such as the development of abnormally rapid cold viscosities. These oat starch gels are reportedly more elastic, adhesive, translucent, and show greater storage stability than corresponding wheat and corn starches (Paton, 1986). The pastes have higher cooling cycle viscosities than comparable concentrations of wheat or corn starches, and cooked oat starch granules are more sensitive to shear. The gels are influenced by salts, sucrose, gums, and mild acids. Industrial applications as ingredients in adhesives, paper products, and pharmaceuticals have been suggested (Paton, 1977,1986; Doublier *et al.*, 1987b).

Oat starch granules have been reported to have a relatively high lipid content, although the values reported depend on the oat varieties studied and the isolation and analysis methods used (Youngs, 1978; Karow *et al.*, 1984; Shamekh *et al.*, 1994). When oat grains are ground and soaked in water most of the lipids are hydrolyzed into free fatty acids (Liukkonen and Laakso, 1992). If oat is fractionated by a water process into fiber, protein and starch, the lipids are carried into each fraction. So, during this fractionation the amount of free fatty acids rises in each fraction and so causes rancidity problems (Liukkonen *et al.*, 1992). When developing good quality starch it is important to know what the contents and compositions of internal and surface lipids are, and how they do change during processing (Liukkonen and Laakso, 1992). Morrison (1988) showed that the lysophospholipid content of the starch of several oat varieties was slightly higher but

in the same range as in normal barley starches, but clearly higher than in normal maize and sorghum starches. The free fatty acid content of the oat starches studied was 0.3-0.4%, and the total lipid content was 1.3-1.5% (Morrison *et al.*, 1984). There was a significant positive correlation between total amylose and lysophospholipid contents. According to Paton (1977) the total phosphorus content of oat starches (0.06-0.08%, db) was higher than that found in other cereal starches such as wheat and maize (0.06 and 0.02%, respectively), indicating a higher lysophospholipid content.

Oat starch had lower iodine affinity (3.2-3.5 at 30°C), lower solubility (17-26% at 95°C) and slightly higher limiting viscosity than wheat and corn starches (Paton, 1977, 1979). The gelatinization enthalpy of oat starch (9.0-9.5 J/g) has been reported to be lower than that of wheat (11-12 J/g), rice (10-14 J/g) (Paton, 1987) and of barley (10.2-10.5 J/g) (Tester and Morrison, 1990; Lauro *et al.*, 1993). On the other hand, the transition enthalpy for dissociation of the amylose-lipid complex (3.6 J/g) was higher than that of wheat (1.9 J/g) or rice starch (1.9 or 3.2 J/g) (Shamekh *et al.*, 1994). Gudmundsson and Eliasson (1989) also reported that the endotherm of the amylose-lipid complex of oat starch (3.7 J/g) was 2-3 times higher than that of wheat or maize (1.6 and 1.2 J/g, respectively). At temperatures approaching 100°C, oat starch granules have been shown to swell more and, therefore, to be more deformable than wheat or maize starches (Gudmundsson and Eliasson, 1989; Hoover and Vasanthan, 1992). The deformability of

starch granules affects the rigidity of starch gels: less swollen starch particles lead to more rigid gels. Both the rigidity and the elasticity of oat and barley starch gels were shown to be greatly dependent on the proceeding heating conditions (Autio, 1990). Doublier *et al.* (1987a,b), Autio (1990) and Virtanen *et al.* (1993) have reported that the pasting behaviour of oat starch differs substantially from that of other cereal starches, such as wheat, barley and maize, by forming during cooling a gel already at higher temperatures (80°C).

During heating of oat starch dispersions, amylose and amylopectin are coleached from the granules, whereas in other cereal starches such as barley and wheat the amylose is solubilized first (Doublier *et al.*, 1987a,b; Autio, 1990; Hoover and Vasanthan, 1992; Virtanen *et al.*, 1993). Microstructural studies of oat starch pastes heated above 95°C showed that amylopectin formed the continuous phase (Autio, 1990). At 95°C, when a large amount of amylopectin was liberated to the paste, the elasticity and rigidity were lowered (Virtanen *et al.*, 1993).

CHAPTER 3

MATERIALS AND METHODS

3.1 Cereal grain

AC Stewart is a spring type oat cultivar (*Avena sativa L.*) that is covered seeded. The pedigree of AC Stewart is Ogle 4/Dumont (Burrows, 1992). NO 753-2 (*Avena nuda L.*) is a naked or hull-free oat. The pedigree of NO 753-2 is as follows: CAV 2700/Gemini/2/Rodney/3/5811 al-8B/4/Gemini/3932-16/2/OA 123-3/3932-16. CAV 2700 is an *Avena byzantina* selection from Bodrum Turkey. Gemini and OA 123-3 were derived from interspecific crosses (*Avena strigosa* X the varieties *Abegwert* and *Victory*). 5811al-8B is a winter type oat from Cornell University and 3932-16 is a naked seeded (hull-free) parent from the Ottawa program. Both cultivars were grown at the Plant Research Centre in Ottawa.

Oat grains were divided into two lots. Each lot was further subdivided into three parts and starch was extracted from them. All experiments were replicated three times.

3.2 Starch isolation and purification

Oat grains (500 g) were steeped overnight in water at room temperature. One part soaked grains was mixed with 3 parts distilled water in a Waring blender for 3 min at low speed followed by another 3 min at high speed. The resultant slurry was passed through a double layer cheese cloth and then centrifuged at 5000 x g for 15 min. The supernatant

was discarded and the sediment suspended in excess 0.02% NaOH to remove the residual proteins. After standing for 1h, the supernatant was removed. This procedure was repeated three times. The final sediment was suspended in distilled water, and then subjected to sequential filtration through 70 and 20 micron polypropylene screens, neutralized to pH 7.0, filtered on a Buchner funnel and thoroughly washed on the filter with distilled water. The filter cake was dried overnight at 30°C (Schoch and Maywald, 1968).

3.3 Chemical composition of starch

3.3.1 Moisture content

Moisture content was determined by drying a preweighed (5.0 g) amount of material in a forced air oven (Fisher scientific, isotemp 615G, USA) at 105°C until it reached a constant weight. The moisture content was calculated as percentage of weight loss of the sample due to drying (AACC, 1983).

3.3.2 Ash content

Samples (2.0 g) were transferred into clean porcelain crucibles, charred using a flame and then placed in a temperature-controlled furnace (Lab Heat, Blue M, IL) which was preheated to 550°C. Samples were held at this temperature until grey ash remained

and then transferred to a desiccator, cooled and weighed immediately. Ash was calculated as percentage weight of the remaining matter (AACC, 1983).

3.3.3 Total protein content

Samples (250 mg) were weighed on nitrogen-free papers and placed in the digestion tubes of a Buchi 430 (Buchi Laboratoriums-Technik AG, Flawil/Schweiz) digester. The samples were digested with two Kjeltabs catalyst pellets (Profamo, Quebec) and 20 ml of concentrated H_2SO_4 in the Kjeldahl digester (Buchi 430) until a clear solution was obtained. Digested samples were diluted with distilled water(50 ml); alkali (150 ml of 25% NaOH) was added and the released ammonia was steam distilled (Buchi 321) into 4% H_3BO_3 (50 ml) containing twelve drops of an end point indicator (N-point indicator, EM Science, Evanston, NJ) until 200 ml distillate was collected. The content of ammonia in the distillate was determined by titrating it against 0.1N H_2SO_4 (AACC, 1983). The content of crude protein in samples was calculated by multiplying the percentage of nitrogen by a factor of 6.25.

3.3.4 Lipid content

In the determination of lipid composition, all starches were subjected to the following extraction procedures: (A) starches (5 g, dry basis) were extracted under

vigorous agitation in a wrist action shaker with 100 ml of chloroform-methanol (2:1 v/v) at 25°C for 1h; (B) the residues from chloroform-methanol extraction were solvent extracted with 100 ml of n-propanol-water (3:1 v/v, 90-100°C) for 7h; (C) lipids were also extracted, after acid hydrolysis of starches with 24% HCl for 30 min at 70-80°C, the hydrolyzate were extracted three times with n-hexane (Goshima *et al.*, 1985).

The crude lipid extracts were then purified by further 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 the addition of chloroform and water (Bligh and Dyer, 1959); the chloroform layer was diluted with benzene and brought to dryness on a rotary evaporator.

3.4 Granule morphology

Granule morphology of native starches was studied by scanning electron microscopy (SEM). Starch samples were mounted on circular aluminum stubs with double sticky tape and then coated with 20 nm of gold and examined and photographed in a Hitachi (S570) scanning electron microscope at an accelerated potential of 20 kV.

3.5 Pasting behaviour

Pasting characteristics of starch slurries at a concentration of 6%(w/v) and pH 5.5 were determined using the Brabender viscoamylograph, Model VA-VE(C.W. Brabender Instruments, Inc., South Hackensack, NJ), equipped with a 700 cm.g sensitivity cartridge, operating at a bowl speed of 75 rpm. The starch slurry was heated from 30 to 95°C at the rate of 1.5°C/min, maintained at 95°C for 30 min, and then cooled to 50°C at the same rate. The viscosity was measured in Brabender units (BU).

3.6 Swelling factor (SF)

The SF of the starches, when heated to 50-80°C in excess water, was measured according to the method of Tester and Morrison (1990). Starch samples (50 mg, db) were weighed into 10 ml screw cap tubes, 5 ml of water was added, and the sealed tubes were incubated with constant shaking in a water bath at the required temperature for 30 min. The tubes were then cooled to 20°C, 0.5 ml of blue dextran (Pharmacia, M_r 2 X 10^6, 5 mg/ml) was added and the contents were mixed gently by inverting the closed tubes several times. After centrifuging at 1,500 X g for 5 min, the absorbence of the supernatant (A_s) was measured at 620 nm. The absorbence of the reference (A_r) sample devoid of any starch was also measured.

Calculation of SF was based on starch weight corrected to 10% moisture, assuming

a density of 1.4 mg/ml.

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

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

A_r and A_s are absorbence of the reference and sample, respectively.

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

$V_o = W/1400$

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

$V_I = 5.0 - FW$

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

$V_f = V_o + V_I$

and $SF = V_f/V_o$

This can also be expressed by the single equation

$SF = 1 + \{(7700/W) X (|A_s - A_r|/A_s)\}$

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

3.7 Extent of amylose leaching

Starch samples (15 mg) in distilled water (10 ml) were heated (50-95°C) in volume calibrated sealed tubes for 30 min. The tubes were then cooled to ambient temperature and centrifuged at 3500 rpm for 10 min. Aliquot (0.1 ml) of the supernatant, was assayed

for solubilized amylose (Chrastil, 1987). Percentage amylose leaching was calculated and represented as mg of amylose leached per 100 mg dry starch.

3.8 Acid hydrolysis

The starches were hydrolysed with 2.2N HCl at 35°C (100 mg starch/4 ml acid) for 20 days. The starch slurries were shaken by hand daily to resuspend the deposited granules. At specific time intervals, aliquots (0.1 ml) of the reaction mixture were neutralized with 2.2N NaOH (0.1 ml) and centrifuged (3500 rpm, for 10 min) and the supernatant liquid was assayed for total carbohydrates (Bruner, 1964). Controls without acid but subjected to the above experimental conditions were run concurrently. The extent of hydrolysis was determined by expressing the solubilized carbohydrates as percentage of the initial dry starch.

3.9 Enzymatic digestibility

Enzymatic digestibility studies on native starches were done using a crystalline suspension of porcine pancreatic α-amylase (EC 3.2.1.1) in 2.9 M saturated sodium chloride containing 3 mM calcium chloride (Sigma Chemical Co., St. Louis, Mo), in which the concentration of α-amylase was 30.0 mg/ml and the specific activity was 790 units per milligram of protein. One unit was defined as the α-amylase activity which

liberated 1 mg maltose in 3 min at 20°C at pH 6.9. The procedure used was essentially that of Knutson *et al.* (1982). However, a higher concentration of enzyme was used in this study. Starch granules (20 mg) were suspended in distilled water (5 ml) and placed in a constant temperature (37°C) shaker water bath. Then 4.0 ml of 0.1M phosphate buffer (pH 6.9), containing 0.006M NaCl were added to the slurry. The mixture was gently stirred before adding 4 μl of α-amylase suspension. The reaction mixtures were shaken by hand daily to resuspend the deposited granules. Then 1.0 ml aliquots were removed at specific time intervals, pipetted into 0.2 ml of 95% ethanol, and centrifuged. Aliquots of the supernatant were analyzed for soluble carbohydrates (Bruner, 1964). Percentage hydrolysis was calculated as the amount (mg) of maltose released per 100 mg of dry starch. Controls without enzyme but subjected to the above experimental conditions were run concurrently. Results used for calculation were means of triplicate measurements.

For DSC analysis, the granular residues were repeatedly washed with ethanol and once with chloroform before air drying. The above experiment was replicated two times.

3.10 Light transmittance of starch pastes

The following procedure, adapted from Craig *et al.* (1989), was used to prepare 1% (w/v) starch pastes. Starch (50 mg, db) was suspended in water (5 ml) in screw cap tubes and the pH adjusted by addition of 0.1N HCl or NaOH as required. The tubes were then

heated in a boiling water bath (with occasional shaking) for 30 min. After cooling to ambient temperature, the percentage transmittance (% T) at 650 nm was determined against a water blank in a Novaspec (Model 4049, LKB Biochrom, Cambridge, England) spectrophotometer.

3.11 Amylose content

The apparent amylose content of native starches were determined by the method of Chrastil (1987), after complete dispersion of samples in 0.5N KOH solution followed by neutralization with HCl at room temperature. The total amylose content of starch sample was determined by the same procedure, but with prior defatting with hot n-propanol-water(3:1 v/v) for 7h.

3.11.1 Preparation of starch dispersions

Starch samples (20 mg, db) were dispersed in 10 ml of 0.5N KOH in 20 ml screw cap glass tubes. The dispersed samples were transferred to 100 ml volumetric flasks and diluted to the mark with distilled water. An aliquot of the test starch solution (10 ml) was pipetted into a 50 ml volumetric flask, and 5 ml 0.1N HCl was added prior to dilution.

3.11.2 Chrastil's method of amylose determination

Sample aliquots(0.1 ml) of the neutralized solution were mixed with 5.0 ml of water and 0.05 ml of 0.01N I_2-KI solution. The absorbence of the blue coloured complex was read at 620 nm (after 30 min at 25°C), in a spectrophotometer (LKB Biochrom, Novaspec). The absorbence of the reaction blanks with water was zero. The amylose content was approximated using the following formula (obtained through a calibration curve) and expressed as mg of amylose per 100 mg dry starch.

