

THE EFFECT OF HEAT-MOISTURE TREATMENT ON THE
STRUCTURE & PHYSICOCHEMICAL PROPERTIES
OF LEGUME STARCHES

CENTRE FOR NEWFOUNDLAND STUDIES

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**THE EFFECT OF HEAT-MOISTURE TREATMENT ON
THE STRUCTURE & PHYSICOCHEMICAL PROPERTIES
OF LEGUME STARCHES**

BY

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A thesis submitted to the School of Graduate Studies
in partial fulfilment of the
requirements for the degree of

MASTER of SCIENCE

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ABSTRACT

Native Green Arrow pea, Eston lentil, Othello pinto bean, black bean and Express field pea starches were heat treated at 100°C for 16h at a moisture content of 30%. The heat treatment did not change granule size or shape. The surfaces of Green Arrow pea and Eston lentil starches were modified after heat treatment. Heat treatment decreased amylose leaching (Green Arrow pea>Express field~Eston lentil>black bean~pinto bean) and the swelling factor (Eston lentil~Express field pea>Green Arrow pea>black bean~pinto bean). The X-ray diffraction intensities increased in Green Arrow pea starch, but decreased in the other starches (Express field pea>black bean>pinto bean>Eston lentil). However, the X-ray pattern of all starches remained unchanged after heat treatment. Differential scanning calorimetry of the heat treated samples showed broadening of the gelatinization temperature range and a shifting of the endothermal transition towards a higher temperature (Eston lentil~Express field pea>black bean~pinto bean). However, the gelatinization enthalpy (ΔH) of all starches remained unchanged. The susceptibility towards hydrolysis by porcine pancreatic α -amylase increased on heat treatment (black bean>Eston lentil>Express field pea>pinto bean>Green Arrow pea). The action of α -amylase on the starches decreased ΔH in Eston lentil and Express field pea starches. However, ΔH decreased only marginally in pinto bean and black bean starches. Acid hydrolysis (2.2N HCl) increased on heat treatment (black bean>Express field pea~Eston lentil~pinto bean>Green Arrow pea). The results showed that bonding forces within the amorphous regions of the granule, crystallite orientation and the granule surface (in Green Arrow pea and Eston lentil) are altered during heat treatment. The magnitude of these changes being dependent upon the starch source.

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LIST OF ABBREVIATIONS

Acid N -	Acid normality (mol/L)
AML -	Amylose leaching
AM-AM -	Amylose-amylose interactions
AM-AMP -	Amylose-amylopectin interactions
CL -	Starch chain length - the number of glucose units per non-reducing end of the starch molecule.
CM -	Chloroform-methanol
db -	Dry weight basis
DMSO -	Dimethylsulfoxide
DNS -	3,5-Dinitrosalicylic acid
DP -	Degree of polymerization - the number of glucose units per reducing end of the starch molecule.
DSC -	Differential scanning calorimetry
HMT -	Heat-moisture treatment
ΔH -	Gelatinization enthalpy (cal/g)
M_r -	Molar mass
MW -	Molecular weight (g/mol)
Mw/Mn -	Molecular weight average/Molecular number average
η -	Limiting viscosity number (mL/g)
PAGE -	Polyacrylamide gel electrophoresis
PW -	Propanol-water
SDS -	Sodium dodecyl sulfate

SEM -	Scanning electron microscope/microscopy
SF -	Swelling factor
To -	Onset temperature of gelatinization ($^{\circ}$ C)
Tp -	Midpoint temperature of gelatinization ($^{\circ}$ C)
Tc -	End temperature of gelatinization ($^{\circ}$ C)
Tc - To -	Gelatinization temperature range
TCA -	Trichloroacetic acid

CHAPTER 1.

INTRODUCTION

Food legumes are the dicotyledonous seeds of plants belonging to the family Leguminosae. There are over 600 genera and 13,000 species belonging to this family of plants (Hoover and Sosulski, 1991). However, only about 20 species are commonly grown throughout the world (Salunkhe & Kadam, 1989). The word legume comes from the latin word "legumen" meaning seeds harvested in pods (Salunkhe & Kadam, 1989). Food legumes are often differentiated on the basis of their fat content: (i) pulses or grain legumes are those which are low in fat (1.0-7.2%), such as lima bean and lentil, (ii) whereas leguminous oilseeds, such as soybean and peanut, contain higher levels of fat (15-50%) (Salunkhe & Kadam, 1989; Salunkhe *et al.*, 1989). Pulses such as lentil, lima bean, and chickpea, are grown as commercial sources of protein which are used for direct consumption (Endres, 1989). Commercial sources of leguminous oilseeds include soybean and peanut, which are grown not only for their protein, but also for their oil content (Endres, 1989).

Food legumes are widely distributed throughout all parts of the world. Legumes are cultivated in India, Japan, China, Korea, Sri Lanka, Mexico, Pakistan, Bangladesh, Africa, Brazil, the United States and Canada, for example. Some of the major food legumes cultivated throughout the world include soybean and peanut (Salunkhe & Kadam, 1989; Endres, 1989), dry bean, smooth pea, wrinkled pea, chickpea and lentil (Hoover & Sosulski, 1991; Bhatti, 1988). Legumes are grown on all continents throughout the world, probably because of their nitrogen fixing ability (Kadam & Salunkhe, 1989). Consequently, nitrogen based fertilizers may not be necessary for legume cultivation.

Food legumes have been historically important protein foods in developing countries (Salunkhe & Kadam, 1989; Hoover & Sosulski, 1991). In fact, legumes are commonly referred to as "poor man's meat" since they are cheap sources of protein. The protein content of food legumes ranges anywhere from 15% (chickpea) to 45% (soybean) (Salunkhe *et al.*, 1985). Although legumes are considered good sources of protein, the value of the protein is somewhat limited since legumes are notably deficient in sulphur-containing amino acids as well as tryptophan (Gupta, 1983). Legumes also contain proteinase inhibitors which, if not destroyed during cooking, lower protein digestibility (Liener, 1983).

Although legume cultivation in developing countries is centuries old, it is relatively new in Canada. Lentil, for example, was first introduced into southern Manitoba and Saskatchewan in the 1970's. The Canadian lentil crop exhibited tremendous growth during the 1970's and 1980's. In 1987, lentil was grown on 200,000 ha in Saskatchewan, compared to only 2000 ha in 1971 (Bhatty, 1988). Today, lentil production in Canada is quite successful. Saskatchewan, for example, produced 425,700 tonnes of lentil in 1995 (Anonymous, 1996). In fact, market opportunities over the next 20 years indicate that there is a potential market for 700,000 MT of green and 700,000 MT of red lentil production in Saskatchewan (Anonymous, 1996). Potential market opportunities also exist for chickpea production in Saskatchewan.

Legume production in Canada is concentrated in the prairie provinces, particularly in Saskatchewan. Most of Canada's legume production consists of pulses (low fat legumes) such as dry pea (wrinkled and smooth), dry bean (white and coloured) and lentil (green, red and gold/yellow). Legume production is becoming increasingly important in Canada for the following reasons: (i) exports of dry peas for livestock feed to the European Economic Community is

increasing, and (ii) people are becoming more aware of the nutritive value of legumes and pulses. Some of the beneficial properties of pulses include their low fat content, high fibre content, gluten-free properties, and high folic acid content (Anonymous, 1996). The starch fraction of legumes, such as lentil, also exhibits a low glycemic index (Jenkins *et al.*, 1980), thus making legumes and legume starches useful in the treatment of diabetes (Bhatty, 1988).

During the 1970's, there was a growing interest in the production of grain legumes in the prairie provinces in order to meet a growing industrial demand, primarily for protein feed (Vose *et al.*, 1976). It was soon discovered that grain legume flours could be air-classified to obtain a fine protein fraction and a coarse starch fraction (Vose, 1977; Vose *et al.*, 1976; Sosulski & Youngs, 1979; Colonna *et al.*, 1980). During the 1970's and early 1980's, extensive investigations were carried out on the structure and functional properties of legume flours, protein isolates and concentrates (Sosulski *et al.*, 1976; D'Appolonia, 1977; Jeffers *et al.*, 1978; Sosulski *et al.*, 1978; Sosulski & Flemming, 1979; Sosulski & Young's, 1979; Vose, 1980; Sumner *et al.*, 1981) in an attempt to meet the industrial demand for protein feed. Until recently, however, there has been very little investigation into the utilization of the starch fraction of grain legumes (Colonna *et al.*, 1982; Colonna & Mercier, 1984; 1985; Singh *et al.*, 1989; Hoover & Sosulski, 1991; Hoover *et al.*, 1991; Gujska, 1994; Hoover & Manuel, 1995). In fact, Colonna and coworkers (1981) have shown that highly purified starch fractions can be isolated from pea and broad bean flours using an alkaline solution to completely remove any residual protein. Since legumes are high in starch (a by-product of protein isolates and concentrates), utilization of the starch fraction will be economically important (Morad *et al.*, 1980). Presently, legume starches have found limited commercial food use, with the exception of mung bean starch which is used in the manufacture of

noodles (Singh *et al.*, 1989). This is probably due to the lack of information concerning the structure and physicochemical properties of these starches, and also to their poor functional properties (Hoover & Sosulski, 1985a; Tjahjadi & Breene, 1984).