Absorbence x 32.5= mg of amylose/litre in cuvette

3.12 X-ray diffraction

X-ray diffractograms were obtained with a Rigaku RU 200 R X-ray diffractometer connected to a data acquisition and processing station. The starch powder was scanned through the 2Θ range of 3-35°. Traces were obtained using Cu-Kα radiation detector with a nickel filter and a scintillation counter operating under the following conditions: 40 kV, 50 mA, 1°/1° divergence slit/scattering slit, 0.3 mm receiving slit, 1s time constant and scanning rate of 3°/min.

3.13 Differential scanning calorimetry (DSC)

DSC measurements on native and enzyme-treated starches were carried out using a Perkin-Elmer DSC-2 (Norwalk, CT) differential scanning calorimeter with a thermal analysis data-station. Water (9.0 μl) was added with a microsyringe to starch (3.0 mg) in DSC pans, which were then sealed and allowed to stand overnight at room temperature to allow better moisture equilibrium. These pans were reweighed before the DSC experiment to ensure that water loss did not occur during storage. The scanning temperature range and the heating rate were 20-120°C and 10°C min $^{-1}$, respectively. The thermogram was recorded with water as reference. The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c). The enthalpy (ΔH) was estimated by integrating the area between the thermogram and a baseline under the peak and was expressed in Joules per unit weight of dry starch (J/g). Fusion of retrograded amylopectin was determined by weighing (3-4 mg dry basis of the stored (at 4°C) gels (50% w/v) into DSC pans which were then sealed and scanned from 20 to 100°C at 10°C/min. All DSC experiments were replicated at least three times.

3.14 Gel preparation

Gels (50% w/v) were prepared as described by Krüsi and Neukom (1984). Oat starches (5 g, db) were carefully weighed into circular aluminum moulds (diameter 3.0

cm, height 3.0 cm) with removable tops and bases and then mixed with 10 ml distilled water containing 0.02% $Na_2S_2O_3$ as preservative. The moulds were then heated in a water bath for 35 min at 95°C. The resulting gels were allowed to cool within the moulds for 30 min at 4°C prior to storage at 25°C for 20 days.

3.15 Gel powder preparation

The procedure (with minor modifications) of Roulet *et al.* (1988) was used to convert the stored gels to a powder prior to examination by DSC and X-ray diffraction. The gels were rinsed with water, cut into small pieces and mixed with 100 ml acetone. After homogenization using a polytron, the mixture was left to decant for 5 min. The liquid was discarded and the rest was transferred to screw cap tubes. Acetone was again added, the mixture centrifuged (3000 x g) and the supernatant discarded. This procedure was repeated three times and the remaining mass was dried in an air-oven for 6h at 30°C.

3.16 Freeze-thaw stability

The gels (6% w/v, db) were subjected to cold storage at 4°C for 16h (to increase nucleation) and then frozen at -16°C. To measure freeze-thaw stability, the gels frozen at -16°C for 24h, were thawed at 25°C for 6h and then refrozen at -16°C. Five cycles of freeze-thaw were performed. The excluded water was determined by centrifuging the

tubes (30 mm diameter x 100 mm) at 1000 x g for 20 min after thawing. Reported values were means of three determinations.

3.17 Gel texture determination

The resistance to penetration of the gel, during storage (2-20 days) at 25°C was determined with a model 6000R Lloyd texture testing machine (Omnitronix Instruments Ltd., Mississauga, ON) equipped with a data acquisition and processing station. The 50 N load cell was used. The gels within the aluminum moulds were placed on the compression table. The cross head of the machine, fitted with the load cell and a cylindrical probe (5 mm diameter) was driven down so as to just touch the gel surface. The probe was then driven at a constant speed (0.5 mm/min) into the gel for a distance of 6 mm. The load at 1 mm compression was termed firmness. The readings were in units of load grams. The reported results were means of three determinations.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Morphological granular characteristics of the starch

Starch granules tended to exist in clusters of individual granules (Figs. 4.1 A,B). The shape of both starch granules tested ranged from polygonal to irregular with an average granule diameter of 5 to 12 μm. Smooth surfaces were observed with no evidence of indentations fissures or pores.

4.2 Chemical composition of the starch

The data on composition and yield of NO 753-2 and AC Stewart starches are presented in Table 4.1. The nitrogen content of the purified oat starches was 0.001% (dry basis), indicating the absence of endosperm proteins and by implication most of the non-starch lipids (Morrison, 1981). The total starch lipids (TSL) obtained by acid hydrolysis were 1.6% in both starches. These values were higher than those reported for AC Hill oat (1.13%) (Hoover and Vasanthan, 1992) and wheat (0.7%) (Vasanthan and Hoover, 1992) starches.

The solvent extracted lipids (SEL) from the combined action of chloroform-methanol 2:1 v/v (CM) and n-propanol-water 3:1 v/v (PW) were 1.63% (NO 753-2) and 1.67% (AC Stewart). These values were higher than those reported for wheat

(0.68%) (Vasanthan and Hoover, 1992) and AC Hill oat starches (1.12%) (Hoover and Vasanthan, 1992), but were within the range (1.3-2.4%) reported by Morrison *et al.* (1984), Doublier *et al.* (1987a,b), Gudmundsson and Eliasson (1989) and Sowa and White (1992) for starches isolated from other varieties of oat grains.

4.3 Amylose content

The total amylose contents in NO 753-2 and AC Stewart starches were 22.7 and 22.9%, respectively (Table 4.1). These values are higher than those reported for AC Hill oat starch (19.4%) (Hoover and Vasanthan, 1992), but lower than that of wheat starch (27.3%) (Vasanthan and Hoover, 1992) and oat starches from other cultivars (22.8-29.4%) (Morrison *et al.*, 1984; Gudmundsson and Eliasson, 1989; Wang and White, 1994). The amount of amylose complexed by native lipids in NO 753-2 and AC Stewart was 14.1 and 15.3%, respectively, . These values were within the range (13.9-32.0%) reported (Morrison *et al.*, 1984; Hoover and Vasanthan, 1992) for oat starches from other cultivars (Morrison *et al.*, 1984; Hoover and Vasanthan, 1992), but lower than that of wheat starch (22.7%) (Vasanthan and Hoover, 1992).

4.4 X-ray diffraction

The X-ray spectra of both oat starches examined were of the A- type representative of cereal starches (Fig. 4.2). At approximately the same moisture content, the intensities of the major diffraction peaks were much higher in NO 753-2 than in AC Stewart (Fig. 4.2 and Table 4.2). The results suggest that crystallites of NO 753-2 are either more closely associated and/or are better orientated to diffract X-rays than those of AC Stewart.

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

The SF and AML of oat starches in the temperature range of 50-80°C are presented in Table 4.3 and 4.4. The SF of both starches increased with increasing temperature. This was most marked between 50 and 60°C (AC Stewart > NO 753-2) (Table 4.3). Furthermore, at all temperatures, the SF of AC Stewart was much higher than that of NO 753-2. For instance, at 80°C the SF values were 22.3 and 9.5 (Table 4.3) for AC Stewart and NO 753-2, respectively. The SF of AC Stewart was comparable to that of wheat (Vasanthan and Hoover, 1992) and AC Hill oat starches (Hoover and Vasanthan, 1992). Tester and Morrison (1990) have shown, by studies on waxy and normal starches, that swelling is a property of amylopectin, and that in

Fig. 4.1. Scanning electron micrographs of native oat starches:

A) NO 753-2; B) AC Stewart

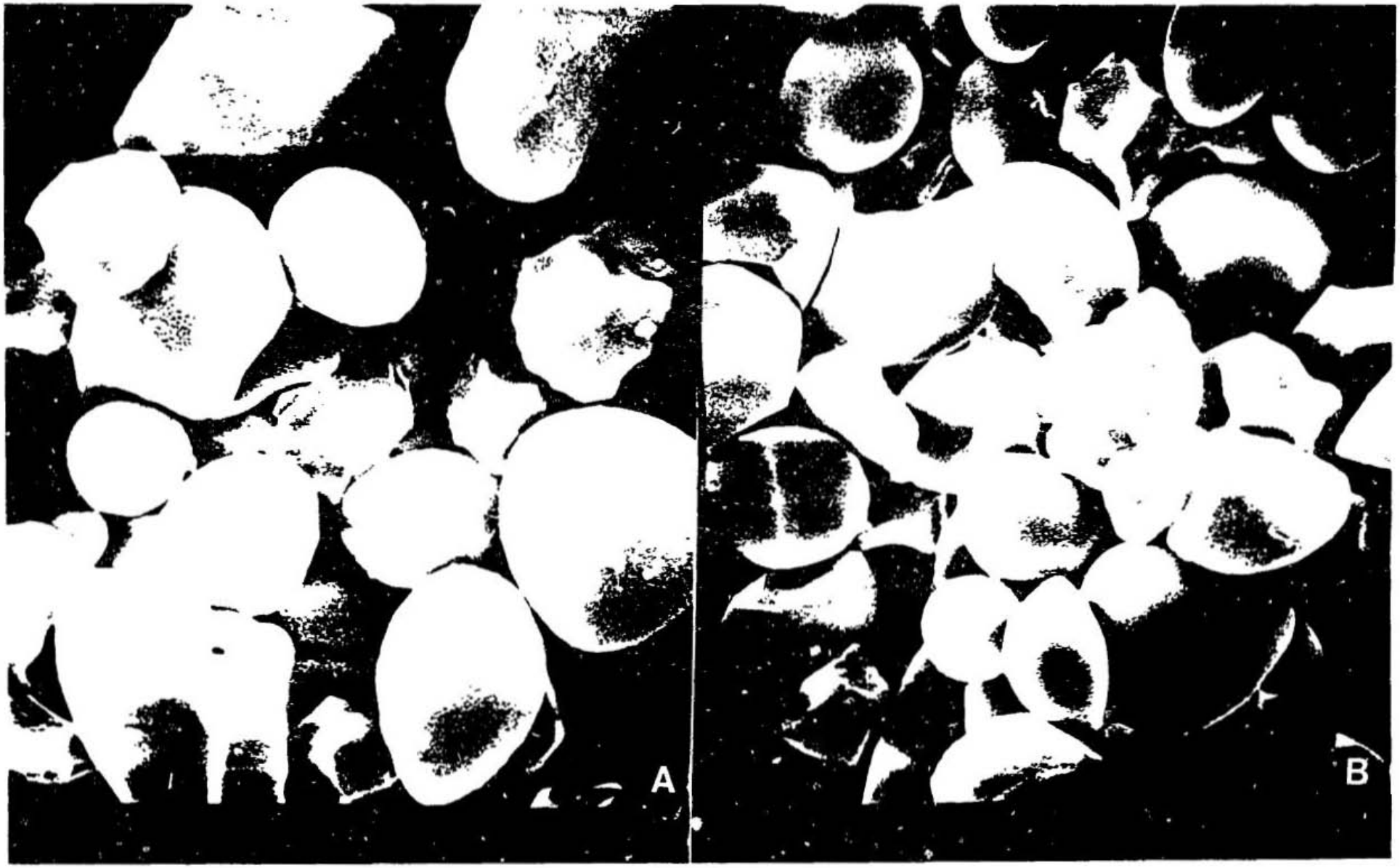

A
B

Table 4.1. Chemical composition (%) of oat starches[1].

Characteristics	Oat cultivar	
	NO 753-2	AC Stewart
Yield (% initial material)	35.8[a] ± 1.5	34.0[a] ± 1.2
Moisture	10.6[a] ± 0.6	10.2[a] ± 0.4
Ash	0.21[a] ± 0.01	0.20[a] ± 0.01
Nitrogen	0.001[a] ± 0.01	0.001[a] ± 0.01
Lipid		
Acid hydrolysed[2]	1.64[a] ± 0.6	1.67[a] ± 0.5
Solvent extracted		
chloroform-methanol[3]	0.36[a] ± 0.01	0.30[b] ± 0.01
n-propanol-water[4]	1.27[b] ± 0.01	1.37[a] ± 0.05
Amylose content (% of total starch)		
Apparent[5]	19.5[a] ± 0.1	19.4[a] ± 0.4
Total[5]	22.7[a] ± 0.6	22.9[a] ± 0.1
Amylose complexed with native lipid[6]	14.1[b] ± 0.1	15.3[a] ± 0.4

[1]All data reported on dry basis and represent means ± standard deviation of triplicate analyses. Mean values in each row sharing the same superscript are not significantly different ($p \geq 0.05$).
[2]Lipids obtained by acid hydrolysis (24% HCl) of the native starch (total lipids).
[3]Lipids extracted from native starch by chloroform-methanol 2:1 (v/v) at 25°C (mainly unbound lipids).
[4]Lipids extracted by hot propanol-water 3:1 (v/v) from the residue left after chloroform-methanol extraction (mainly bound lipids).
[5]Apparent and total amylose determined by I_2-binding before and after removal of bound lipids by hot propanol-water extraction.
[6]$\frac{\text{Total amylose - apparent amylsee}}{\text{Total amylose}} \times 100$

Fig. 4.2. X-ray diffraction patterns of native oat starches:

A) AC Stewart; B) NO 753-2

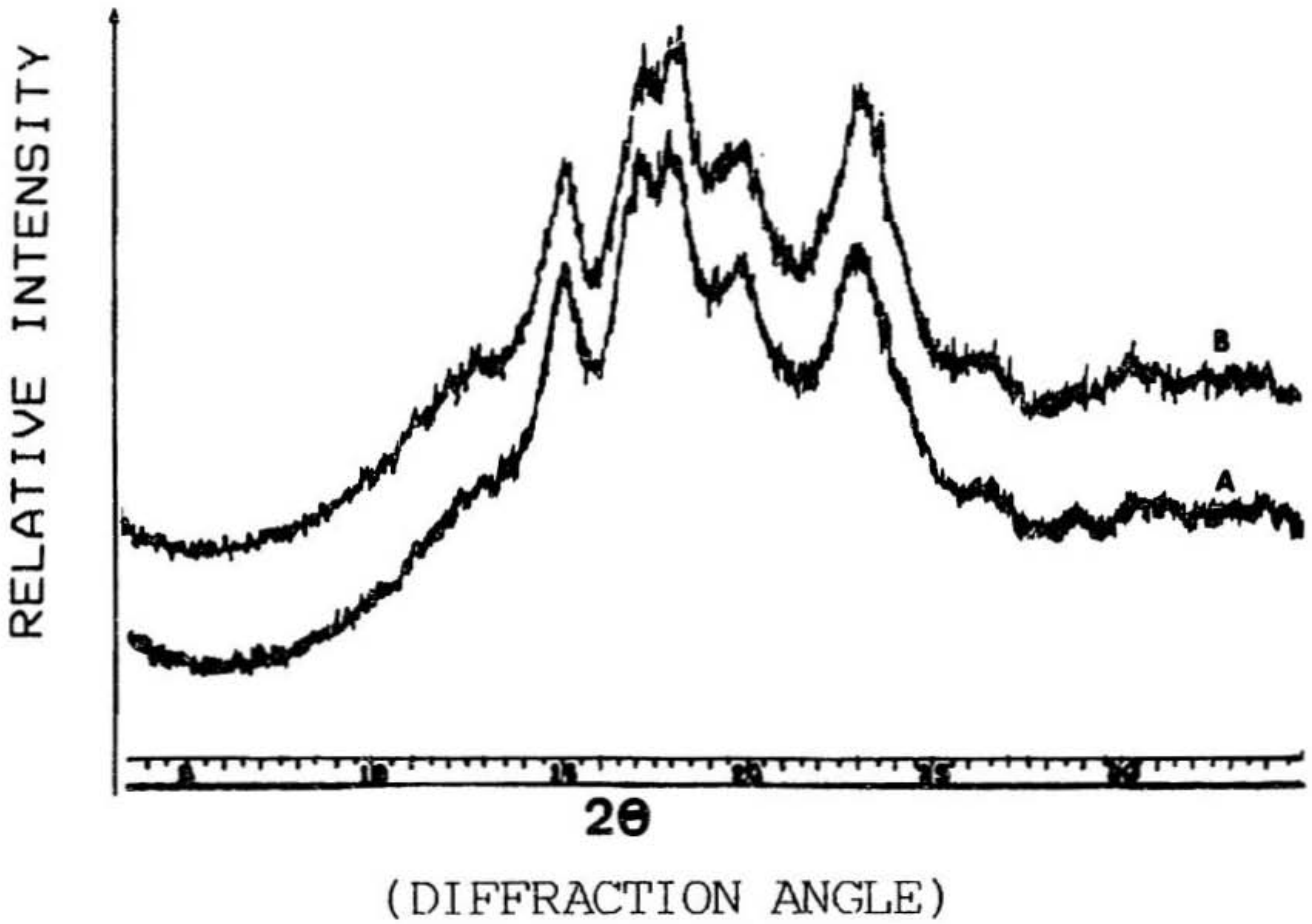

RELATIVE INTENSITY
B
A
2θ
(DIFFRACTION ANGLE)

Table 4.2. X-ray diffraction spacings and intensities of the major peaks of oat starches [1].

Oat cultivar	Moisture content (%)	Interplanar spacings (d) in Å with intensities (CPS)[2]			
NO 753-2	10.6[a]	5.8 (1849 [a] ± 0.8)	5.2 (2571 [a] ± 0.7)	4.9 (2698 [a] ± 0.9)	3.8 (954 [a] ± 1.0)
AC Stewart	10.2[a]	5.9 (1085 [b] ± 0.7)	5.3 (1085 [b] ± 0.7)	5.2 (1494 [b] ± 0.7)	3.8 (495[b] ± 1.5)

[1] Values represent means ± standard deviation of triplicate analyses.
Mean values in each column not followed by the same superscript are significantly different ($p \leq 0.05$).
[2] Counts per second

normal cereal starches amylose and lipid actively inhibit swelling under conditions where amylose-lipid complexes are likely to form. The present results suggest that the SF of both starches is probably influenced mainly by differences in the degree of association between amylopectin chains (NO 753-2 > AC Stewart).

The extent of AML in both starches was nearly similar (Table 4.4) and much lower than those reported for other oat starch cultivars (Paton, 1979; Hoover and Vasanthan, 1992). These differences probably reflect the lower amylose content of AC Stewart and NO 753-2 starches (Table 4.1).

4.6 Pasting properties

The pasting properties of the oat starches are shown in Table 4.5. NO 753-2 exhibited a higher pasting temperature, a lower viscosity at 95°C , a higher resistance to shear (during the holding cycle at 95°C) than AC Stewart. It has been postulated (Doublier *et al.*, 1987a,b; Hoover and Vasanthan, 1992; Hoover *et al.*, 1993; Wang and White, 1994), that pasting properties are influenced by the magnitude of associative bonding forces within the granule interior of starch. The difference in pasting properties (Table 4.5) is probably due to stronger bonding forces within the granule interior of NO 753-2 starch.

Table 4.3. Effect of temperature on swelling factor of oat starches [1].

Oat cultivar	Temperature (°C)			
	50	60	70	80
NO 753-2	$3.5^{b} \pm 0.2$	$7.3^{b} \pm 0.7$	$8.6^{b} \pm 0.9$	$9.5^{b} \pm 0.3$
AC Stewart	$6.3^{a} \pm 0.4$	$12.9^{a} \pm 0.9$	$16.7^{a} \pm 0.9$	$22.3^{a} \pm 0.9$

[1]Values represent means ± standard deviation of triplicate analyses.

Mean values in each column not followed by the same superscript are significantly different ($p \leq 0.05$).

Table 4.4. Effect of temperature on amylose leaching of oat starches [1].

	Temperature (°C)			
Oat cultivar	50	60	70	80
NO 753-2	–	0.7[a] ± 0.1	1.6[a] ± 0.2	2.2[a] ± 0.2
AC Stewart	–	0.7[a] ± 0.1	1.8[a] ± 0.1	2.5[a] ± 0.5

[1] Values represent means ± standard deviation of triplicate analyses.
Mean values in each column sharing the same superscript are not significantly different ($p \geq 0.05$).

Table 4.5. Pasting characteristics of oat starches[1].

Oat cultivar	Pasting temperature (°C)	Viscosity at 95°C (BU)[2]	Viscosity after 30 min at 95°C (BU)[2]	Viscosity at 50°C (BU)[2]
NO 753-2	$95.7^{a} \pm 0.1$	$139.0^{b} \pm 1.4$	$112.5^{b} \pm 1.0$	$445.0^{b} \pm 2.5$
AC Stewart	$94.7^{b} \pm 0.2$	$162.5^{a} \pm 1.6$	$117.5^{a} \pm 1.0$	$490.0^{a} \pm 2.5$

[1]Values represent means ± standard deviation of triplicate analyses.

Mean values in each column not followed by the same superscript are significantly different ($p \leq 0.05$).

[2]Brabender units.

4.7 Gelatinization temperatures

The DSC thermograms of the two oat starches are presented in Table 4.6. The transition temperatures (T_o (onset), T_p (peak), T_c (conclusion)) and the gelatinization enthalpy (ΔH) of AC Stewart were lower than those of NO 753-2 by 8.4°C, 9.4°C, 10.7°C and 2.5 J/g, respectively. Furthermore, the gelatinization temperature range (T_c-T_o) of NO 753-2 was higher than that of AC Stewart by 2.3°C (Table 4.6). The T_o, T_p and T_c of the above starches were within the range reported for other oat cultivars. However, ΔH values were lower than the reported range (9.4-10.6 J/g) (Paton, 1987; Gudmundsson and Eliasson, 1989; Hoover and Vasanthan, 1992; Sowa and White, 1992; Wang and White, 1994). The higher T_o, T_p and T_c of NO 753-2 suggest that the crystallite size and/or crystallite association within its granules are of a higher order of magnitude than AC Stewart.

Kugimiya *et al.* (1980) showed that lysolecithin complexed potato starch exhibited a lower ΔH value than did native potato starch. Biliaderis *et al.* (1986) noted that waxy rice starches (devoid of lipids) exhibited higher ΔH values than those of their non-waxy counterparts. The latter authors postulated that the lower ΔH values for non-waxy starches were the net result of two competing processes: melting of starch crystallites (endothermic) and crystallization (exothermic) of amylose-lipid complexes.

Table 4.6. Differential scanning calorimetry of oat starches[1].

Oat cultivar	T_o[2] (°C)	T_p[2] (°C)	T_c[2] (°C)	$T_c - T_o$[3] (°C)	ΔH[4] (J/g)	T_{cx}[5] (°C)	ΔH_{cx}[6] (J/g)
NO 753-2	$60.4^a \pm 0.1$	$67.0^a \pm 0.2$	$74.2^a \pm 0.1$	$13.8^a \pm 0.1$	$8.4^a \pm 0.2$	$98.5^b \pm 0.3$	$3.0^b \pm 0.1$
AC Stewart	$52.0^b \pm 0.1$	$57.6^b \pm 0.1$	$63.5^b \pm 0.4$	$11.5^b \pm 0.5$	$5.9^b \pm 0.1$	$105.0^a \pm 0.3$	$3.5^a \pm 0.2$

[1]Starch:water ratio 1:3, Values represent means ± standard deviation of triplicate analyses.

[2]T_o, T_p and T_c indicate the temperatures of the onset, midpoint and end of gelatinization.

[3]Gelatinization temperature range.

[4]Enthalpy of gelatinization.

[5]Amylose-lipid complex transition temperature.

[6]Amylose-lipid complex transition enthalpy.

Mean values in each column not followed by the same superscript are significantly different ($p \leq 0.05$).

Biliaderis *et al.* (1986) also showed that ΔH values decrease with increase in bound lipid content for 8 cultivars of rice starch. Thus, the difference in ΔH values between NO 753-2 and AC Stewart starches (Table 4.6) is probably due to the higher bound lipid content of the latter (Table 4.1).

The transition enthalpy for the melting of the amylose-lipid complex (ΔH_{cx}) was 3.0 and 3.5 J/g in NO 753-2 and AC Stewart starches, respectively (Table 4.6). These values were within the range (0.84-3.70 J/g) reported for other oat starch cultivars (Paton, 1987; Gudmundsson and Eliasson, 1989; Hoover and Vasanthan, 1992; Sowa and White, 1992). Furthermore, the ΔH_{cx} of both starches were higher than those reported for wheat starch (1.30 J/g) (Hoover and Vasanthan, 1992). This suggests that ΔH_{cx} is influenced by the amount of amylose complexed lipids (AC Stewart > NO 753-2 > wheat). It was interesting to observe that although the amount of lipid complexed amylose in NO 753-2 (14.1%) and AC Stewart (15.5%) starches was only slightly higher than the value reported for AC Hill oat starch (13.1%) (Hoover and Vasanthan, 1992), the ΔH_{cx} of the latter (0.8 J/g) was much lower than those of NO 753-2 (3.1 J/g) and AC Stewart (3.5 J/g) starches. This indicates that lipids of AC Hill oat starch are probably less strongly bound to the amylose helix than those of NO 753-2 and AC Stewart starches.

4.8 Light transmittance

The percentage light transmittance (% T) of the oat starches at different pH values are presented in Table 4.7. Above pH 6, the clarity of the starch paste was higher in AC Stewart. This difference was most pronounced at pH values between 6 and 9. At pH 12, the % T was 91 and 83% in AC Stewart and NO 753-2 starches, respectively. The % T of AC Stewart at pH 12 was comparable to that reported for AC Hill oat starch (90) and wheat starch (94) (Hoover and Vasanthan, 1992).

Swinkels (1985) and Craig *et al.* (1989) showed that amylose-lipid complexes decrease the % T of starch paste. The latter authors also reported that % T increases with the degree of swelling. Our results showed that the difference in % T between the two oat starches (Table 4.7) is probably influenced by differences in swelling factor (Table 4.3) (AC Stewart > NO 753-2).

4.9 *In vitro* digestibility of native starches by porcine pancreatic α-amylase

The extent of α-amylase hydrolysis of the oat starches is presented in Table 4.8. During the first 12h, AC Stewart was more extensively hydrolysed than NO 753-2. Thereafter, differences in hydrolysis were only marginal (Table 4.8). For instance, after 12h of hydrolysis AC Stewart and NO 753-2 starches were hydrolysed by 35 and 28%, respectively. However, the corresponding values after 48h of hydrolysis

Table 4.7 Effect of pH on light transmittance (%) of native oat starches [1].

Oat cultivar	pH							
	2	3	4	6	7	8	9	12
	Transmittance (%)							
NO 753-2	2.5[a]±0.4	6.1[a]±0.4	11.8[a]±0.5	20.0[b]±0.3	22.4[b]±0.2	27.6[b]±0.3	40.0[b]±0.2	83.2[b]±0.3
AC Stewart	2.2[a] ±0.3	5.5[a] ±0.7	10.7[a]±0.4	24.2[a]±0.4	34.9[a]±0.4	39.4[a]±0.7	49.1[a]±0.3	91.2[a]±0.2

[1] Values represent means ± standard deviation of triplicate analyses.

Mean values in each column not followed by the same superscript are significantly different ($p \leq 0.05$)

were 43 and 41 %, respectively. The above values were comparable to that of wheat starch (42% after 24h) (Hoover and Vasanthan, 1992), but higher than that of AC Hill oat starch (31.6% in 24h) (Hoover and Vasanthan, 1992). Studies have shown that amylose helices complexed with monoacyl lipids (Van Lonkhuysen and Blankestijn, 1976; Larrson and Meizis, 1979; Holm *et al.*, 1983) are resistant to hydrolysis by α-amylase, probably due to a decreased solubility of the complex. In addition, factors such as degree of crystallinity (Dreher *et al.*, 1984; Ring *et al.*, 1988; Lauro *et al.*, 1993), amylose/amylopectin ratio (Dreher *et al.*, 1984; Holm and Björck, 1988), and particle size (Ring *et al.*, 1988) have also been shown to influence starch digestibility. Marsden and Gray (1986) and Franco *et al.* (1988) postulated that α-amylase initially hydrolyses the amorphous regions of the granule. Furthermore, Williamson *et al.* (1992) showed by means of DSC and X-ray diffraction studies that in A-type granules, hydrolysis occurs predominantly in the amorphous regions of the granule. The differences in the degree of susceptibility of the two starches towards α-amylase hydrolysis suggest that starch chain associations within the amorphous regions of the granule are probably more extensive in NO 753-2 than in AC Stewart. This seems plausible, since difference in granule size (Fig. 4.1), between the two starches is only marginal.

Table 4.8 Time course of hydrolysis of native oat starches by porcine pancreatic α-amylase [1].

Oat cultivar	Hydrolysis time (h)					
	3	6	9	12	24	48
	Hydrolysis (%)					
NO 753-2	14.6[b]±0.1	20.0[b]±0.7	25.4[b]±0.3	28.3[b]±0.2	40.0[a]±0.9	41.3[a]±1.5
AC Stewart	21.0[a]±0.1	27.2[a]±0.2	30.9[a]± 0.5	35.3[a]±0.6	42.5[a]±0.8	42.6[a]±1.2

[1] Values represent means ± standard deviation of triplicate analyses.

Mean values in each column not followed by the same superscript are significantly different ($p \leq 0.05$).

The granular residues left after enzyme hydrolysis (2-20h) were analyzed by DSC (Table 4.9). Both starches exhibited only a small reduction in T_o, T_p, T_c and ΔH. This suggests that the perfecting and ordering of the amylopectin crystallites in the granular residues are very nearly the same as in the native granule.