Heat-moisture treatment of starches at restricted moisture levels (18-30%) and high temperature (100°C) for 16 hours has been shown to alter the structure and physicochemical properties of normal maize, waxy maize, high amylose maize, wheat oat, barley, potato, yam, pigeon pea and laird lentil starches (Sair & Fetzer, 1944; Sair, 1967; Donovan *et al.*, 1983; Kulp & Lorenz, 1981; Lorenz & Kulp, 1981, 1982; Hagiwara *et al.*, 1991; Stute, 1992; Hoover & Vasanthan, 1994; Hoover & Manuel, 1996; Franco *et al.*, 1995; Kawabata *et al.*, 1994; Maruta *et al.*, 1994; Hoover *et al.*, 1994). Most of the above studies have been on cereal and tuber starches, hence only limited information is available on structural changes within the amorphous and crystalline regions during heat-moisture treatment of legume starches.

Legume starches are known to vary in their amylose content, levels of amylose-lipid complexes, and in the extent of interaction between the starch components within the native granule (Hoover & Sosulski, 1991). Therefore, studies on legume starches would provide a deeper insight into the nature and extent of starch chain interactions during heat-moisture treatment.

Lorenz and Kulp (1981) have shown that heat-moisture treatment changes the functionality (thickening power, baking and cake making potential) of wheat and potato starches. However, no work has been reported on the influence of heat-moisture-treatment on the functionality of legume starches. Such a study is important, since legume starches are presently not utilized in foods due to their poor functional properties.

1.1 Purpose

Presently, legume starches have found limited use in foods due to their poor functional properties. Heat-moisture treatment is one method of physical modification which may improve the functional properties of legume starches. However, in order to understand the effect of heat-moisture treatment on the functionality of legume starches, researchers must first understand how such a treatment influences the structure and physicochemical properties of these starches. In this study, changes in amylose leaching, granular swelling, X-ray intensities, gelatinization characteristics and granular susceptibility towards hydrolysis by acid and α -amylase following heat-moisture treatment (30% moisture, 100°C, 16 hours) of Green Arrow pea (wrinkled pea), black bean, pinto bean, Express field pea (smooth pea) and Eston lentil starches were investigated. Therefore, the information gained from this study would form the basis for future investigation on the influence of heat-moisture treatment on the functionality of legume starches.

CHAPTER 2.

LITERATURE REVIEW

2.1 Starch

Commercial sources of starch include potato, corn, waxy corn, wheat, tapioca and arrowroot. Commercial starch has many industrial applications such as its use in the manufacture of textiles, paper, adhesives, pharmaceuticals, building materials and various food stuffs. Starch has found a wide variety of uses in the manufacture of foods primarily because of its ability to produce viscous pastes when heated in the presence of excess water (Swinkels, 1985). Some of the functional properties of starch in foods include the gelling of puddings, the thickening of gravies, the setting of cakes, moisture retention in cake toppings, and colloidal stabilizers in salad dressings (Hoseney, 1994).

Starch, a homopolysaccharide of α -D-glucose, is the major storage carbohydrate in most higher plants. In plant reserve organs, such as seeds, tubers, stems and leaves, starch is deposited in the form of granules that range in size from 1 to 100 μ m and vary in shape depending on the botanical source (Biliaderis, 1991). Granule composition, morphology and supermolecular organization are essentially genetically determined. However, the mechanism by which plant genetic information is translated into a specific granule composition, morphology and organization is not well understood (Biliaderis, 1991).

2.2 Components of Starch

Starch granules are mainly composed of α -D-glucan polymers, linked via α -(1-4) and α -(1-6) glucosidic bonds, and minor non-carbohydrate constituents which include proteins and

lipids. The functional properties of starch are mostly due to the two major carbohydrate macromolecules, amylose and amylopectin, and to the physical organization of these macromolecules within the starch granule (Biliaderis, 1991). Therefore, the structure of starch must be regarded at two distinct levels: (i) the molecular level and (ii) the organizational level of the starch granule.

2.2.1 Carbohydrate Components

As previously stated, the carbohydrate fraction of starch consists of two major glucose macromolecules, amylose and amylopectin (Swinkels, 1985; Bornet, 1993; Biliaderis, 1991; Manners, 1985). Amylose (Fig. 2.1) is essentially composed of long, linear glucose chains which are linked through the α -(1-4) glucosidic bond. Amylopectin (Fig. 2.2), however, consists of short linear segments which are α -(1-4) linked with numerous branch points occurring at the α -(1-6) linkages. The amount of each starch fraction present will vary depending on the origin of the starch. Most starches generally contain 20-30% amylose and 70-80% amylopectin (Manners, 1985). The properties of amylose and amylopectin vary widely and are compared in Table 2.1.

2.2.1.1 Amylose

The view that amylose is essentially a linear homopolymer of α -D-glucose linked via α -(1-4) glucosidic bonds is well documented (Davis, 1994; Bornet, 1993; Biliaderis, 1991; Manners, 1985; Eliasson & Larsson, 1993; Swinkels, 1985; Hosenev, 1994; Curá & Krisman, 1990; Krisman & Curá, 1991). The molecular weight of amylose ranges from 50,000 to 1,000,000 and its average chain length is \sim 1000 (Biliaderis, 1991; Davis, 1994), but it may range from 600-6000 glucose units (Bornet, 1993). Amylose molecules have a reducing end and a non-reducing end, and may be present as

FIG. 2.1 **Structure of the amylose component of starch. A - diagram of a portion of an amylose molecule. B - enlarged view of A, showing the chemical formula.**

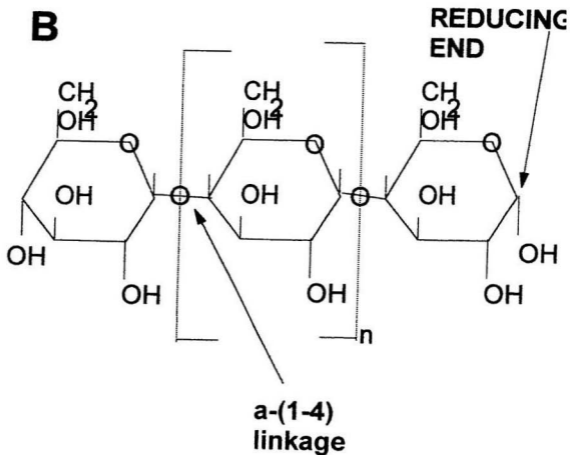
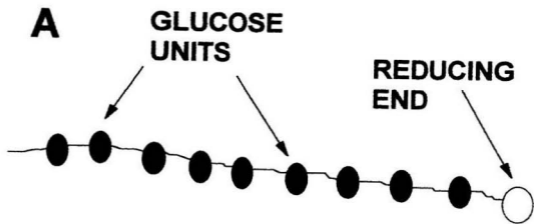


FIG. 2.2 Structure of the amylopectin component of starch. A - diagram of a portion of an amylopectin molecule. B - enlarged view of a portion of A showing the chemical formula, including the branch point.