4.10 Acid hydrolysis

The solubilization patterns of the two oat starches are presented in Table 4.10. A relatively higher rate of solubilization was observed during the first 9 and 12 days for AC Stewart and NO 753-2 starches, respectively, followed by a slower rate thereafter (Table 4.10). After 20 days, the extent of hydrolysis was 86.5 (AC Stewart) and 79.0% (NO 753-2). During the same period, corn, wheat, and AC Hill oat starches were hydrolysed by 76 (unpublished results), 82 (Hoover and Vasanthan, 1994) and 80% (Hoover and Vasanthan, 1992), respectively. The faster rate during the early stages of hydrolysis (9 days (AC Stewart), 12 days (NO 753-2)) corresponded to the destruction of the amorphous regions of the granule. During the final stage, the crystalline region is slowly degraded (Kainuma and French, 1971; Robin *et al.*, 1974; French, 1984). It is evident from the results presented in Table 4.10, that the amorphous and crystalline regions are more highly ordered in NO 753-2 than in AC Stewart.

Table 4.9. DSC gelatinization parameters of enzyme treated granular residues following hydrolysis with porcine pancreatic α-amylase[1].

		Hydrolysis time (h)			
Starch source	DSC parameter	2	4	10	20
NO 753-2	Tp[2]	69.0[a] ± 0.2	68.0[a] ± 0.1	69.0[a] ± 0.2	70.0[a] ± 0.1
	ΔH[3]	6.7[c] ± 1.0	5.9[c] ± 1.0	5.9[c] ± 1.0	5.5[c] ± 1.0
AC Stewart	Tp[2]	65.0[b] ± 0.2	63.0[b] ± 0.2	65.0[b] ± 0.1	67.0[b] ± 0.1
	ΔH[3]	5.9[c] ± 0.1	5.9[c] ± 0.1	5.8[c] ± 0.1	5.7[c] ± 0.1

[1]Values represent means ± standard deviation of triplicate analyses.

Mean values in each column sharing the same superscript are not significantly different ($p \geq 0.05$).

[2]Peak temperature (°C)

[3]Enthalpy of gelatinization (J/g)

Table 4.10 Heterogenous acid hydrolysis of native oat starches in 2.2N HCl at 35°C [1].

Oat cultivar	Hydrolysis time (days)									
	1	3	5	7	9	12	13	15	17	20
	Hydrolysis (%)									
NO 753-2	3.5^{b}±0.1	12.2^{b}±0.2	19.1^{b}±0.1	25.6^{b}±0.5	42.3^{b}±0.4	59.5^{b}±0.2	67.0^{b}±0.3	72.4^{b}±0.2	75.8^{b}±0.2	79.0^{b}±0.3
AC Stewart	4.0^{a}±0.2	14.2^{a}±0.2	21.8^{a}±0.4	30.8^{a}±0.1	55.6^{a}±0.2	64.2^{a}±0.1	70.2^{a}±0.1	76.6^{a}±0.2	81.0^{a}±0.2	86.5^{a}±0.2

[1] Values represent the means ± standard deviation of triplicate analyses.

Mean values in each column not followed by the same superscript are significantly different ($p \leq 0.05$).

4.11 Retrogradation of oat starch gels

The extent of retrogradation during gel storage was monitored by determining changes in freeze-thaw stability, gel strength, retrogradation enthalpy and X-ray intensities.

4.11.1 Freeze-thaw stability

The freeze-thaw stability of a starch gel is evaluated by the amount (%) of water released (syneresis) when starch chains retrograde (reassociate) during the freeze-thaw cycle. The degree of syneresis of the starch gels are presented in Fig. 4.3. The extent of syneresis has been shown to be influenced by the amount of amylose complexed by native lipids (Hoover and Vasanthan, 1992), and by the degree of polymerization of the A chains of amylopectin (Wu and Seib, 1990). The results showed that more starch chains (which separated out as individual units during gelatinization) reassociate during frozen storage in AC Stewart than in NO 753-2 starch. This is probably due to more extensive interaction between starch chains within native granules of NO 753-2 (since differences in the extent of amylose leaching (Table 4.4) and lipid content (Table 4.1) between the starches are only marginal). Both oat starches showed a higher degree of syneresis than AC Hill oat starch (Hoover and Vasanthan, 1992). However, in comparison with wheat starch (Hoover and Vasanthan, 1992), the % syneresis was

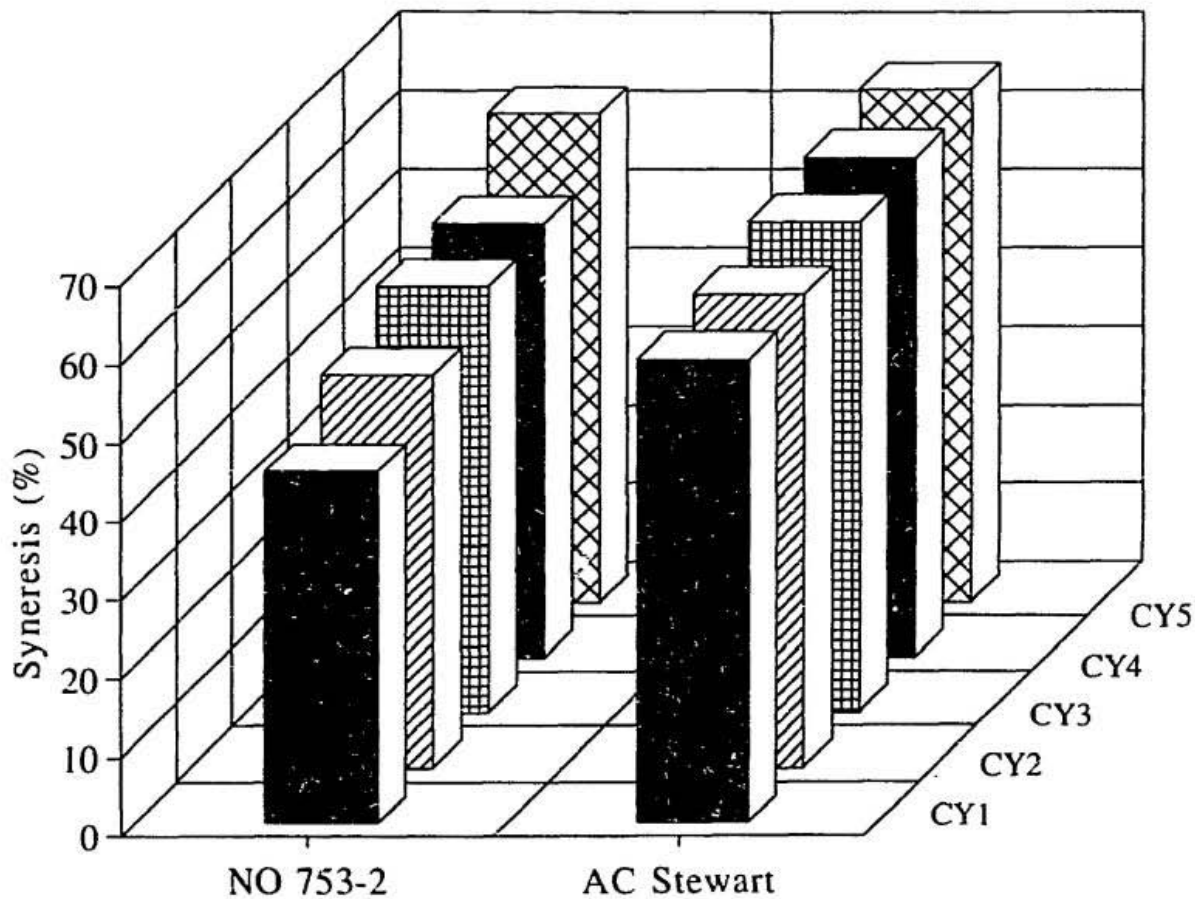
lower in NO 753-2, but higher in AC Stewart. For instance, at the same concentration (6% w/v) and storage temperature (-16°C), the % syneresis were 62.2, 65.3, 52.0 and 60.1% respectively, in NO 753-2, AC Stewart, AC Hill and wheat starches.

4.11.2 Differential scanning calorimetry

In both starches, the retrogradation endotherm was observed after the 16 days of storage (at 4°C). The transition temperatures (T_o, T_p, T_c) of the retrogradation endotherm of NO 753-2 (45, 48, 54°C) and AC Stewart (42, 44, 55°C) starch gels showed only marginal changes during the time course of retrogradation. Furthermore, T_c-T_o for retrogradation [NO 753-2 (17°C), AC Stewart (13.5°C)] were broader than for gelatinization [NO 753-2 (13.8°C), AC Stewart (5.9°C)]. In both oat starch gels, the enthalpy of retrogradation (ΔH_R) increased gradually until the 24th day of storage (Table 4.11). Thereafter, ΔH_R increased rapidly (NO 753-2 > AC Stewart). At the end of the storage period (32 days), the increase in ΔH_R was 6.7 and 5.0 J/g in NO 753-2 and AC Stewart starch gels, respectively.

The broadening of the crystallite melting endotherm on retrogradation, probably reflects melting of crystallites of different stability, size or perfection formed by different types of starch chain associations (amylose-amylopectin and/or amylopectin-

Fig. 4.3. Freeze-thaw stability of native oat starches. CY1-CY5 represent the number of freeze-thaw cycles.The data are presented as means of three determinations.


Syneresis (%)
70
60
50
40
30
20
10
0
NO 753-2
AC Stewart
CY5
CY4
CY3
CY2
CY1

amylopectin) during gel storage. Gudmundsson and Eliasson (1989) showed that maize starch exhibited higher ΔH_R values on storage than oat starches (which had higher lipid contents). However, the oat starch cultivar *Chicauhua*, when defatted with 75% isopropanol, showed a higher increase in ΔH_R when compared with its native counterpart. Furthermore, in the oat cultivar *Svea*, the difference in ΔH_R between native and defatted starches was only marginal (Gudmundsson and Eliasson, 1989). Wang and White (1994) reported that in three oat cultivars (E 77, Dal, L 996), the percentage retrogradation (% R) decreased with decreased starch lipid content. The E 77 starch, with the least starch lipid content had the lowest % R, whereas L 996 starch with the highest starch-lipid content, had the highest % R. Our results showed that ΔH_R values are not influenced by the lipid content, since both NO 753-2 and AC Stewart starches with identical total lipid contents (Table 4.1) showed wide differences in their ΔH_R (Table 4.11). The differences in ΔH_R observed by Gudmundsson and Eliasson (1989) between native and defatted oat starches probably reflect structural changes on defatting (Vasanthan and Hoover, 1992), rather than lipid removal. Yuan *et al.* (1993) reported that the chain length and chain length distribution of amylopectin could influence the extent of retrogradation of maize starch by forming a mixture of crystallites of different sizes, with longer chain lengths forming longer double helices. Wang and White (1994) showed that in oat starch cultivars, a close relationship exists

Table 4.11 The enthalpy of retrogradation of native oat starch gels [1] .

Oat cultivar	Time (days)				
	16	20	24	28	32
	Enthalpy (J/g)				
NO 753-2	2.3[a]±0.05	2.6[a]±0.04	3.2[a]±0.02	5.0[a]±0.01	6.7[a]±0.02
AC Stewart	1.4[b]±0.02	1.8[b]±0.05	2.5[b]±0.04	3.5[b]±0.05	5.0[b]±0.05

[1] Values represent means ± standard deviation of triplicate analyses.

Mean values in each column not followed by the same superscript are significantly differe9nt ($p \leq 0.05$).

between the percentage retrogradation, weight average chain length and the degree of multiple branching. Since ΔH_R values reflect the melting of amylopectin crystallites formed (by association between adjacent double helices) during gel storage, the difference in the extent of retrogradation between the oat starches (NO 753-2 > AC Stewart), suggest that amylopectin chains of NO 753-2 are probably less branched and/or are of a longer chain length than those of AC Stewart.

4.11.3 X-ray diffraction

The X-ray intensity patterns of NO 753-2 starch gels stored for periods ranging from 2-20 days (at 25°C) are presented in Fig. 4.4. The X-ray intensities of the major peaks of the two oat starch gels stored for 6 and 10 days (at 25°C) are presented in Table 4.12.

The intensities of the peaks gradually increased (AC Stewart > NO 753-2) during storage (Fig. 4.4, Table 4.12). This suggests that more crystallites form during retrogradation in AC Stewart than in NO 753-2. This seems to contradict the data obtained by DSC (Table 4.11) which showed that NO 753-2 retrograded faster than AC Stewart. This is not surprising, since DSC selectively examines only the crystallization of amylopectin, whereas X-ray diffraction examines crystallization between amylose-amylose, amylose-amylopectin and amylopectin-amylopectin. Interaction between amylose chains can extend over many more residues (due to its linearity) than those

between amylopectin chains. Consequently, amylose crystallites would be thermally more stable than amylopectin crystallites. Thus, it would not be possible to monitor amylose crystallization by DSC, since pan failure is common at temperatures exceeding 140°C. The X-ray data suggests that the amylose chains of AC Stewart are probably longer (leading to the formation of more crystallites during storage) than those of NO 753-2. A comparison of the data obtained by X-ray (Table 4.12) and DSC (Table 4.11), suggests that crystallization between chains of amylose (AC Stewart > NO 753-2) are more extensive than between amylopectin chains (NO 753-2 > AC Stewart).

4.11.4 Gel firmness

The gel firmness of the starch pastes after storage at 25°C for periods ranging from 2-20 days are presented in Fig.4.5. The magnitude and the extent of increase in gel firmness during storage were more pronounced in AC Stewart. Gudmundsson and Eliasson (1989) and Takahashi and Seib (1988) have shown that starch lipids influence gel firmness. The former authors reported that among oat cultivars, the starch which gave the stiffest gel had the highest lipid content. Whereas, the latter authors

Fig.4. 4. X-ray diffraction patterns of retrograded NO 753-2 starch gels as a function of storage time (at 25°C)

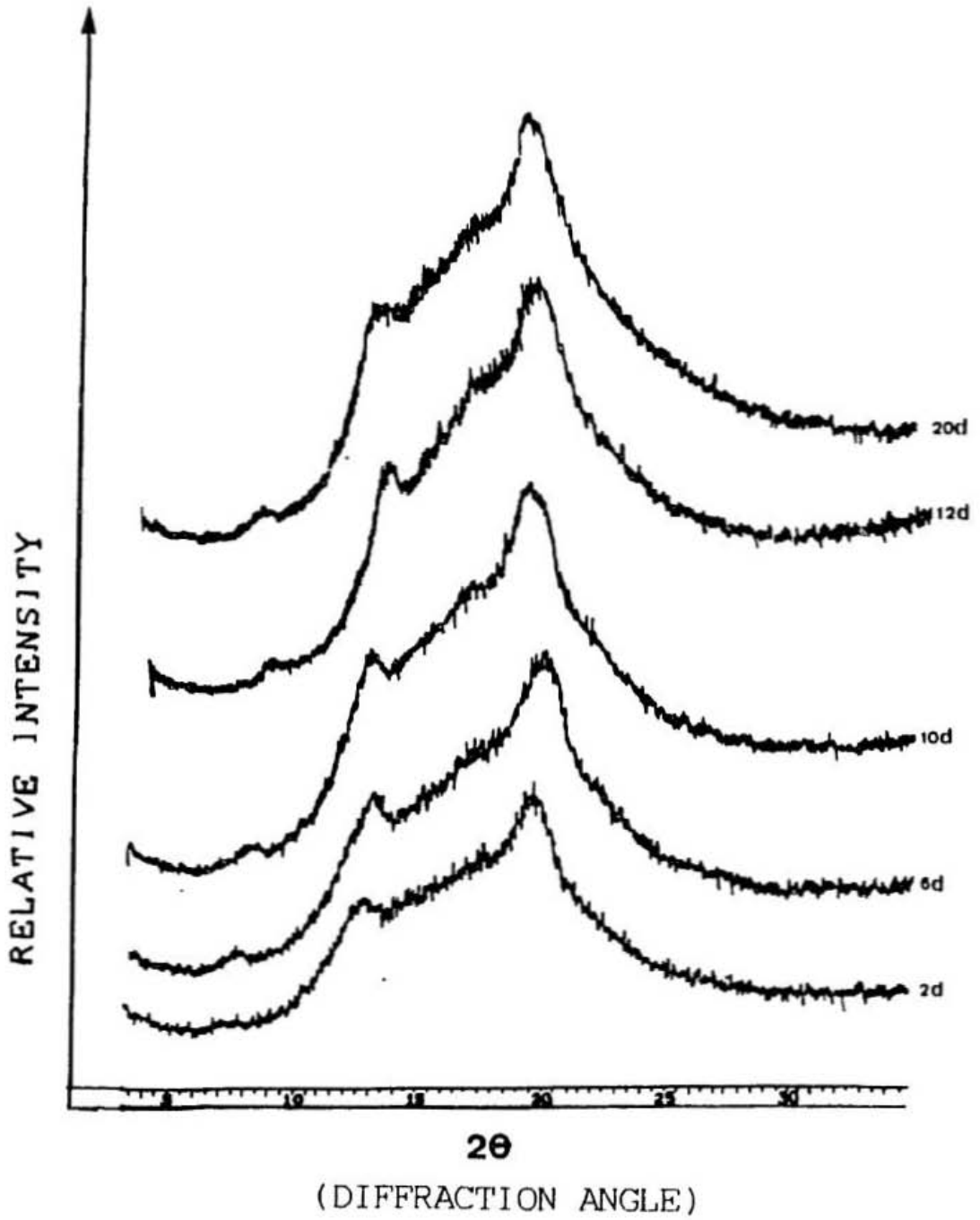

RELATIVE INTENSITY
20d
12d
10d
6d
2d
5
10
15
20
25
30
2θ
(DIFFRACTION ANGLE)

Table 4.12. X-ray diffraction spacings and intensities of the major peaks of retrograded oat starch gels [1].

Oat cultivar	Time of storage at 25°C	Interplanar spacings (d) in Angstroms (Å) with intensities (CPS)[2]			
NO 753-2	6	6.5 (1166^{b} ± 0.8)	5.0 (1724 ^{b} ± 0.5)	4.6 (1764 ^{b} ± 0.5)	4.4 (2597 ^{b} ± 0.8)
	10	6.6 (1226 ^{a} ± 0.7)	5.5 (1769 ^{a} ± 0.6)	5.2 (2206 ^{a} ± 0.4)	4.4 (2839^{a} ± 0.7)
AC Stewart	6	6.9 (608^{d} ± 0.9)	5.0 (734 ^{d} ± 0.7)	4.1 (1171 ^{d} ± 0.8)	4.3 (1880 ^{d} ± 0.8)
	10	6.5 (1092 ^{c} ± 1.0)	5.5 (1526 ^{c} ± 0.6)	5.2 (1692 ^{c} ± 0.7)	4.9 (1961^{c} ± 0.4)

[1] Values represent means ± standard deviation of triplicate analyses.

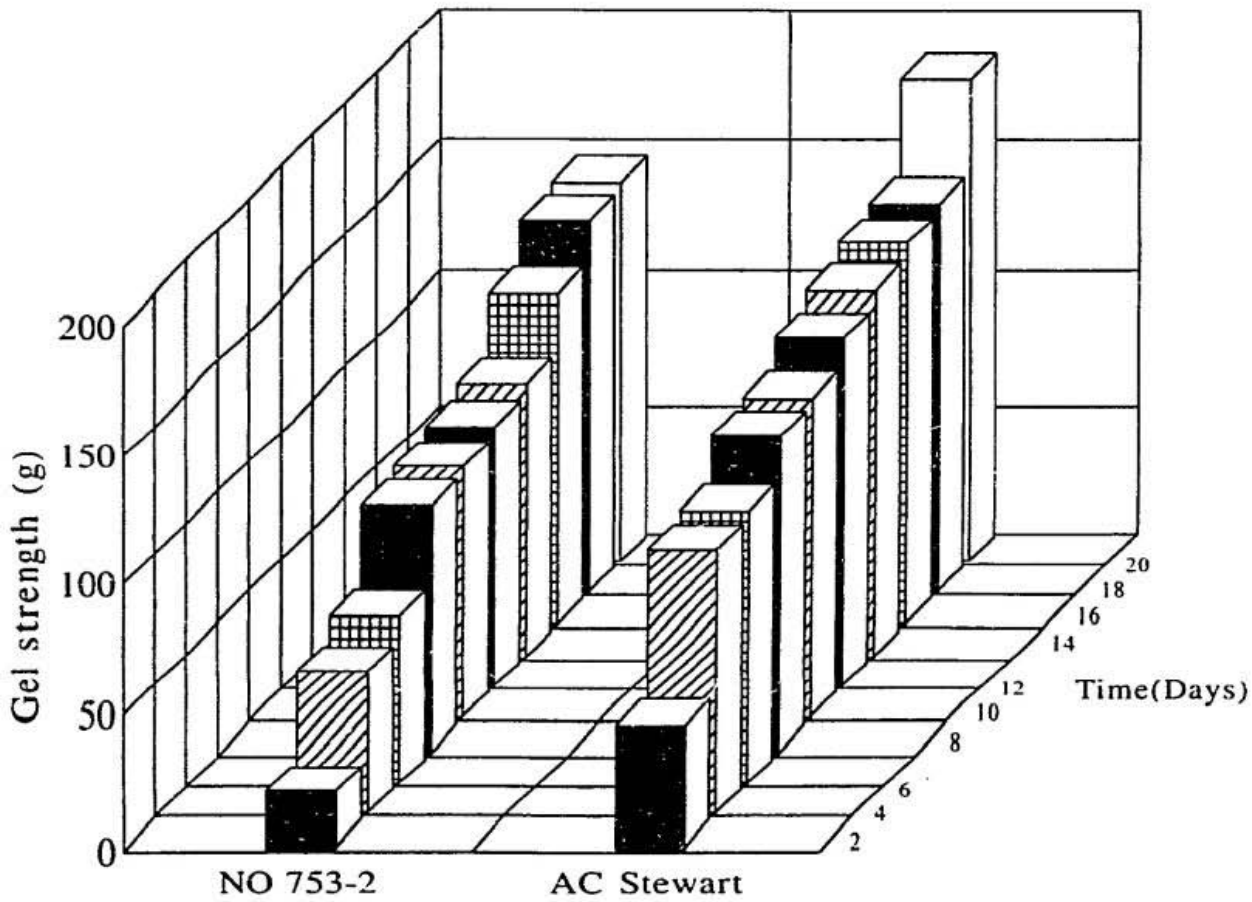
Mean values in each column not followed by the same superscript are significantly different ($p \leq 0.05$).

[2] Counts per second.

reported that starch lipids in native corn and wheat starches showed reduced gel strength (~50%) in comparison with defatted starches. This was attributed to a decrease in amylose concentration in the continuous phase (resulting from amylose-lipid interactions during gelatinization).

In the starches examined, the differences in total lipid content (Table 4.1) and amylose leaching (Table 4.4) were only marginal. Therefore, the difference in gel firmness (Fig. 4.5) is probably a reflection of the extent of amylose crystallization (AC Stewart > NO 753-2) in the continuous phase.

Fig. 4.5 Gel strength of oat starches as a function of storage time (at 25°C). The data are presented as means of three determinations.


Gel strength (g)
200
150
100
50
0
NO 753-2
AC Stewart
2
4
6
8
10
12
14
16
18
20
Time(Days)

CONCLUSIONS

This study has demonstrated that starches from two new oat varieties, NO 753-2 and AC Stewart, differ widely in their composition and physicochemical properties. AC Stewart had a higher swelling factor than that of NO 753-2. The X-ray spectra of both oat starches examined had A-type pattern representative of cereal starches. However, intensities of major diffraction peaks were higher in NO 753-2 than in AC Stewart. The pasting temperature and shear resistance of NO 753-2 were higher than those of AC Stewart. The transition temperatures and the gelatinization enthalpy of AC Stewart were lower than those of NO 753-2. The AC Stewart had a higher clarity of starch paste than NO 753-2.

The susceptibility of AC Stewart towards hydrolysis by α-amylase and acid was higher than that of NO 753-2. Thus, the present results show that the amorphous and crystalline regions of NO 753-2 are ordered to a much greater extent than AC Stewart. The extent of retrogradation during storage was more pronounced in AC Stewart than in NO 753-2. These differences are probably influenced by existing differences in the magnitude of interaction between and among starch chains within the amorphous and crystalline regions of the native granule, and by the chain lengths of amylose and amylopectin. However, further studies are needed to characterize the structure and properties of starch components fractionated from these two starches in order to

obtain a more precise insight into their granular structures. An understanding of the variation in physicochemical properties among oat starches would form the basis for future investigations on chemical modifications to improve functionality of oat starches.

REFERENCES

AACC (1983). *Approved Methods of the AACC.* (8th edition). American Association of Cereal Chemists. St. Paul, MN, 08-18, 41-60, 46-50.

Atwell, W.A., Hood, L.F., Lineback, D.R., Varriano Marston, E., and Zobel, H.F. (1988). The terminology and methodology associated with basic starch phenomena. *Cereal Foods World*, **33**, 306-11.

Autio, K. (1990). Rheological and microstructural changes of oat and barley starches during heating and cooling. *Food Structure*, **9**, 297-304.

Badenhuizen, N.P.(1965). Occurrence and development of starch in plants. In *Starch: Chemistry and Technology*. (eds. Whistler, R.L. and Paschall, E.F.) Vol.1. Academic Press, NY. pp. 65-103.

Banks, W., Greenwood, C.T. and Khan, K.M. (1970). Studies on starch degrading enzymes. Part XII. The initial stages of the action on amylose of the α-amylases from *B. subtilis*, human saliva, malted rye and porcine pancreas. *Carbohydr. Res.*, **12**, 79-87.

Banks, W. and Greenwood, C.T. (1975). The structure and biosynthesis of the starch granule. In *Starch and its Components.* (eds. Banks, W. and Greenwood, C.T.). Edinburgh University Press. pp. 242-73.

Batres, L.V. and White, P.J. (1986). Interaction of amylopectin with monoglycerides in model systems. *J. Am. Oil Chem. Soc.*, **63**, 1537-40.

Bean, M.M. and Yamazaki, W.T. (1978a). Wheat starch gelatinization in sugar solutions. I. Sucrose: microscopy and viscosity effects. *Cereal Chem.*, **55**, 936-44.

Bean, M.M. and Yamazaki, W.T. (1978b). Wheat starch gelatinization in sugar solutions. II. Fructose, glucose, and sucrose: cake performance. *Cereal Chem.*, **55**, 945-52.

Bello-Pérez, L. A. and Paredes-Lopez, O. (1995). Effects of solutes on retrogradation of stored starches and amylopectins: A calorimetric study. *Starch/Stärke*, **47**, 83-6.

Biliaderis, C.G., Page, C.M., Slade, L. and Sirett, R.R. (1985). Thermal behaviour of amylose-lipid complexes. *Carbohydr. Polymers*, **5**, 367-89.

Biliaderis, C.G., Page, C.M., Maurice, T.J., and Juliano, B.O. (1986). Thermal characterization of rice starches: a polymeric approach to phase transitions of granular starch. *J. Agric. Food Chem.*, **34**, 6-14.

Biliaderis, C.G. and Zawistowski, J. (1990). Viscoelastic behavior of aging starch gels: Effects of concentration, temperature, and starch hydrolysates on network properties. *Cereal Chem.*, **67**, 240-46.

Biliaderis, C.G. (1991). The structure and interactions of starch with food constituents. *Can. J. Physiol. Pharmacol.*, **69**, 60-78.

Birnbaum, H. (1977). Interactions of surfactants in bread making. *Baker's Dig.*, **51**, 17-24.

Björck, I., Asp, N.-G., Birkhed, D. and Lundquist, I. (1984). Effects of processing on availability of starch for digestion *in vitro* and *in vivo*. I. Extrusion cooking of wheat flours and starch. *J. Cereal Sci.*, **2**, 91-103.

Björck, I., Gunnarsson, A. and Ostergard, K. (1989). A study of native and chemically modified potato starch. Part II. Digestibility in the rat intestinal tract. *Starch/Stärke*, **41**, 128-34.

Blanshard, J.M.V. (1978). Physicochemical aspects of starch gelatinization. In *Polysaccharides in food*. (eds. Blanshard, J.M.V. and Mitchell, J.R.). Butterworths, London. pp.139-52.

Bligh, E.G. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.*, **37**, 911-7.

Bonne, O.T. (1961). Morphology and development. In *Oats and Oat Improvement*, (ed. Coffman, F.A.). American Society of Agronomy, Madison, WI. pp. 41-74.

Bornet, F. (1993). Technological treatments of cereals. Repercussions on the physiological properties of starch. *Carbohydr. Polymers*, **21**, 195-203.

Botlan, D. Le and Desbois, P. (1995). Starch retrogradation study in the presence of sucrose by low-resolution Nuclear Magnetic Resonance. *Cereal Chem.*, **72**, 191-93.

Braga, D., Ferracini, E., Ferrero, A., Ripamonti, A., Brant, D.A., Bulga, G.S. and Cesaro, A. (1985). Amylose conformation in aqueous solution: a small angle X-ray scattering study. *Int. J. Biol. Macromol.*, **7**, 161-65.

Bruner, R.L. (1964). Determination of reducing value. In *Methods in Carbohydrate Chemistry*, Vol. 4 (ed. Whistler, R.L.) Academic Press, NY. pp. 67-71.

Burrows, V.D. (1992). AC Stewart oat. *Can. J. Plant Sci.*, **72**, 447-49.