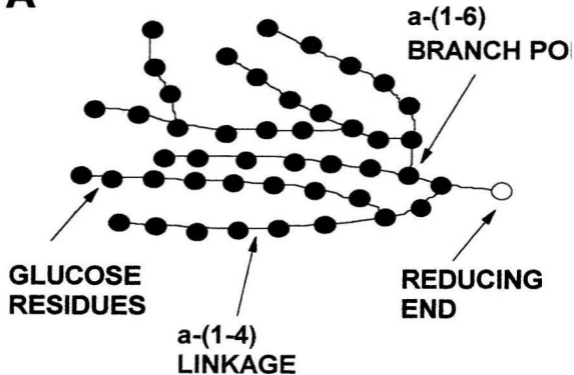
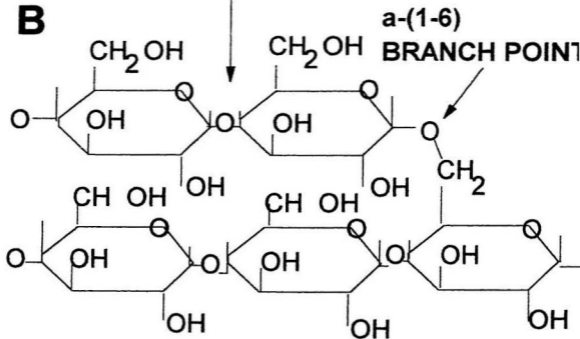
A**B**

Table 2.1 Properties of Amylose and Amylopectin

PROPERTY	AMYLOSE	AMYOPECTIN
Molecular Weight (g/mol)	50,000 - 10 ⁶	10 ⁶ -10 ⁸
Glycosidic Linkages	Mainly α -(1-4)	α -(1-4) and α -(1-6)
Molecular Shape	Essentially linear, Slightly branched	Highly Branched; Short linear segments
Degree Polymerization (# glucose units)	$\sim 10^3$	10 ⁴ -10 ⁵
Average Chain Length (# glucose units)	$\sim 10^3$	20-25
Iodine Colouration	Deep Blue	Purple/Red Brown
λ_{\max}	600-660	530-550
Stability of Aqueous solution	Retrogrades (unstable)	Stable
DIGESTIBILITY		
β -amylase	~ 70	~ 55
α -amylase	$\sim 100-110$	$\sim 90-100$
Debranching enzyme + β -amylase	~ 100	~ 75

(Manners, 1985; Biliaderis, 1991; Davis, 1994).

single or double helices that can contain lipid inclusions (Davis, 1994). The characteristic blue colour given by iodine and starch is also thought to be due to the formation of an amylose inclusion complex. The secondary and tertiary structures of amylose molecules are stabilized by hydrogen bonds and van der Waals forces (Davis, 1994).

A slight degree of branching has been found in some amyloses (Manners, 1985, 1989). The branched nature of amylose accounts for its incomplete β -amylase degradation (Curá *et al.*, 1995). The β -amylolysis limit of wheat amylose, for example, is only 77-79% (Lii & Lineback, 1977). β -amylase hydrolyses α -(1-4) linkages but cannot hydrolyse nor bypass α -(1-6) linkages. Therefore, the incomplete hydrolysis of amylose by this enzyme indicates that amylose must also contain α -(1-6) linkages (Eliasson & Larsson, 1993).

The incomplete degradation of amylose by β -amylase was first illustrated by Peat and coworkers (1952) who proposed the presence of α -(1-3) linkages to explain the results. However, Banks and Greenwood (1966) later illustrated, using bacterial pullulanase, that amylose contains α -(1-6) linkages. Based on studies of various starches, Hizukuri and coworkers (1981) determined that amylose contains between 9 and 20 branch points per molecule. More recent evidence (Curá & Krisman, 1990) indicates that approximately 1.0-2.0% of the glucose in amylose is contained in α -(1-6) branch points. Fractionation studies (Krisman & Curá, 1991) indicate that the amylose population may vary between strictly linear and somewhat branched in various starches. Using both enzyme and chemical (methylation) analyses, Curá and coworkers (1995) confirmed that amylose is slightly branched, and that the branch points are α -(1-6) linked.

Although amylose is slightly branched, it behaves essentially as an unbranched entity (Eliasson & Larsson, 1993; Hoseney, 1994). Amylose exhibits this type of behaviour since its

branches are quite long and very few (Hoseney, 1994). X-ray scattering data indicate that amylose chains are highly disordered but short range helical structures might exist (Braga *et al.*, 1985). In solution, amylose behaves as a random coil which may form single or double helices (Davis, 1994) which are capable of forming helical inclusion complexes. Each amylose molecule may actually contain several of these inclusion complexes (Eliasson & Larsson, 1993). It is the long linear nature of amylose which is responsible for its ability to form helical inclusion complexes with iodine, organic acids and alcohols, fatty acids, lipids and hydrocarbons (Hoseney, 1994; Swinkels, 1985). The characteristic blue colour given by iodine and starch is thought to be due to polyiodide ions (eight ions) residing in the central core of the amylose helix (Hoseney, 1994).

Amylose solutions are not very stable since the amylose has a strong tendency to retrograde/crystallize by forming intramolecular bonds (Eliasson & Larsson, 1993). In fact, retrograded starch material is predominantly composed of amylose. The term retrogradation as used to refer to starch solutions means a return from a solvated, dispersed amorphous state to an insoluble, aggregated or crystalline condition (Swinkels, 1985). The tendency of amylose to retrograde is mostly due to its long linear nature (Hoseney, 1994), which allows dissolved amylose molecules to align themselves in a parallel fashion, thereby facilitating the formation of interchain hydrogen bonds between hydroxyl groups on adjacent molecules (Swinkels, 1985). Amylose also retrogrades in starch gels during storage of the gel. Amylose molecules laterally associate, as they do in solution, to form aggregated, crystalline regions.

2.2.1.1.1 Legume Amyloses

The amylose content of starch will vary depending on the botanical origin. Generally, cereal starches contain 15-26% amylose, although some high amylose cereal starches are available which contain 50 to 70% amylose (Bornet, 1993; Hoover & Manuel, 1996). Legume starches contain anywhere from 24 to 64% amylose (Hoover & Sosulski, 1991). Table 2.2 summarizes the properties of legume and cereal amyloses. The data shown for legume amyloses may not be accurate, since many researchers have experienced considerable difficulties in isolating homogeneous fractions of amylose and amylopectin. Recently, Oates (1990) showed that addition of bromelain to isolated mung bean amylose (extraction performed by the procedure of Takeda *et al.*, 1986) resulted in a pure sample of amylose, free of any contaminating high molecular weight material. Therefore, in light of these findings, the molecular structure of legume amyloses must be re-examined.

Information on the molecular weight (MW), degree of polymerization (DP) and chain length (CL) of legume amyloses is limited. Legume amyloses have molecular weights ranging from 165,000 to 312,000, compared to 260,000 and 200,000 for wheat and corn amyloses, respectively, (Table 2.2). The degree of polymerization of amylose has been determined only on a few legume starches (DP = 578 - 1300), and is generally higher than those of wheat (DP = 270 - 1300) and corn (DP = 295 - 960) amyloses (Biliaderis *et al.*, 1979, 1981; Banks & Greenwood, 1967; Naivikul & D'Appolonia, 1979; Kawamura, 1969; Lai & Varriano-Marston, 1979) (Table 2.2). The β -amylolysis limits of most legume amyloses exceed 85% (Biliaderis *et al.*, 1981) (higher than for cereal amyloses), the exception being amyloses of wrinkled pea (79-84.7%) (Biliaderis *et al.*, 1979, 1980, 1981; Banks & Greenwood, 1967) and mung bean (78.4%)

Table 2.2 Properties of legume and cereal amyloses.

PROPERTY	STARCH SOURCE			
	LEGUMES ^a	WRINKLED PEA ^a	WHEAT ^b	CORN ^c
Molecular Weight (g/mol)	165, 000 to 312, 000	125, 000	260, 000	200, 000
Iodine Binding Capacity (%)	16 to 22	17.99 to 19.20	18.5 to 19.9	19.2 (amylomaize)
Iodine Colouration λ_{\max} (nm)	625 to 630	625	660	~660
Degree Polymerization (# glucose units)	578 to 1900	1000 to 1100	270 to 1300	295 to 960
β -amylolysis Limit (%)	> 85	79.0 to 84.7	77.0 to 79.0	77 (amylomaize)
Limiting Viscosity Number [η] (mL/g)	> 180	136 to 150	330	170

^a Banks & Greenwood, 1967; Biliaderis *et al.*, 1979, 1980, 1981; Naivikul & D'Appolonia, 1979; Lai & Varriano-Marsten, 1979; Kawamura, 1969; Colonna *et al.*, 1981b.

^b Medcalf & Gilles, 1965; Lii & Lineback, 1977; Banks *et al.*, 1973; Lineback & Rasper, 1988; Sterling 1978; Banks & Greenwood, 1975; Fleche 1979; Schoch, 1969; Melvin, 1979; Glicksman, 1969; Greenwood & Thomson, 1962).

^c Banks *et al.*, 1973, 1975; Sterling 1978; Banks & Greenwood, 1975; Fleche 1979; Schoch, 1969; Melvin, 1979; Glicksman, 1969; Greenwood & Thomson, 1962.