Burt, D.J. and Russell, P.L. (1983). Gelatinization of low water content wheat starch-water mixtures. A combined study by differential scanning calorimetry and light microscopy. *Starch/Stärke*, **35**, 354-60.

Camire, M.E. and Camire, A.L. (1994). Enzymatic starch hydrolysis of extruded potato peels. *Starch/Stärke*, **46**, 308-11.

Camire, M.E., Camire, A. and Krumhar, K. (1990). Chemical and nutritional changes in foods during extrusion. *CRC Crit. Rev. Food Sci. Nutr.*, **29**, 35-55.

Cameron, R.E., Durrani, C.M., and Donald, A.M. (1994). Gelation of amylopectin without long range order. *Starch/Stärke*, **46**, 285-7.

Cameron, R.E. and Donald, A.M. (1992). A small-angle x-ray scattering study of the annealing and gelatinization of starch. *Polymer*, **33**, 2628-35.

Carlson, T.L.-G., Larsson, K., Dinh-Nguyen, N. and Krog, N. (1979). A study of the amylose-monoglyceride complex by Raman spectroscopy. *Starch/Stärke*, **31**, 222-4.

Chandrashekar, A. and Kirleis, A.W. (1988). Influence of protein on starch gelatinization in sorghum. *Cereal Chem.*, **65**, 457-62.

Chungcharoen, A. and Lund, D.B. (1987). Influence of solutes and water on rice starch gelatinization. *Cereal Chem.*, **64**, 240-3.

Chrastil, J. (1987). Improved calorimetric determination of amylose in starches or flours. *Carbohydr. Res.*, **159**, 154-8.

Ciacco, C.F. and Fernandes, J.L.A. (1979). Effect of various ions on the kinetics of retrogradation of concentrated wheat starch gels. *Starch/Stärke*, **31**, 51-5.

Cluskey, J.E., Wu, Y.V., Wall, J.S. and Inglett, G.E. (1973). Oat protein concentrates from a wet-milling process: Preparation. *Cereal Chem.*, **50**, 475-81.

Collison, R. and Chilton, W.G. (1974). Starch gelation as a function of water content. *J. Food Technol.*, **9**, 309-15.

Colwell, K.H., Axford, D.W.E., Chamberlain, N. and Elton, G.A.H. (1969). Effect of storage temperature on the ageing of concentrated wheat starch gels. *J. Sci. Food Agric.*, **20**, 550-5.

Coma, V., Copinet, A., Couturier, Y. and Prudhomme, J. (1995). Biofragmentation of acetylated starch by the α-amylase of *Aspergillus oryzae*. *Starch/Stärke*, **47**, 100-7.

Craig, S.A.S., Maningat, C.C., Seib, P.A. and Hoseney, R.C. (1989). Starch paste clarity. *Cereal Chem.*, **66**, 173-82.

Curà, J.A. and Krisman, C.R. (1990). Cereal grains: a study of their α-1,4-α-1,6 glucopolysaccharide composition. *Starch/Stärke*, **42**, 171-5.

D'Appolonia, B.L. (1972). Effect of bread ingredients on starch-gelatinization properties as measured by the amylograph. *Cereal Chem.*, **49**, 532-43.

Derby, R.I., Miller, B.S., Miller, B.F. and Trimbo, H.B. (1975). Visual observations of wheat-starch gelatinization in limited water systems. *Cereal Chem.*, **52**, 702-13.

Donovan, J.W. (1979). Phase transitions of the starch-water system. *Biopolymers*, **18**, 263-75.

Doublier, J.L. (1981). Rheological studies on starch -Flow behaviour of wheat starch pastes. *Starch/Stärke*, **33**, 415-20.

Doublier, J.L., Llamas, G.L. and LeMeur, M. (1987a). A rheological investigation of cereal starch pastes and gels. Effect of pasting procedure. *Carbohydr. Polymers*, **7**, 251-75.

Doublier, J.L., Paton, D. and Llamas, G. (1987b). A rheological investigation of oat starch pastes. *Cereal Chem.*, **64**, 21-6.

Dreher, M.L., Dreher, C.J. and Berry, J.W. (1984). Starch digestibility of foods: a nutritional perspective. *CRC Crit. Rev. Food Sci. Nutr.*, **20**, 47-71.

Eberstein, K., Höpcke, R., Koniecyny-Janda, G. and Stute, R. (1980). DSC-Untersuchungen an Stärken. I. Möglichkeiten thermoanalytischer Methoden zur Stärke-charakterisierung. *Starch/Stärke*, **12**, 397-404.

Eerlingen, R.C., Van Den Broeck, I., Delcour, J.A., Slade, L. and Levine, H. (1994). Enzyme-resistant starch. VI. Influence of sugars on resistant starch formation. *Cereal Chem.*, **71**, 472-6.

Eliasson, A.-C. (1980). Effect of water content on the gelatinization of wheat starch. *Starch/Stärke*, **32**, 270-2.

Eliasson, A.-C. (1983). Differential scanning calorimetry studies on wheat starch-gluten mixtures. II. Effects of gluten and sodium stearoyl lactylate on starch crystallization during ageing of wheat starch gels. *J. Cereal Sci.*, **1**, 207-13.

Eliasson, A.-C. (1985). Retrogradation of starch as measured by differential scanning calorimetry. In *New Approaches to Research on Cereal Carbohydrates.* (ed. Hill, R.D. and Munck, L.). Elsevier Science, Inc., Amsterdam. pp. 93-98.

Eliasson, A.-C. (1986a). Viscoelastic behaviour during the gelatinization of starch. *J. Texture Stud.*, **17**, 253-65.

Eliasson, A.-C. (1986b). On the effects of surface active agents on the gelatinization of starch -a calorimetric investigation. *Carbohydr. Polymers*, **6**, 463-76.

Eliasson, A.-C. and Krog, N. (1985). Physical properties of amylose-monoglyceride complexes. *J. Cereal Sci.*, **3**, 239-48.

Eliasson, A.-C. and Ljunger, G. (1988). Interactions between amylopectin and lipid additives during retrogradation in a model system. *J. Sci. Food Agric.*, **44**, 353-61.

Eliasson, A. -C. (1988). On the thermal transitions of the amylose-cetyltrimethylammonium bromide complex. *Carbohydr. Res.*, **172**, 83-95.

Ellis, H.S. and Ring, S.G. (1985). A study of some factors influencing amylose gelation. *Carbohydr. Polymers*, **5**, 201-13.

Evans, I.D. (1986). An investigation of starch/surfactant interactions using viscometry and differential scanning calorimetry. *Starch/Stärke*, **38**, 227-35.

Franco, C.M.L., Preto, S.J. doR, Ciacco, C.F. and Tavares, D.Q. (1988). Studies on the susceptibility of granular cassava and corn starches to enzymatic attack. Part 2. Study of the granular structure of starch. *Starch/Stärke*, **40**, 29-32.

Freeman, J.E. and Verr, W.J. (1972). A rapid procedure for measuring starch paste development and its application to corn and sorghum starches. *Cereal Sci. Today*, **17**, 46-53.

French, D.(1972). Fine structure of starch and its relationship to the organization of the granules. *J. Jpn. Soc. Starch Sci.*, **19**, 8-33.

French, D. (1975). Chemistry and biochemistry of starch. In *Biochemistry of Carbohydrates*. Vol. 5, (ed. Whelan, W.J.). Butterworth and Co. Ltd., London. pp. 267-335.

French, A. D. and Murphy, V.G. (1977). Computer modelling in the study of starch. *Cereal Foods World*. **22**, 61-70.

French, D. (1984). Organisation of starch granules. In *Starch: Chemistry and Technology (II)*. (eds. Whistler, D.M., BeMiller, J.N., and Paschall, E.F.) Academic Press, NY. pp. 188-200.

Fulcher, R.G. (1986). Morphological and chemical organization of the oat kernel. In *Oats: Chemistry and Technology*. (ed. Webster, F.H.). AACC, St. Paul, MN. pp. 47-74.

Galliard, T. and Bowler, P. (1987). Morphology and composition of starch. In *Starch: properties and potential*. (ed. Galliard, T.). John Wiley and Sons, NY. pp. 55-78.

Germani, R., Ciacco, C.F., and Rodriguez-Amaya, D.B. (1983). Effect of sugars, lipids, and type of starch on the mode and kinetics of retrogradation of concentrated corn starch gels. *Starch/Stärke*, **35**, 377-81.

Ghiasi, K., Hoseney, R.C. and Varriano-Marston, E. (1982). Gelatinization of wheat starch. IV. Amylograph viscosity. *Cereal Chem.*, **59**, 262-5.

Gibinski, M., Palasinski, M. and Tomasik, P. (1993). Physicochemical properties of defatted oat starch. *Starch/Stärke*, **45**, 354-7.

Gidley, M.J. (1987). Factors affecting the crystalline type (A-C) of native starches and model compounds: a rationalization of observed effects in terms of polymorphic structures. *Carbohydr. Res.*, **161**, 301-4.

Gidley, M.J. and Bulpin, P.V. (1987). Crystallization of malto-oligosaccharides as models of the crystalline forms of starch: minimum chain-length requirement for the formation of double helices. *Carbohydr. Res.*, **161**, 291-300.

Gorinstein, S. (1993). Kinetic studies during enzyme hydrolysis of potato and cassava starches. *Starch/Stärke*, **45**, 91-95.

Goshima, G., Abe, M., Sato, N., Ohashi, K. and Tsuge, H. (1985). Amylographic reproducibility of defatted potato starch by the reintroduction of lipid. *Starch/Stärke*, **37**, 10-14.

Gough, B.M., Greenwell, P. and Russell, P.L. (1985). On the interaction of sodium dodecyl sulphate with starch granules. In *New Approaches to Research on Cereal Carbohydrates*. (eds. Hill, R.D. and Munck, L.). Elsevier Science, Inc., Amsterdam. pp. 99-105.

Gracza, R. (1965). Minor constituents of starch. In *Starch : Chemistry and Technology, Vol.I. Fundamental Aspects* (eds. R. L. Whistler and Paschall, E.F.). Academic Press, NY, pp. 105-31.

Gray, V.M. and Schoch, T.J.(1962). Effect of surfactants and fatty adjuncts on the swelling and solubilization of granular starches. *Starch/Stärke*, **14**, 239-46.

Greenwood, C.T. and Milne, E.A. (1968a). Starch degrading and synthesizing enzymes: A discussion of their properties and action pattern. *Adv. Carbohydr. Chem.*, **23**, 281-366.

Greenwood, C.T. and Milne, E.A. (1968b). Studies on starch degrading enzymes. Part VII. Properties and action pattern of the α-amylases from barley, oats, rye and wheat. *Starch/Stärke*, **20**, 101-7.

Gudmundsson, M. and Eliasson, A.-C. (1989). Some physico-chemical properties of oat starches extracted from varieties with different oil content. *Acta Agric. Scand.*, **39**, 101-11.

Gudmundsson, M. and Eliasson, A.-C. (1991). Thermal and viscous properties of rye starch extracted from different varieties. *Cereal Chem.*, **68**, 172-7.

Gudmundsson, M. and Eliasson, A.-C. (1992). Comparison of thermal and viscoelastic properties of four waxy starches and the effect of added surfactant. *Starch/Stärke*, **44**, 379-85.

Hahn, D.E. and Hood, L.F. (1987). Factors influencing corn starch-lipid complexing. *Cereal Chem.*, **64**, 81-5.

Hansen, L.M., Sester, C.S., and Paukstelis, J.V. (1989). Investigation of sugar-starch interactions using carbon-13 nuclear magnetic resonance. I. Sucrose. *Cereal Chem.*, **66**, 411-15.

Hase, S., Kawamura, U. and Yasui, T. (1981). Effects of sugars and sugar alcohols on the gelatinization and retrogradation of starch. *Rept. Natl. Food Res. Inst.*(Tsukuba, Japan), **38**, 73-84.

Hashim, D.B.M., Moorthy, S.N., Mitchell, J.R., Hill, S.E., Linfoot, K.J. and Blanshard, J.M.V. (1992). The effect of low levels of antioxidants on the swelling and solubility of cassava starch. *Starch/Stärke*, **44**, 471-5.

Hellman, N.N., Fairchild, B. and Senti, F.R. (1954). The bread staling problem. Molecular organization of starch upon aging of concentrated starch gels at various moisture levels. *Cereal Chem.*, **31**, 495-505.

Hill, R.D. and MacGregor, A.W. (1987). Cereal α-amylases in grain research and technology. In *Advances in Cereal Science and Technology*. Vol. IX. (ed. Pomeranz, Y.). AACC, St. Paul, MN. pp. 217-61.

Hizukuri, S., Kaneko, T. and Takeda, Y. (1983). Measurement of the chain length of amylopectin and its relevance to the origin of crystalline polymorphism of starch granules. *Biochim. Biophys. Acta*, **760**, 188-91.

Hizukuri, S., Takeda, Y., and Yasuda, M. (1981). Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydr. Res.*, **94**, 205-13.

Hizukuri, S. (1985). Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydr. Res.*, **141**, 295-306.

Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydr. Res.*, **147**, 342-47.

Holm, J., Björck, I. and Eliasson, A.-C. (1988). Effects of thermal processing of wheat on starch. I. Physico-chemical and functional properties. *J. Cereal Sci.*, **8**, 249-60.

Holm, J., Björck, I, Ostrowska, S., Eliasson, A.C., Asp, N.G., Larrson, K. and Lundquist, I. (1983). Digestibility of amylose-lipid complexes *in vitro* and *in vivo*. *Starch/Stärke*, **35**, 294-7.

Holm, J. and Björck, I. (1988). Effect of thermal processing of wheat on starch. II. Enzymic availability. *J. Cereal Sci.*, **8**, 261-8.

Hoover, R. (1995). Starch retrogradation. *Food Reviews International*, **11**, 331-46.

Hoover, R. and Vasanthan, T. (1992). Studies on isolation and characterization of starch from oat (*Avena nuda*) grains. *Carbohydr. Polymers*, **19**, 285-97.

Hoover, R., Swamidas, G. and Vasanthan, T. (1993). Studies on the physicochemical properties of native, defatted and heat-moisture treated pigeon pea (*Cajanus cajan L*) starch. *Carbohydr. Res.*, **246**, 185-203.

Hoover, R. and Vasanthan, T. (1994). Effect of heat-moisture treatment on the structure and physicochemical properties of cereal, legume, and tuber starches. *Carbohydr. Res.*, **252**, 33-53.

Hoover, R. and Vasanthan, T. (1994). The effect of annealing on the physicochemical properties of wheat, oat, potato and lentil starches. *J. Food Biochem.*, **17**, 303-25.

Hoover, R., and Hadziyev, D. (1981). Characterization of potato starch and its monoglyceride complexes. *Starch/Stärke*, **33**, 290-300.

Hoover, R. and Sosulski, F.W. (1985). Studies on the functional characteristics and digestibility of starches from *Phaseolus vulgaris* biotypes. *Starch/Stärke*, **37**, 181-91.

Hoseney, R.C., Atwell, W.A., and Lineback, D.R. (1977). Scanning electron microscopy of starch isolated from baked products. *Cereal Foods world*, **22**, 56-60.

Huang, R., Chang, W., Chang, Y. and Lii, C. (1994). Phase transitions of rice starch and flour gels. *Cereal Chem.*, **71**, 202-7.

I'anson, K.J., Miles, M.J., Morris, V.J., Besford, L.S., Jarvis, D.A. and Marsh, R.A. (1990). The effects of added sugars on the retrogradation of wheat starch gels. *J. Cereal Sci.*, **11**. 243-8.

Imberty, A., Chanzy, H., Perez, S., Buleon, A. and Tran, V. (1987). Three-dimensional structure analysis of the crystalline moiety of A-starch. *Food Hydrocolloids*, **1**, 455-9.

Imberty, A., Buléon, A., Tran, V. and Pérez, S. (1991). Recent advances in knowledge of starch structure. *Starch/Stärke*, **43**, 375-84.

Jenkins, P.J., Cameron, R.E., and Donald, A.M. (1993). A universal feature in the structure of starch granules from different botanical sources. *Starch/Stärke*, **45**, 417-20.

Johnson, J.M., Davis, E.A. and Gordon, J. (1990a). Lipid binding of modified corn starches by electron spin resonance. *Cereal Chem.*, **67**, 236-40.

Johnson, J.M., Davis, E.A. and Gordon, J. (1990b). Interactions of starch and sugar water measured by electron spin resonance and differential scanning calorimetry. *Cereal Chem.*, **67**, 286-91.

Kainuma, K. and French, D. (1971). Nageli amylodextrin and its relationship to starch granule structure I. Preparation and properties of amylodextrins from various starch types. *Biopolymers*, **10**, 1673-7.

Kalichevsky, M.T., Orford, P.D. and Ring, S.G. (1990). The retrogradation and gelation of amylopectins from various botanical sources. *Carbohydr. Res.*, **198**, 49-55.

Karow, R.S., Forsberg, R.A. and Peterson, D.M. (1984). A comparison of two rapid oat-lipid extraction procedures in terms of fatty-acid profile. *Cereal Chem*, **61**, 196-7.

Katsuta, K., Miura, M. and Nishimura, A. (1992a). Kinetic treatment for rheological properties and effects of saccharides on retrogradation of rice starch gels. *Food Hydrocolloids*, **6**, 187-98.

Katsuta, K., Nishimura, A. and Miura, M. (1992b). Effects of saccharides on stabilities of rice starch gels. 1. Mono- and disaccharides. *Food Hydrocolloids*, **6**, 387-98.

Katsuta, K., Nishimura, A. and Miura, M. (1992c). Effects of saccharides on stabilities of rice starch gels. 2. Oligosaccharides. *Food Hydrocolloids*, **6**, 399-408.

Katz, J.R. and Van Itallie, T.B. (1930). Abhandlungen zuv physikalischen chemie der stärke und eler brotbereitung. V. Alle starkearten haben das gleiche. Retrogradations spektrum. *Z. Phys. Chem.*, **150**, 90-100.

Knutson, C.A., Khoo, U., Cluskey, J.E. and Inglett, G.E. (1982). Variation in enzyme digestibility and gelatinization behavior of corn starch granule fractions. *Cereal Chem.*, **59**, 512-5.

Koepsel, K.M. and Hoseney, R.C. (1980). Effects of corn syrups in layer cakes. *Cereal Chem.*, **57**, 49-53.

Konik, C.M., Miskelly, D.M. and Gras, P.W. (1993). Starch swelling power, grain hardness and protein: Relationship to sensory properties of japanese noodles. *Starch/Stärke*, **45**, 136-39.

Kowblansky, M. (1985). Calorimetric investigation of inclusion complexes of amylose with long-chain aliphatic compounds containing different functional groups. *Macromolecules*, **18**, 1776-79.

Krog, N. and Jensen, N. B. (1970). Interaction of monoglycerides in different physical states with amylose and their anti-firming effects in bread. *J. Food Technol.*, **5**, 77-87.

Krog, N. (1971). Amylose complexing effect of food grade emulsifiers. *Starch/Stärke*, **23**, 206-10.

Krog, N. and Lauridsen, J.B. (1976). Food emulsifiers and their associations with water. In *Food Emulsions*. (ed. Friberg, S.). Marcel Dekker, Inc., NY. pp. 67-135.

Krog, N., Olesen, S.K., Toernaes, H. and Joensson, T. (1989). Retrogradation of the starch fraction in wheat bread. *Cereal Foods World*, **34**, 281-5.

Krüsi, Von H. and Neukom, H. (1984). Untersuch unger über die retrogradation der stärke in konzentrierten weizestärkegelen. Teil. I. Herstellung konzentrierter gele, einflub der stärke koncentration and herstellungsbedingungen auf die retrogradation der stärke. *Starch/Stärke*, **36**, 40-5.

Kugimiya, M., Donovan, J.W. and Wong, R.Y. (1980). Phase transitions of amylose-lipid complexes in starches: a calorimetric study. *Starch/Stärke*, **32**, 265-70.

Kulp, K. and Lorenz, K. (1981). Heat-moisture treatment of starches. 1. Physicochemical properties. *Cereal Chem.*, **58**, 46-8.

Kulp, K. and Ponte, Jr., J.G. (1981). Staling of white pan bread: fundamental causes. *Crit. Rev. Food Sci. Nutr.*, **15**, 1-48.

Lagendijk, J. and Pennings, H.J. (1970). Relation between complex formation of starch with monoglycerides and the firmness of bread. *Cereal Sci. Today*, **15**, 354-70.

Larsson, K. and Miezis, Y. (1979). On the possibility of dietary fiber formation by interaction in the intestine between starch and lipids. *Starch/Stärke*, **31**, 301-2.

Launay, B., Doublier, J.L. and Cuvelier, G.(1986). Flow properties of aqueous solutions and dispersions of polysaccharides. In *Functional properties of food macromolecules*. (eds. Mitchell, J.R., and Ledward, D.A.), Elsevier Applied Sci. Publ., NY. pp. 56-78.

Lauro, M., Suortti, T., Autio, K., Linko, P. and Poutanen, K. (1993). Accessibility of barley starch granules to α-amylase during different phases of gelatinization. *J. Cereal Sci.*,**17**, 125-36.

Leach, H.W., McCowen, L.D. and Schoch, T.J. (1959). Structure of the starch granule. 1. Swelling and solubility patterns of various starches. *Cereal Chem.*, **36**, 534-44.

Lee, S.-J., Yoo, S.H., Kim, M.J., Kim, J.W., Seok, H.M. and Park, K.H. (1995). Production and characterization of branched oligosaccharides from liquefied starch by the action of *B. licheniformis* amylase starch. *Starch/Stärke*, **47**, 127-34.

Leegwater, D.C., Zeist, and Luten, J.B. (1971). A study on the in vitro digestibility of hydroxypropyl starches by pancreatin. *Starch/Stärke*, **23**, 430-2.

Leloup, V. M., Colonna, P., Ring, S.G., Roberts, K. and Wells, B. (1992). Microstructure of amylose gels. *Carbohydr. Polymers*, **18**, 189-97.

Lineback, D.R. (1984). The starch granule organization and properties. *Baker's Dig.*, **58**, 16-8.

Lineback, D.R. and Rasper, V.F. (1988). Wheat carbohydrates. In *Wheat: Chemistry and Technology*. (ed. Pomeranz, Y.). AACC, St. Paul, MN. pp. 277-372.

Liukkonen, K. and Laakso, S. (1992). Characterization of internal and surface lipids of oat starches from two isolation processes. *Starch/Stärke*, **44**, 128-32.

Liukkonen, K.H., Montfoort, A., Laakso, S.V. (1992). Water-induced lipid changes in oat processing. *J. Agric. Food Chem.*, **40**, 126-30.

Longton, J. and LeGrys, G.A. (1981). Differential scanning calorimetry studies on the crystallization of ageing wheat starch gels. *Starch/Stärke*, **33**, 410-4.

Lund, D.(1984). Influence of time, temperature, moisture, ingredients, and processing conditions on starch gelatinization. *CRC Crit. Rev. Food Sci. Nutr.*, **20**, 249-72.

MacArthur, L.A. and D'Appolonia, B.L. (1979). Comparison of oat and wheat carbohydrates. II. Starch. *Cereal Chem.*, **56**, 458-61.

MacMasters, M.M., Wolf, M.J. and Seckinger, H.L. (1947). The possible use of oat and other cereal grains for starch production. *Am. Miller Process*, **75**, 82-83.

Mäkelä, M.J. and Laakso, S. (1984). Studies on oat starch with celloscope: granule size and distribution. *Starch/Stärke*, **36**, 159-63.

Manley, R.S.J. (1946). Chain folding in amylose crystals. *J. Polym. Sci.*, **2**, 4503-6.

Manners, D.J. (1962). Enzymic synthesis and degdradation of starch and glycogen. *Adv. Carbohydr. Chem.*, **17**, 371-430.

Manners, D.J. (1974). Some aspects of the enzymic degradation of starch. In *Plant Carbohydrate Chemistry*. (ed. Pridham, J.B.). Academic Press, NY. pp. 109-25.

Manners, D.J. and Matheson, N.K. (1981). The fine structure of amylopectin. *Carbohydr. Res.*, **90**, 99-110.

Manners, D.J. (1985a). Some aspects of the structure of starch. *Cereal Foods World*, **30**, 461-7.

Manners, D.J. (1985b). Some aspects of the metabolism of starch. *Cereal Foods World*, **30**, 722-7.

Manners, D.J. (1985c). Starch. In *Biochemistry of Storage Carbohydrates in Green Plants*. (eds. Dey, P.M. and Dixon, R.A.). Academic Press, NY. pp. 149-203.

Manners, D.J. (1989). Recent developments in our understanding of amylopectin structure. *Carbohydr. Polymers*, **11**, 87-112.

Marchant, J.L. and Blanshard, J.M.V. (1978). Studies of the dynamics of the gelatinization of starch granules employing a small angle light scattering system, *Starch/Stärke*, **30**, 257-64.

Marsden, W.L. and Gray, P.P. (1986). Enzymatic hydrolysis of cellulose in lignocellulosic materials. *CRC Crit. Rev. Biotechnol.*, **3**, 235-76.

Maurice, T.J., Slade, L., Page, C.M. and Sirett, R.R. (1985). Polysaccharide-water interactions-Thermal behavior of rice starch. In *Properties of Water in Foods*, (eds. Simatos, D. and Multon, J.L.). Martinus Nijhoff, Dordrecht, The Netherlands. pp. 211-27.

Maxwell, J.L. and Zobel, H.F. (1978). Model studies on cake staling. *Cereal Foods World*, **23**, 124-8.

McBurney, M.I. (1991). Passage of starch into the colon of humans: quantitation and implications. *Can. J. Physiol. Pharmacol.*, **69**, 130-36.

McMullen, M.S. (1991). Oats. In *Handbook of Cereal Science and Technology*. (eds. Lorenz, K.J. and Kulp. K.) Marcel Dekker, Inc. NY. pp. 199-232.

Medcalf, D. G. and Gilles, K. A. (1965). Wheat starches. 1. Comparison of physicochemical properties. *Cereal Chem.*, **42**, 558-68.

Meyer, K.H. and Bernfeld, P. (1940). Recherches sur l'amidon. V.L'Amylopectine. *Helv. Chim. Acta*, **23**, 875-85.

Mikus, F.F., Hixon, R.M. and Rundle, R.E. (1946). The complex of fatty acids with amylose. *J. Am. Chem. Soc*, **68**, 1115-23.

Miles, M.J., Morris, V.J., and Ring, S.G. (1984). Some recent observations on the retrogradation of amylose. *Carbohydr. Polymers*, **4**, 73-7.

Miles, M.J., Morris, V.J., Orford, P.D., and Ring, S.D. (1985a). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydr. Res.*, **135**, 271-81.

Miles, M.J., Morris, V.J., and Ring, S.G. (1985b). Gelation of amylose. *Carbohydr. Res.*, **135**, 257-69.

Minitab, Inc. (1991). Minitab computer supplement: Statistics for business and economics. 5th. edition. Minitab, Inc., State College, PA. pp. 299-319.

Miura, M., Nishimura, A. and Katsuta, K. (1992). Influence of addition of polyols and food emulsifiers on the retrogradation rate of starch. *Food Structure*, **11**, 225-36.

Morris, V.J. (1990). Starch gelation and retrogradation. *Trends in Food Science and Technology*, **July**, 2-6.

Morrison, W.R., Mann, D.L., Wong, S. and Conventry, A.M. (1975). Selective extraction and quantitative analysis of non-starch and starch lipids from wheat flour. *J. Sci. Food Agric.*, **26**, 507-21.

Morrison, W.R. (1981). Starch lipids: a reappraisal. *Starch/Stärke*, **33**, 408-10.

Morrison, W.R., Milligan, T.P. and Azudin, M.N. (1984). A relationship between the amylose and lipid contents of starches from diploid cereals. *J. Cereal Sci.*, **2**, 257-71.

Morrison, W.R. (1988). Lipids in cereal starches: a review. *J. Cereal Sci.*, **8**, 1-15.

Muhrbeck, P. and Eliasson, A.-C. (1987). Influence of pH and ionic strength on the viscoelastic properties of starch gels. A comparison of potato and cassava starches. *Carbohydr. Polymers*, **7**, 291-6.

Myrbäck, K. and Neumuller, G. (1950). Amylases and the hydrolysis of starch and glycogen. In *The Enzymes, Chemistry and Mechanism of Action*. 1st ed. (eds. Sumner, J.B. and Myrbäck, K.). Academic Press, NY. pp. 653-724.

Olkku, J. and Rha, C. (1978). Gelatinization of starch and wheat flour starch.-A review. *Food Chem.*, **3**, 293-317.

Oosten, B.J. (1984). Effects of organic molecules on the gelatinization temperature of starch. *Starch/Stärke*, **36**, 18-23.

Orford, P.D., Ring, S.G., Carroll, V., Miles, M.J., and Morris, V.J. (1987). The effect of concentration and botanical source on the gelation and retrogradation of starch. *J. Sci. Food Agric.*, **39**, 169-77.

Osman, E.M., Leith, S.J. and Fles, M. (1961). Complexes of amylose with surfactants. *Cereal Chem*, **38**, 449-53.

Paredes-Lopez, O. and Hernàndez-Lopez, D. (1991). Application of differential scanning calorimetry to amaranth starch gelatinization -Influence of water, solutes and annealing. *Starch/Stärke*, **43**, 57-61.