(Naivikul & D/Appolonia, 1979; Biliaderis *et al.*, 1979). This indicates that legume amyloses are less branched than wheat (77-79%) and corn amyloses (~77%) (Table 2.2). The limiting viscosity numbers (η) of legume amyloses are generally higher (> 180 mL/g) than that of corn amylose (179 mL/g) (Table 2.2), the exception being amyloses of wrinkled pea (136-150 mL/g) (Biliaderis *et al.*, 1979, 1980, 1981; Banks & Greenwood, 1967) and navy bean (174 mL/g) (Naivikul & D/Appolonia, 1979; Biliaderis *et al.*, 1979) starches.

2.2.1.2 Amylopectin

Amylopectin, although similar to amylose in composition, both being primarily composed of α -(1-4) linked D-glucose molecules, varies significantly from amylose in molecular size, shape and structure. Amylopectin, having a molecular weight of $\sim 10^7$ - 10^8 (Banks & Greenwood, 1975), is much larger than amylose. In fact, it is one of the largest known molecules in nature. Macromolecules of amylopectin may contain up to 10^6 glucose units (Bornet, 1993). Like amylose, amylopectin is largely composed of linear α -(1-4) linked D-glucose chains with branching occurring at the α -(1-6) glucosidic linkages. The difference in the two macromolecules is that amylopectin contains 4-6% (Bornet, 1993; Hosoney, 1994) of these branch points versus amylose which contains only 1-2%. The average chain length of amylopectin is 20-25 glucose units (Manners, 1985; Hosoney, 1994), but may be greater than 200 in some wheat varieties (Kobayashi *et al.*, 1986). Most legume amylopectins have an average chain length in the range of 20-24 (Biliaderis *et al.*, 1981). Colonna and Mercier (1984) have shown that legume amylopectins have longer external chain lengths than cereal amylopectins.

The main structural features of amylopectin were determined by methylation analysis between 1935 and 1940. However, the fine structure of amylopectin is still not completely

understood. This may be due to variations in amylopectin molecules isolated from different sources. In any case, amylopectin molecules differ with respect to their molecular weight and molecular shape (Lelievre *et al.*, 1986), the number of branches per molecule, the length of the branches, and the branching pattern (French, 1972).

The molecular weight of amylopectin will vary depending on the botanical source (Eliasson & Larsson, 1993). However, variations in amylopectin molecular weight will also occur depending on the method of determination used. Lelievre and coworkers (1986) used the Svedberg equation to determine the molecular weight of wheat starch amylopectin in dimethylsulfoxide (DMSO) and in water. In DMSO, the molecular weight was $\sim 1 \times 10^7$ compared to 6×10^7 in water. However, light-scattering measurements in aqueous solvents indicate the molecular weight of amylopectin to be $\sim 4 \times 10^8$ (Whelan, 1971).

Molar mass studies of various sources of amylopectin report that the ratio of Mw/Mn (weight average molecular weight/number average molecular weight) is ~ 300 , which indicates that a wide range of molecular sizes exist (Lelievre *et al.*, 1986). The molecular weight differences observed by the above methods may be due to the shape and behaviour of amylopectin in the different solvents. Pulsed field gradient nuclear magnetic resonance measurements indicate that wheat starch amylopectin molecules in DMSO are highly planar and behave as oblate ellipsoids (Lelievre *et al.*, 1986). The same amylopectin molecules in aqueous systems tend to aggregate into a more spherical shape with a volume increase ~ 400 times that of a single molecule (Callaghan & Lelievre, 1985).

The fine structure of amylopectin is not clearly understood. When one considers the size

of the molecule it is easy to comprehend why. Amylopectin is one of the largest known molecules in nature, with a molecular weight of $\sim 10^7$ to 10^8 and an average CL of 20-25, and, therefore, several thousand chains per molecule. Consequently, it is not possible to accurately determine its structure. Between 1935 and 1940, however, three models describing possible structures of amylopectin were proposed: (i) the Haworth structure; (ii) the Staudinger structure, and; (iii) the Meyer structure (Fig. 2.3). These models represent possible structures for small segments within a much larger molecule and, therefore, are not accurate depictions of amylopectin molecules.

Several years after the above models were proposed, Peat and coworkers (1956) proposed that all three structures contained three different types of chains: (i) A chains, which are linked to the molecule through α -(1-6) linkages, while all other linkages are α -(1-4) linked; (ii) B chains, which are linked in the same manner as A chains, but also carry other chains (A &/or B) through α -(1-6) linkages and are designated as Ba or Bb chains; and (iii) C chains, of which there is only one per molecule and which carries the only reducing end group in the molecule (Fig. 2.4). The A/B chain ratio in the molecule can be estimated by treating amylopectin with β -amylase, followed by debranching with a combination of isoamylase and pullulanases, and subjecting the remnants of hydrolysis to chromatography (Ring *et al.*, 1993). The A/B chain ratio has recently been determined to be $\sim 1/1$ to $1.5/1$ for several amylopectins (Manners & Matheson, 1981; Manners, 1985, 1989).

The most recent structural models of amylopectin molecules include a revised Meyer model, or Whelan elongated model, by Whelan and coworkers (1970), and several cluster-type models (French, 1972; Robin *et al.*, 1974; Manners & Matheson, 1981). The Whelan elongated model is a combination of the Haworth and Staudinger structures. It is consistent with an A/B

FIG. 2.3 Three models describing possible structures of amylopectin. A - the Haworth structure, B - the Staudinger structure, and C - the Meyer structure. (Adapted from Banks & Greenwood, 1973; Manners, 1985).

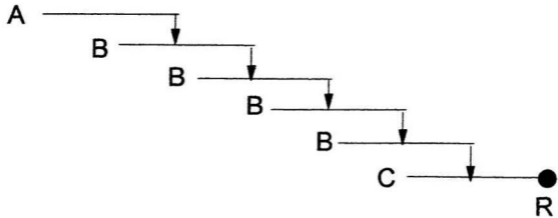
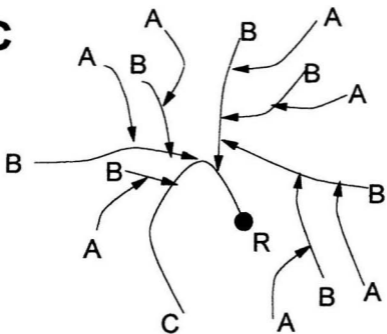
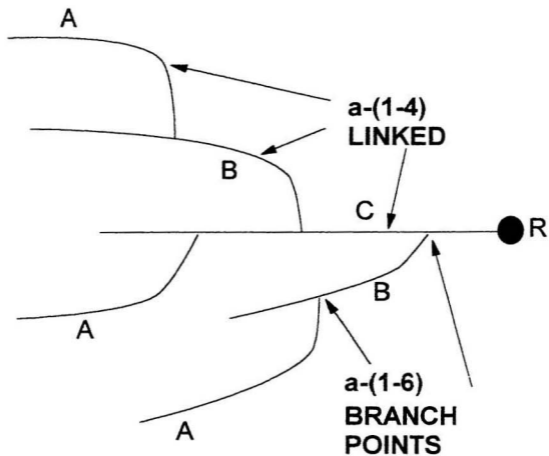
A**B****C**

FIG. 2.4 Identification of A, B, and C chains of amylopectin. (Adapted from Hosney, 1994).

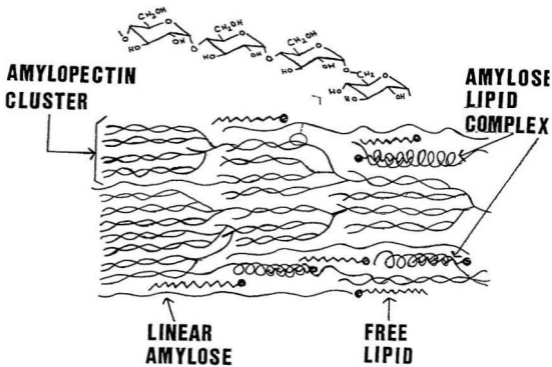


chain ratio of 1/1, and is assymetrical and can therefore explain some of the differences in physical properties between amylopectin and glycogen, such as the higher viscosity of amylopectin and its behaviour on ultracentrifugation (Manners & Matheson, 1981). However, studies on the debranching of amylopectin with pullulanase indicate that amylopectin has a relatively compact, assymetrical tree-like structure rather than an open elongated structure (Whelan, 1976; Rovsky *et al.*, 1979).

The most widely accepted model of amylopectin is the cluster-type model (Fig. 2.5) of French (1972), Robin and coworkers (1974), and Manners & Matheson (1981). All three cluster-type models indicate that amylopectin is composed of compact parts of oriented chains, or clusters, which are randomly or somewhat regularly branched, and that the clusters are linked by long chains which extend into two or more clusters (Hizukuri, 1986). Current evidence on the structural characteristics of amylopectin best support the cluster-type models. The A/B chain ratio of many amylopectin molecules is 1/1. This ratio is more consistent with a cluster-type model as opposed to an elongated model (Manners & Matheson, 1981). The cluster-type model also supports the physical properties, observed structural features and the mode of biosynthesis in the starch granules (Manners & Matheson, 1981). Gel permeation high performance liquid chromatography studies on debranched amylopectin (Hizukuri, 1986) reveal a polymodal chain length distribution of amylopectin which is consistent with that depicted in the cluster models. Hizukuri (1986) also showed that the distribution characteristics of chain lengths are inconsistent with the Haworth, Staudinger, and Meyer models of amylopectin (Fig. 2.3), but are consistent with the cluster model of French (Fig. 2.5).

In comparison to amylose, amylopectin solutions do not exhibit a strong tendency to

FIG. 2.5 The cluster model of amylopectin showing the distribution of amylopectin clusters, linear amylose, free lipids and amylose-lipid complexes within the starch granule. (Adapted from French, 1972; Robin *et al.*, 1974; Manners & Matheson, 1981).



retrograde; however, crystallization will occur under the right conditions (Eliasson & Larsson, 1993). Amylopectin binds very little iodine (Table 2.1) due to its short average chain length (Ring *et al.*, 1987) and, therefore, forms a red-brown complex with iodine which has a λ_{\max} of 530-540 nm (Banks & Greenwood, 1975). Thus, the iodine binding behaviour of amylopectin provides a convenient method for assessing the purity of amylopectin (Ring *et al.*, 1987).

2.2.1.2.1 Legume Amylopectins

The characteristics of legume and cereal amylopectins are characterized in Table 2.3. The overall chain length of most legume amylopectin is in the range of 20-24. However, that of wrinkled pea amylopectin has been reported to be 34 (Table 2.3). The iodine binding capacity of legume amylopectins is higher than that of cereal amylopectins (Table 2.3). Colonna and Mercier (1984) have shown, in their studies on amylopectins from smooth pea and wrinkled pea starches, that differences between cereal and legume amylopectins are more probably due to the external chain lengths being longer in the latter (since the λ_{\max} of the iodine complexes of the respective β -limit dextrans are identical) than to contaminant amylose (since the debranched material from the legume amylopectins gave only one peak at the elution volume on sepharose CL 2B in contrast to amylose). The above authors have also shown the existence of a high percentage (18.9%) of an intermediate material (branched) of low molecular weight in wrinkled pea starch. This was present only in trace quantities (0.4%) in smooth pea starch. The ratio of short (DP 15) to long (DP 45) linear chains in the intermediate fraction was 3.6, in contrast to 9.6 for wrinkled pea and 8.1 for smooth pea amylopectins.

Table 2.3 Properties of legume and cereal amylopectins.

PROPERTY	STARCH SOURCE		
	LEGUMES ^a	WHEAT ^b	CORN ^c
Molecular Weight (g/mol)	80.6 X 10 ⁶ (smooth pea) 19.4 X 10 ⁶ (wrinkled pea)	~250 X 10 ⁶	-
Iodine Binding Capacity (%)	0.9 (smooth pea) 1.7 (wrinkled pea)	<1.0 - 2.0	-
Iodine Colouration λ_{max} (nm) of	540 to 560	530 to 550	~570 (waxy)
Degree of Polymerization (# glucose units)	Not reported	2 X 10 ⁶	2 X 10 ⁶
Chain Length (# glucose units)	20 to 24 34 (wrinkled pea)	17 to 25	28 ~19 (waxy) 44 (amylomaize)
β -amylolysis Limit (%)	56.1 to 66.5	~55.0	~57.2 (waxy)
Limiting Viscosity Number [η] (mL/g)	126 to 131 114 (wrinkled pea)	90 to 150	35 to 100 (waxy)

^a Colonna & Mercier, 1984; Colonna *et al.*, 1981; Biliaderis *et al.*, 1981.

^b Banks & Greenwood, 1967b, 1973, 1975; Lii & Lineback, 1977; Hizukuri *et al.*, 1983; Lelievre *et al.*, 1986; Kobayashi *et al.*, 1986.

^c Banks & Greenwood, 1967b, 1973, 1975.

2.2.2 Non-carbohydrate Components

Starch contains non-carbohydrate components, which are often referred to as "minor" constituents because they are present at levels of 1% or less (Eliasson & Larsson, 1993). The most important minor non-carbohydrate constituents of starch include proteins and lipids (Lineback & Rasper, 1988). The origin of these non-carbohydrate components is not clear, but it is thought that they may originate from two possible sources: (i) as trace components of the starch itself, or (ii) as contaminants that are not completely removed during starch isolation (Lineback & Rasper, 1988). Although the non-carbohydrate components are present in very small amounts, they can still greatly affect the properties of starch.

2.2.2.1 Proteins

Nitrogen present in starch is generally considered to be present as protein, but it may also be part of the starch lipids (Lineback & Rasper, 1988). Therefore, the protein content of purified starch can be estimated from the non-lipid nitrogen content (Eliasson & Larsson, 1993). In wheat starch, the protein content has been estimated to be 0.1-0.25% (Eliasson & Larsson, 1993), whereas a broader range has been reported for legume starches, 0.05-1.12% (El Faki *et al.*, 1983a; Hoover & Sosulski, 1985a; Lai & Varriano-Marston, 1979). Generally, as the purity of the starch increases, the nitrogen and/or protein content decreases (Lineback & Rasper, 1988).

The protein composition of starch will vary with the starch source, but is mainly dependent on the method of starch preparation (Eliasson & Larsson, 1993). Starch proteins may be present in starch in various forms: (i) as enzymes trapped within the starch granule, which may be remnants of starch biosynthesis, or enzymes needed for starch hydrolysis during seed germination; (ii) as granule surface proteins; or (iii) as internal granule proteins, (Eliasson &

Larsson, 1993; Lineback & Rasper, 1988; Lowy *et al.*, 1981).

The enzymes present as starch proteins include α - and β -amylases. Alpha-amylase is an endoenzyme which randomly breaks α -(1-4) glucosidic linkages, thereby quickly reducing the size of the starch molecules and, consequently, the viscosity of starch suspensions is rapidly decreased (Eliasson & Larsson, 1993). Beta-amylase is an exoenzyme which breaks α -(1-4) glucosidic linkages by hydrolyzing starch from the nonreducing end, and thus releasing maltose. Beta-amylase, unlike α -amylase, cannot bypass α -(1-6) glucosidic linkages, and will, therefore, leave β -limit dextrins (Eliasson & Larsson, 1993).

Starch granule surface proteins are easily extracted with salts such as sodium dodecyl sulphate (SDS) (Lowy *et al.*, 1981; Gough *et al.*, 1985). Greenwell and Schofield (1986) extracted such a protein from several wheat starches. The protein had a molecular weight of 15,000 and was positively correlated with endosperm softness in wheat. A different granule surface protein, which is also found in wheat starch, has a molecular weight of 30,000, no enzyme activity, and represents 8% of the total protein in wheat starch (Eliasson & Larsson, 1993).

Internal granule proteins can only be extracted after the starch has been gelatinized (Lowy *et al.*, 1981) These proteins have been shown (by gel electrophoresis with SDS-PAGE) to have molecular weights ranging from 30,000 to 57,200 (Seguchi & Yamada, 1989).

2.2.2.2 Lipids

Lipids associated with starch granules have been differentiated on the basis of selective solvent extraction methods (Morrison, 1983). Starch granule lipids can be classified either as non-starch lipids or as starch lipids. The non-starch lipids can be easily extracted from ungelatinized starch granules by a cold solvent (Morrison, 1981), such as water-saturated butanol

at ambient temperature. However, true starch lipids, which are much more difficult to extract, can only be completely extracted from gelatinized starch granules using hot solvent, such as n-propanol-water (3:1) at 90-100°C (Morrison, 1981).

The non-starch lipids are surface lipids which occur as spherosomes and membrane lipids which may be present on the granule surface *in situ* within the plant tissue (Vasanthan & Hoover, 1992) (prior to starch extraction), or they may be monoacyl non-starch lipids which have become bound to the carbohydrate surface of starch granules, possibly as amylose-inclusion complexes (Morrison, 1983). Surface lipids (easily extracted) include both non-starch as well as starch lipids. However, solvent extraction techniques do not distinguish between the two types of surface lipids. Therefore, all lipids found on the surface of starch granules are usually considered to be starch lipids (Galliard & Bowler, 1987). Surface lipids consist of triglycerides, free fatty acids, glycolipids and phospholipids (Morrison, 1981; Galliard & Bowler, 1987).