Paton, D. (1977). Oat Starch. I. Extraction, purification and pasting properties. *Starch/Stärke*, **29**, 149-53.

Paton, D. (1979). Oat Starch. Some recent developments. *Starch/Stärke*, **31**, 184-7.

Paton, D. (1986). Oat Starch: Physical, chemical and structural properties. In *Oats: Chemistry and Technology*. (ed. Webster, F.H.). AACC, St. Paul, MN. pp. 93-120.

Paton, D. (1987). Differential scanning calorimetry of oat starch pastes. *Cereal Chem.*, **64**, 394-9.

Pazur, J.H. (1965). Enzymes in synthesis and hydrolysis of starch. In *Starch Chemistry and Technology. Vol. 1. Fundamental Aspects*. (eds. Whistler, R.L. and Paschall, E.F.). Academic Press, NY. pp. 133-75.

Preiss, J. and Levi, C. (1980). Starch biosynthesis and degradation. In *The Biochemistry of Plants: a comprehensive treatise*. Vol. 3. (ed. Preiss, J.). Academic Press, NY. pp. 371-423.

Raphaelides, S. and Karkalas, J. (1988). Thermal dissociation of amylose-fatty acid complexes. *Carbohydr. Res.*, **172**, 65-8.

Riisom, T., Krog, N. and Eriksen, J. (1984). Amylose complexing capacities of cis- and trans-unsaturated monoglycerides in relation to their functionality in bread. *J. Cereal Sci.*, **2**, 105-18.

Ring, S.G. (1985). Some studies on starch gelation. *Starch/Stärke*, **37**, 80-3.

Ring, S.G., l'Anson, J. and Morris, V.J. (1985). Static and dynamic light scattering studies of amylose solutions. *Macromolecules*, **18**, 182-5.

Ring, S.G. (1987). Molecularm interactions in aqueous solutions of the starch polysaccharides: a review. *Food Hydrocolloids*, **1**, 449-54.

Ring, S.G., Colonna, P., l'Anson, K.J., Kalichevsky, M.T., Miles, M.J., Morris, V.J., and Orford, P.D. (1987). The gelation and crystallization of amylopectin. *Carbohydr. Res.*, **162**, 277-93.

Ring, S.G., Gee, J.M., Whittam, M., Orford, P. and Johnson, I.T. (1988). Resistant starch: its chemical form in foodstuffs and effect on digestibility *in vitro*. *Food Chem.*, **28**, 97-109.

Robin, J.P., Mercier, C., Charbonniere, R., and Guilbot, A. (1974). Lintnerized starches. Gel filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch. *Cereal Chem.*, **51**, 389-406.

Robin, J.P., Mercier, C., Duprat, F., Charbonniere, R. and Guilbot, A. (1975). Amidons lintnérisés. Etudes chromatographique et enzymatique des résidus insolubles provenant de l'hydrolyse chlorhydrique d'amidons de céréles, en particulier de maïs cireux. *Starch/Stärke*, **27**, 36-45.

Robyt, J.F. and French, D. (1967). Multiple attack hypothesis of α-amylase action: Action of porcine pancreatic, human salivary and *Aspergillus oryzae* α-amylases. *Arch. Biochem. Biophys.*, **122**, 8-16.

Robyt, J.F. and Whelan, W.J. (1968a). The α-amylases. In *Starch and Its Derivatives*. (ed. Radley, J.A.). Chapman and Hall Ltd. London. pp. 430-76.

Robyt, J.F. and Whelan, W.J. (1968b). The β-amylases. In *Starch and Its Derivatives*. (ed. Radley, J.A.). Chapman and Hall Ltd. London. pp. 477-97.

Robyt, J.F. (1984). Enzymes in the hydrolysis and synthesis of starch. In *Starch: Chemistry and Technology*. (eds. Whistler, R.L., BeMiller, J.N. and Paschall, E.F.). Academic Press, Orlando, FL. pp. 87-123.

Roos, Y.H. (1992). Phase transitions and transformations in food systems. In *Handbook of Food Engineering*. (eds. Heldman, D.R. and Lund, D.B.). Marcel Dekker, Inc., NY. pp. 145-97.

Rosario, R.R. del and Pontiveros, C.R. (1983). Retrogradation of some starch mixtures. *Starch/Stärke*, **35**, 86-92.

Roulet, P.H., MacInnes, W.M., Wursch, P., Sanchez, R.M. and Raemy, A. (1988). A comparative study of the retrogradation kinetics of gelatinized wheat starch in gel and powder form using x-rays, differential scanning calorimetry and dynamic mechanical analysis. *Food Hydrocolloids*, **2**, 381-96.

Russell, P.L. (1983). A kinetic study of bread staling by differential scanning calorimetry and compressibility measurements. The effect of added monoglyceride. *J. Cereal Sci.*, **1**, 297-303.

Russell, P.L. and Oliver, G. (1989). The effect of pH and NaCl content on starch gel ageing. A study by differential scanning calorimetry and rheology. *J. Cereal Sci.*, **10**, 123-38.

Sarko, A. and Wu, H.C.H. (1978). The crystal structures of A-, B- and C- polymorphs of amylose and starch. *Starch/Stärke*, **30**, 73-78.

Savage, H.L. and Osman, E.M. (1978). Effects of certain sugars and sugar alcohols on the swelling of corn starch granules. *Cereal Chem.*, **55**, 447-54.

Schoch, T.J. and Maywald, E.C. (1968). Preparation and properties of various legume starches. *Cereal Chem.*, **45**, 564-73.

Shamekh, S., Forssell, P. and Poutanen, K. (1994). Solubility pattern and recrystallization behavior of oat starch. *Starch/Stärke*, **46**, 129-33.

Shannon, J. C. and Garwood, D.L.(1984). Genetics and physiology of starch development. In *Starch Chemistry and Technology*.(eds. Whistler, R.L., BeMiller, J. N. and Paschall, E. F.), Academic Press Inc, NY. pp. 26-86.

Sievert, D. and Wursch, P. (1993). Amylose chain association based on differential scanning calorimetry. *J. Food Sci.*, **58**, 1332-35.

Slade, L. and Levine, H.(1987). Recent Advances in Starch Retrogradation. In *Industrial Polysaccharides: The Impact of Biotechnology and Advanced Methodologies*.(eds. Stivala, S.S., Crescenzi, V. and Dea, I.C.M.) Gordon and Breach Science Publishers, NY. pp.387-430.

Slade, L. and Levine, H. (1988). Non-equilibrium melting of native granular starch. Part I. Temperature location of the glass transition associated with gelatinization of A-type cereal starches. *Carbohydr. Polymers*. **8**, 183-208.

Sollars, W.F. (1973). Water-retention properties of wheat flour fractions. *Cereal Chem.*, **50**, 717-22.

Soni, P.L., Sharma, H., Dun, D. and Gharia, M.M. (1993). Physicochemical properties of *Quercus leucotrichophora* (oak) starch. *Starch/Stärke*, **45**, 121-7.

Sowa, S.M. and White, P.J. (1992). Characterization of starch isolated from oat groats with different amounts of lipids. *Cereal Chem.*, **69**, 521-7.

Spies, R.D. and Hoseney, R.C. (1982). Effect of sugars on starch gelatinization. *Cereal Chem.*, **59**, 128-31.

Sreenath, H.K. (1992). Studies on starch granules digestion by α-amylase. *Starch/Stärke*, **44**, 61-3.

Svegmark, K. and Hermansson, A.-M. (1993). Microstructure and rheological properties of composites of potato starch granules and amylose: A comparison of observed and predicted structures. *Food Structure*, **12**, 181-93.

Swinkles, J.J.M. (1985). Composition and properties of commercial native starches. *Starch/Stärke*, **37**, 1-5.

Takahashi, S. and Seib, P.A. (1988). Paste and gel properties of prime corn and wheat starches with and without native lipids. *Cereal Chem.*, **65**, 474-83.

Tester, R.F. and Morrison, W.R. (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chem.*, **67**, 551-7.

Thoma, J.A., Spradlin, J.E. and Dygert, S. (1971). Plant and animal amylases. In *The Enzymes*. 3rd Ed., Vol.5. (ed. Boyer, P.D.). Academic Press, NY. pp.115-89.

Thoma, J.A. (1976). Models for depolymerizing enzymes. Application to α-amylases. *Biopolymers*, **15**, 729-40.

Tomasik, P., Wang, Y.-J. and Jane, J.L. (1995). Complexes of starch with low-molecular saccharides. *Starch/Stärke*, **47**, 185-91.

Tsuge, H., Tatsumi, E., Ohtani, N. and Nakazima, A. (1992). Screening of α-amylase for evaluating the degree of starch retrogradation. *Starch/Stärke*, **44**, 29-32.

Vaaler, S., Hanssen, K.F., and Aagenaes, O. (1984). The effect of cooking upon the blood glucose response to ingested carrots and potatoes. *Diabetes Care*, **7**, 221-3.

Van Lonkhuysen, H. and Blankestijn, J. (1976). Influence of monoglycerides on the gelatinization and enzymatic breakdown of wheat and cassava starch. *Starch/Stärke*, **28**, 227-31.

Vasanthan, T. and Hoover, R. (1992). Effect of defatting on starch structure and physicochemical properties. *Food Chem.*, **45**, 337-47.

Wang, L.Z. and White, P.J. (1994). Functional properties of oat starches and relationships among functional and structural characteristics. *Cereal Chem.*, **71**, 451-58.

Ward, K.E.J., Hoseney, R.C. and Seib, P.A. (1994). Retrogradation of amylopectin from maize and wheat starches. *Cereal Chem.*, **71**, 150-5.

Williams, P.C., Kuzina, F.D. and Hlynka, I. (1970). A rapid colorimetric procedure for estimating the amylose content of starches and flours. *Cereal Chem.*, **47**, 411-20.

Williamson, G., Belshaw, N.J., Self, D.J., Noel, T.R., Ring, S.G., Cairns, P., Morris, V.J., Clark, S.A. and Parker, M.L. (1992). Hydrolysis of A- and B-type crystalline polymorphs of starch by α-amylase, β-amylase and glucoamylase I. *Carbohydr. Polymers*, **19**, 179-87.

Wolever, T.M.S. (1991). Small intestinal effects of starchy foods. *Can. J. Physiol. Pharmacol.*, **69**, 93-99.

Wolters, M.G.E. and Cone, J.W. (1992). Prediction of degradability of starch by gelatinization enthalpy as measured by differential scanning calorimetry. *Starch/Stärke*, **44**, 14-8.

Wood, P.J., Weisz, J. and Fedec, P. (1987). Oat beta glucan-Potential value as dietary fiber. In *Industrial Polysaccharides: The Impact of Biotechnology and Advanced Methodologies.*(eds. Stivala, S.S., Crescenzi, V. and Dea, I.C.M.) Gordon Breach Science Publishers, NY. pp. 215-27.

Wootton, M. and Bamunuarachchi, A. (1979). Application of differential scanning calorimetry to starch gelatinization. II. Effect of heating rate and moisture level. *Starch/Stärke*, **31**, 262-4.

Wootton, M. and Bamunuarachchi, A. (1980). Application of differential scanning calorimetry to starch gelatinization. III. Effects of sucrose and sodium chloride. *Starch/Stärke*, **32**, 126-9.

Wootton, M. and Chaudhry, M.A. (1979). Enzymic digestibility of modified starches. *Starch/Stärke*, **31**, 224-8.

Wootton, M. and Chaudhry, M.A. (1980). Gelatinization and *in vitro* digestibility of starch in baked products. *J. Food Sci.*, **45**, 1783-4.

Wootton, M. and Mahdar, D. (1993). Properties of starches from australian wheats. Part 3: *In vitro* digestibility and hydroxypropyl derivatives. *Starch/Stärke*, **45**, 337-41.

Wu, Y.V., Cluskey, J.E., Wall, J.S. and Inglett, G.E. (1973). Oat protein concentrates from a wet milling process: Composition and properties. *Cereal Chem.*, **50**, 481-88.

Wu, Y.V. and Stringfellow, A.C. (1973). Protein concentrates from oat flours by air classification of normal and high-protein varieties. *Cereal Chem.*, **50**, 489-96.

Wu, H.C.H. and Sarko, A. (1978a). The double-helical molecular structure of crystalline B-amylose. *Carbohydr. Res.*, **61**, 7-26.

Wu, H.C.H. and Sarko, A. (1978b). The double-helical molecular structure of A-amylose. *Carbohydr. Res.*, **61**, 27-40.

Wu, Y. and Seib, P.A. (1990). Acetylated and hydroxypropylated distarch phosphates from waxy barley, paste properties and freeze-thaw stability. *Cereal Chem.*, **67**, 202-8.

Yamishita, Y.-H., Ryugo, J. and Monobe, K. (1973). An electron microscopic study on crystals of amylose V complexes. *J. Electron Microscopy*, **22**, 19-23.

Youngs, V.L. (1972). Protein distribution in the oat kernel. *Cereal Chem.*, **49**, 407-11.

Youngs, V.L. (1978). Oat lipids. *Cereal Chem.*, **55**, 591-7.

Yuan, R.C., Thompson, D.B. and Boyer, C.D. (1993). Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three wx-containing genotypes in two inbred lines. *Cereal Chem.*, **70**, 81-9.

Zeleznak, K.J. and Hoseney, R.C. (1986). The role of water in the retrogradation of wheat starch gels and bread crumb. *Cereal Chem.*, **63**, 407-11.

Zeleznak, K.J. and Hoseney, R.C. (1987). Characterization of starch from bread aged at different temperature. *Starch/Stärke*, **39**, 231-5.

Zobel, H.F., French, A.D. and Hinkle, M.E. (1967). X-ray diffraction of oriented amylose fibers. II. Structure of V-amyloses. *Biopolymers*, **5**, 837-45.

Zobel, H.F. (1984). Gelatinization of starch and mechanical properties of starch pastes. In "*Starch Chemistry and Technology*. (eds. Whistler, R.L., Paschall, E.F. and BeMiller, J.N.). Academic Press, NY. pp. 285-309.

Zobel, H.F. (1988a). Starch crystal transformations and their industrial importance. *Starch/Stärke*, **40**, 1-7.

Zobel, H.F. (1988b). Molecules to granules: a comprehensive starch review. *Starch/Stärke*, **40**, 44-50.

PUBLICATIONS

Hoover, R., Vasanthan, T., Senanayake, N. J. and Martin, A.M. (1994). The effects of defatting and heat-moisture treatment on the retrogradation of starch gels from wheat, oat, potato, and lentil. *Carbohydr. Res.*, **261**, 13-24.

Hoover, R. and Senanayake, S.P.J.N. (1995). Composition and physicochemical properties of oat starches. *Food Research International* (Accepted for publication, August 9, 1995).