Internal starch lipids are the true starch lipids, and are very difficult to extract. Internal lipids reside within the starch granule interior, and are thought to exist as amylose-inclusion complexes (Morrison, 1983; Acker, 1977), or may reside in the spaces between amylose and amylopectin. In order to completely extract the internal lipids, the granule must be sufficiently swelled. Internal starch lipids are predominantly monoacyl lipids, which primarily include lysophospholipids and free fatty acids (Hargin & Morrison, 1980; Morrison, 1983).

Both surface and internal lipids may be present in starch in two forms: (i) the free state or (ii) the bound state. Free lipids are easily extracted by solvent systems at ambient temperatures (Morrison, 1981). Bound lipids are linked to starch components, either as amylose-inclusion complexes (Acker, 1977) or via ionic or hydrogen bonding to hydroxyl groups of the starch

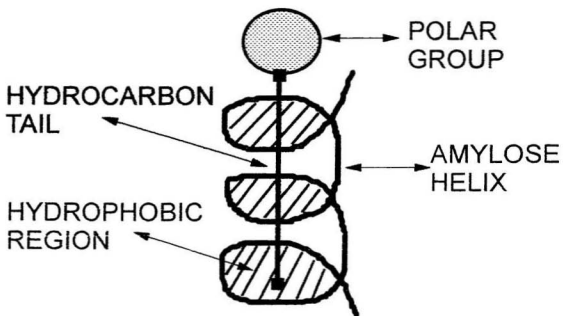
components (Vasanthan & Hoover, 1992). Bound lipids are difficult to extract and their removal from starch may require prolonged extraction with hot aqueous alcoholic solvent (Morrison, 1981), or disruption of the granular structure by acid hydrolysis (Goshima *et al.*, 1985).

2.3 The Amylose-Lipid Complex

Amylose is known to form crystalline complexes with numerous polar and non-polar compounds; these complexes exhibit a characteristic V-amylose structure (Biliaderis & Galloway, 1989). Amylose can form strong helical inclusion complexes (Fig. 2.6) with a variety of compounds in aqueous solution (Banks & Greenwood, 1975; Kugimiya & Donovan, 1981) such as iodine, alcohols and fatty acids (Kugimiya & Donovan, 1981). However, it is the interaction of starch with lipids which is of most interest to food processors since interactions can significantly alter the properties of food products. Formation of the amylose-lipid complex, for example, has been used to slow down the rate of staling in bread (Krog & Jensen, 1970), to prevent stickiness in mashed potatoe granules (Hoover & Hadziyev, 1981), and to control texture in extruded starch-containing products (Meuser *et al.*, 1984). The formation of amylose inclusion complexes has also been used to separate amylose from amylopectin (Schoch, 1945), and to estimate the amylose content of starch (Banks & Greenwood, 1975).

The dominant interaction between starch and lipids gives the amylose-lipid complex (Eliasson & Larsson, 1993) (Fig. 2.6). In cereal starches, monoacyl lipids are usually present as inclusion complexes with the amylose component of the starch (Acker & Becker, 1971; Morrison, 1978). In fact, all long chain monoacyl lipids are probably capable of forming inclusion complexes with amylose, under suitable conditions (Morrison, 1988). Amylose-lipid complexes

FIG. 2.6 Schematic illustration of an amylose-lipid inclusion complex with the hydrocarbon tail of the lipid (monoacyl lipid) residing within the hydrophobic interior of the amylose helix.



may be naturally present in starch, or may be formed between amylose and an added lipid.

2.3.1 Structure

The helical structure of amylose provides the basis for starch-lipid interactions. The helical structure of amylose was first suggested by Hanes (1937) as an explanation for the development of the characteristic blue colour of the starch-iodine reaction. Hanes (1937) suggested that the blue colour was due to the formation of a complex between a helical polysaccharide molecule and iodine, with the iodine occupying the helical cavity. Freudenberg (1939) also proposed that amylose possessed a helical structure. However, it was not until some years later (1944) that the helical structure of amylose was confirmed by X-ray diffraction studies of the amylose-iodine complex (Rundle *et al.*, 1944). Banks and Greenwood (1975) provide a detailed discussion of the starch-iodine interaction.

The amylose helical structure generated by the glucose residues possesses a hydrophobic interior (Krog, 1971) referred to as the helical cavity. Amylose molecules form a single helix and the complexing ligand occupies the hydrophobic helical cavity (Eliasson & Larsson, 1993) (Fig. 2.6). Ligands that complex with amylose include lipophilic substances such as aliphatic alcohols and fatty acids (Acker & Becker, 1971; Krog, 1971), monoglycerides, sodium dodecyl sulphate, lysophospholipids, lecithin, carboxylic acids and cyclic compounds (eg. cyclohexanol) (Eliasson & Krog, 1985; Eliasson, 1986; Collison *et al.*, 1960; Evans, 1986; Mikus *et al.*, 1946; Osman *et al.*, 1961; Legendijk & Pennings, 1970; Eberstein *et al.*, 1980; Gough *et al.*, 1985; Kowblansky, 1985; Eliasson, 1988). Perhaps the best known amylose complex is that of amylose and iodine which has been subjected to detailed X-ray structural analysis (Rundle *et al.*, 1944).

The structure of complexes between amylose and polar lipids has been referred to as V-

amylose (Eliasson, 1988) because, in the crystalline form the amylose complex gives a V-type X-ray diffraction pattern (Osman *et al.*, 1961; Zobel, 1964; Zobel *et al.*, 1976). X-ray diffraction studies indicate that the unit cell of the complex is orthorhombic with the dimensions $a = 13.0\text{\AA}$, $b = 23.0\text{\AA}$ and $c = 8.05\text{\AA}$ (Mikus *et al.*, 1946). X-ray diffraction studies have also revealed that the number of glucose residues per turn of the helix can vary with different ligands (Rundle, 1947; Zaslow, 1963; Yamashita, 1966). A six-fold helix, for example, is obtained with linear alcohols, but seven and eight-fold helices may form to accommodate bulkier ligands (Yamishita *et al.*, 1973; French & Murphy, 1977) such as branched alcohols (Zaslow, 1963; Yamishita, 1966). Normally, for a polar lipid (eg. monoglyceride), each turn of the amylose helix consists of six anhydroglucose residues with two to three turns per helix and a pitch of 8\AA (Eliasson & Larsson, 1993). Approximately two thirds of the lipid hydrocarbon chain is involved in the helix (Carleson *et al.*, 1979). The diameter of the helix is controlled by the size of the complexing ligand (Biliaderis & Galloway, 1989). The interior diameter of the helical cavity, for instance, increases with the number of glucose residues per turn of the helix. A helix which has six glucose residues per turn has a diameter of 4.8\AA compared to 7.8\AA for a helix containing eight glucose residues (Yamishita *et al.*, 1973). It is thought that the amylose helix is stabilized by hydroxyl groups (Kowblansky, 1985) which are oriented outward into the aqueous medium, while the hydrophobic groups of the helix are hidden within its central core.

2.3.2 Formation

Native starch very rarely shows the characteristic V-pattern of amylose-lipid complexes, although, in order to form complexes amylose must be in the V-crystalline form (Banks & Greenwood, 1975). It appears that amylose-lipid complexes are usually formed during swelling

and gelatinization of the starch granules (Morrison & Milligan, 1982; Eliasson & Larsson, 1993). Differential scanning calorimetry indicates the presence of amylose-lipid complexes only after heating to temperatures above the gelatinization temperature of the starch (Eliasson & Larsson, 1993).

In order to form complexes, it is best if both the amylose and the lipid (monoacyl lipid) are in solution. If not, the complex will take longer to form and may not form to the same extent (Eliasson & Larsson, 1993). Therefore, lipids must be in a suitable dispersed state to form complexes with amylose. For monoglycerides, the best aqueous dispersions for complex formation are the lamellar phase and liposomes (Morrison, 1988). Monoglycerides in the cubic phase are poor complexing ligands (Riisom *et al.*, 1984).

Complex formation is affected by several factors such as the pH, temperature and ionic strength at which complexation occurs (Hahn & Hood, 1987). Generally, complex formation is favoured by neutral or slightly acidic pH levels (Morrison, 1988). In fact, the same complexing ligand can form two different complexes with amylose, depending on the conditions at which complexation occurs (Kowblansky, 1985). Perhaps the most important factor affecting complex formation is temperature. Both high and low temperature complexes may be formed (Biliaderis & Galloway, 1989; Kowblansky, 1985) which exhibit high and low melting temperatures, respectively (Biliaderis & Galloway, 1989). Kowblansky (1985) has suggested that the high and low temperature complexes may both be helical inclusion complexes that differ in the number of glucose units per turn of the amylose helix.

Steric factors related to the structure of the ligand are of critical importance in complex formation (Bear, 1942). Complex forming ability differs among monoglycerides. However, the

best complex forming ability, as measured by a decrease in iodine binding capacity of starch, is exhibited by saturated monoglycerides (Osman *et al.*, 1961; Lagendijk & Pennings, 1970; Krog, 1971). Monoglycerides form complexes more readily than diglycerides, and saturated monoglycerides form complexes more easily than unsaturated monoglycerides (Eliasson & Larsson, 1993). Generally, as the chain length of the saturated fatty acid increases, the amount of fatty acid bound by amylose also increases. The same result is obtained by increasing the molecular weight of the saturated fatty acid (Hahn & Hood, 1987). However, increasing the unsaturation of the fatty acid causes a decrease in the amount of the fatty acid bound by amylose (Hahn & Hood, 1987). Differences in complex-forming ability between saturated and unsaturated monoglycerides may be attributed to differences in the shapes of the molecules (Hahn & Hood, 1987; Eliasson & Larsson, 1993). Saturated fatty acids with straight hydrocarbon chains may fit into the amylose helical cavity more easily than unsaturated fatty acid hydrocarbon chains which may be bent at various angles. Unsaturated fatty acids also have greater solubility in water and may therefore exist in the free state in higher amounts than saturated fatty acids which are less soluble (Hahn & Hood, 1987).

Although many authors have suggested that the amylose-lipid complex does not exist in native starch, but rather is formed during swelling and gelatinization of the starch granules, this may not be entirely true. Eliasson and Larsson (1993) have suggested that the amylose-lipid complex may be present in native starch, but that it may not be properly oriented in starch domains to diffract X-rays. This seems plausible since naturally occurring starch lipids are not easily removed with the usual organic solvents (Morrison, 1978). This is an indication that in starch, the lipid acyl chain is tightly bound to the hydrophobic amylose helical cavity.

2.3.3 Thermal Behaviour

The thermal behaviour of amylose-lipid complexes has been extensively studied by differential scanning calorimetry (DSC). Thermal properties of amylose-lipid complexes depend largely on the solvent used, the temperature at which the reactants are mixed, and the rate of cooling during preparation of the complex (Raphaelides & Karkalas, 1988). Other factors affecting the endothermic transition of the amylose-lipid complex include water content, chain length of the fatty acid involved in the complex, the type of polar group on the fatty acid, and the environment of the complex (Eliasson & Larsson, 1993).

Amylose-lipid complexes exhibit endothermic transitions at temperatures above the temperature range for starch gelatinization (Eliasson, 1988). The exact temperature range of the endothermic transition of the complex depends largely on the water content and the monoacyl lipid involved in the complex (Eliasson & Larsson, 1993). Biliaderis and Galloway (1989) observed small endothermic transitions at 45–47°C which they attributed to the melting of uncomplexed lipid (monoglyceride).

The thermal transition of the complex is reversible and on cooling, an exothermic transition occurs at ~20° below the endothermic transition. It is thought that thermal transitions may involve dissociation of the amylose-lipid complex (Eliasson & Larsson, 1993). Multiple endotherms have been observed when the water content of the complex is decreased (Biliaderis *et al.*, 1985; Raphaelides & Karkalas, 1988). This has been attributed to the formation of different forms of the complex during crystallization procedures (Biliaderis & Galloway, 1989).

Thermal transitions also depend on the characteristics of the complexing ligand. Dissociation temperatures of complexes between amylose and saturated fatty acids increase with

increasing chain length of the acid (Raphaelides & Karkalas, 1988; Biliaderis & Galloway, 1989), but complexes formed between amylose and unsaturated fatty acids exhibit an increase in dissociation temperature as the degree of unsaturation decreases (Raphaelides & Karkalas, 1988).

2.4 The Structure of Starch

2.4.1 Characteristics of the Starch Granule

Starch occurs naturally in plants as discrete, semi-crystalline granules (Lineback & Rasper, 1988). The size, shape, chemical composition and morphology of the starch granule will vary depending on its botanical source (Banks *et al.*, 1973). However, the exact structure of the starch granule, as well as the arrangement of amylose and amylopectin molecules at the surface of the granule, is not well understood (Lineback & Rasper, 1988; Eliasson & Larsson, 1993).

Microscopic examination of starch granules reveals that the granules tend to possess radial symmetry, and often exhibit well defined rings or lamellae, often referred to as "growth rings", especially after treatment with acid or amylolytic enzymes (Banks *et al.*, 1973; Eliasson & Larsson, 1993; Jenkins *et al.*, 1993). Growth rings (Fig. 2.7) are concentric shells surrounding the hilum in alternating layers of high and low refractive index, density, crystallinity, and resistance or susceptibility to chemical and enzymatic attack (French, 1984). Growth rings are oriented parallel to the outer surface of the starch granule (French, 1984) and range in size from 1200 to 4000Å (Yamaguchi *et al.*, 1979; Davis, 1994). Growth rings are composed of alternating layers of semi-crystalline and amorphous regions (Jenkins *et al.*, 1993) (Fig. 2.7). The semi-crystalline regions are in turn composed of stacks of alternating crystalline and amorphous lamellae (Yamaguchi *et al.*, 1979; Kassenbeck, 1979). The crystalline regions contain a high degree of order. Birefringence studies, as well as X-ray diffraction studies, indicate that the

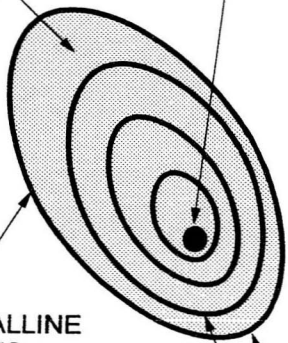
FIG. 2.7 Schematic illustration of the concentric growth rings of the starch granule, showing the alternating layers of crystalline and amorphous regions.

AMORPHOUS
REGIONS

HILUM

CRYSTALLINE
REGIONS

GROWTH RINGS



highly ordered crystalline regions are composed of amylopectin molecules (Meyer, 1942; Montgomery & Senti, 1958). Various starches have also been shown to retain their crystallinity even after all of the amylose has been leached from the granules (Eliasson & Larsson, 1993). The amorphous regions are less organized and are more susceptible to acid and enzymatic attack than the crystalline regions (Lineback & Rasper, 1988). The amorphous regions contain both amylose and amylopectin, and are rich in α -(1-6) branch points (Eliasson & Larsson, 1993; Jenkins *et al.*, 1993).

The most current models of the starch granule are based on the structure of amylopectin proposed by French (1984), in which the amylopectin molecules are oriented perpendicularly to the granule surface (Fig. 2.8). In current models, the crystalline structure of the starch granule consists of a radial arrangement of amylopectin clusters (Jenkins *et al.*, 1993). However, the exact arrangement of the polysaccharide chains within the granule is not clearly understood. Lineback (1984) proposed a model of the organization of the starch granule (Fig. 2.9) which incorporates the cluster model of amylopectin. In Lineback's (1984) model, amylose is intermingled through the amylopectin structure, the amylopectin clusters form the growth rings (crystalline regions), and the areas rich in branch points form the amorphous regions (Davis, 1994).

Fig. 2.8 Schematic illustration of the perpendicular orientation of amylopectin molecules within 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. (Adapted from French, 1984).

(a)



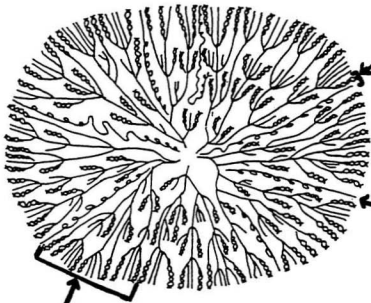
(b) Amorphous shell
of growth ring



Amorphous shell
of growth ring

Fig. 2.9 Schematic representation of the organization of starch components within a starch granule (Adapted from Lineback, 1984).

**GRANULE
SURFACE**



**AMP
DOUBLE
HELIX**

**AMYLOSE
HELIX**

**AMP
CLUSTER**

2.4.2 The Crystalline Structure of Starch

2.4.2.1 General

Starch granule crystallinity has been extensively studied with the X-ray diffraction technique. The classic X-ray diffraction studies of Katz and coworkers (Katz & van Itallie, 1930; Katz & Derksen, 1933; Katz, 1937) have led to the widespread acceptance that starch is a semi-crystalline material. The crystalline structures of native starches have since been classified into three forms: (i) the A pattern, characteristic of most cereal starches, (ii) the B pattern, characteristic of root, tuber and wrinkled pea starches, and (iii) the C pattern, characteristic of legume starches and certain root and tuber starches (Katz & van Itallie, 1930; Zobel, 1988; Colonna *et al.*, 1981; Hoover & Sosulski, 1985a; Gernat *et al.*, 1990) (Fig. 2.10). The A polymorph is composed of A-type unit cells (Fig. 2.11A), and the B polymorph is composed of B-type unit cells (Fig. 2.11B). The C polymorph, once thought to be a structure of its own (Zobel, 1988), has recently been shown to be a mixture of A and B-type unit cells (Gernat *et al.*, 1990). This is consistent with earlier studies which indicate that the C pattern is intermediate between the A and B patterns (Hizukuri, 1969; Hizukuri *et al.*, 1965). The different X-ray diffraction patterns (A, B, C) result from the way in which the amylopectin double helices are packed into the unit cells (Eliasson & Larsson, 1993). Thus, starch crystallinity is due to the packing arrangement of double helices (Wu & Sarko, 1978a,b).

The unit cells of the A and B polymorphs are structurally very similar. Both contain amylopectin double helices arranged in pairs to minimize the energy of the interaction, with the starch chains arranged in parallel fashion (Hoover & Vasanthan, 1994). The A-type unit cell (Fig. 2.11A) has been described as a centred orthorhombic with the dimensions $a=11.90\text{\AA}$,

Fig. 2.10 Typical X-ray diffraction spectra for starches exhibiting A-, B-, and C-type X-ray patterns (Adapted from Banks & Greenwood, 1975).

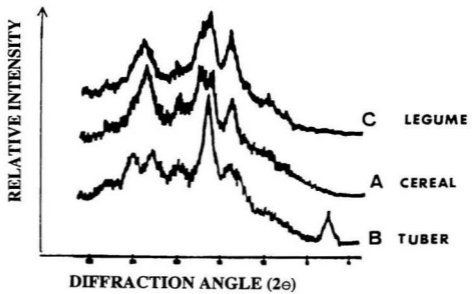
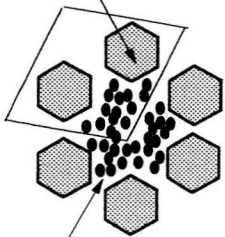
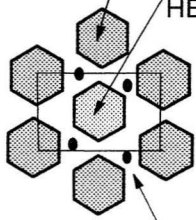


Fig. 2.11 Double helix packing arrangements in A- and B-type unit cells. A - the A-type unit cell. B - the B-type unit cell. (Adapted from Wu & Sarko, 1978a, b)

PAIRS OF AMYLOPECTIN
DOUBLE HELICES

AMYLOSIC
HELIX



A-TYPE

B-TYPE

WATER MOLECULES

$b=17.70\text{\AA}$ and $c=10.52\text{\AA}$ (Wu & Sarko, 1978b). The A-type unit cell has twelve double helices, arranged in pairs, and four to eight water molecules, with the central cavity occupied by an amylosic double helix. The water molecules are situated between the pairs of double helices. Adjacent double helices are linked by direct hydrogen bonds (Hoover & Vasanthan, 1994). The A-type polymorph has recently been characterized by the packing of left-handed parallel-stranded double helices (Imberty *et al.*, 1987). The B-type unit cell (Fig. 2.11B) is hexagonal with the following dimensions: $a=b=18.50\text{\AA}$ and $c=10.40\text{\AA}$ (Wu & Sarko, 1978a). Like the A-type unit cell, the B-type unit cell also contains twelve double helices arranged in pairs. However, it contains thirty-six water molecules which are present in the centre of six hexagonally arranged pairs of double helices. Adjacent double helices are predominantly linked by hydrated water bridges and some hydrogen bonds (Hoover & Vasanthan, 1994).

Recent studies indicate that the A polymorph is the most thermodynamically stable form, however, the B polymorph is the kinetically favoured form (Gidley, 1987; Gidley & Bulpin, 1987). Thus, the stability of the different polymorphs follows the order: $A > C > B$. Several researchers (Hizukuri, 1986; Hizukuri *et al.*, 1983) have suggested that the average chain length of amylopectin may determine the type of crystal polymorph observed among native starches. The average chain length of amylopectin in A-type starches is 25, that of B-type starches is 34, while the average chain length of the C-type starches is 28 (intermediate between the A- and B-types) (Eliasson & Larsson, 1993).

2.4.2.2 Legume Starches

Legume starches exhibit a C-type X-ray diffraction pattern (Fig. 2.10) which is a mixture of A and B type unit cells (Gernat *et al.*, 1990). In legumes, the starch granules consist of pure

A-type as well as pure B-type unit cells in varying proportions (Gernat *et al.*, 1990). It has been shown that crystallites of pea starch are composed of 38.6% type B and 61.4% type A, while in broad bean starch the corresponding values are 17 and 83% (Gernat *et al.*, 1990). The tendency toward A type structure for broad bean and B type structure for pea starch was also reported by Colonna *et al.* (1981) who showed by means of X-ray diffraction studies on Linternized starches from pea and broad bean that the proportion of crystalline fraction (50%) of these starches were higher than that of potato starch (Robin *et al.*, 1974), but were comparable to those of cereals (Robin *et al.*, 1975). Furthermore, the above legume starches were shown to retain their C-type spectra even after Linternization. However, in wheat starches, a change in X-ray spectra (A-type - B-type) has been observed during Linternization. Hoover and Sosulski (1985) showed that the X-ray intensities of the major peaks varied widely, even among legume starches belonging to the same biotype. It is likely that this could be due either to differences in crystallite orientation or to the amount of crystallites, or both.

2.5 Gelatinization

It is well known that starch granules are insoluble in cold water. Their insolubility is due to an extensive network of hydrogen bonding between neighbouring hydroxyl groups of "individual" starch molecules. Although hydrogen bonding forces are weak, they are so numerous that they prevent the granules from dissolving (in cold water). When starch is heated in excess water to a temperature just below its gelatinization temperature, the granules swell reversibly (Banks & Greenwood, 1975). However, when starch granules are heated in excess water above the gelatinization temperature, swelling is irreversible and is accompanied by several changes within the starch granule.

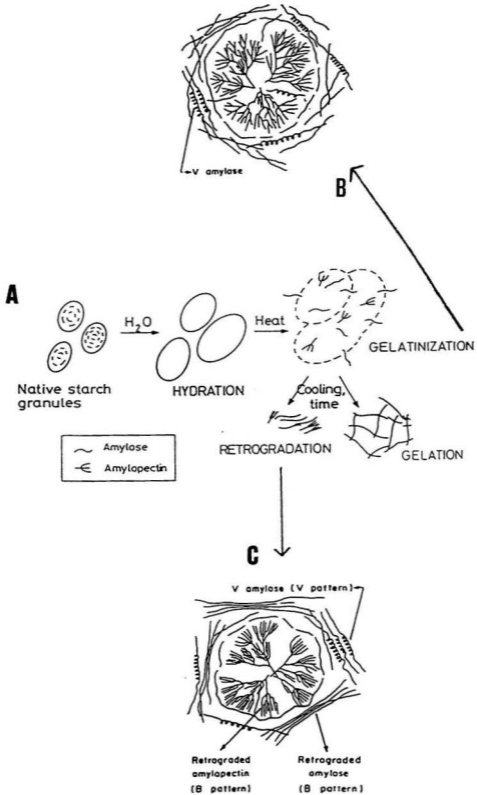
When aqueous suspensions of starch granules in excess water are heated, a temperature is reached (the "gelatinization temperature") at which the hydrogen bonds holding the starch granules together are weakened and water can then be absorbed by the granules (Glicksman, 1969). This aqueous suspension then undergoes an order-disorder, non-equilibrium phase transition called "gelatinization" (Donovan, 1979; Biliaderis *et al.*, 1980) over a temperature range (the "gelatinization temperature range") which is characteristic of the starch source. During gelatinization, numerous irreversible changes occur in the starch granule: water diffuses into the granule; the granule becomes hydrated and swells; heat is absorbed; crystallinity (birefringence) is lost; and amylose leaches out of the granule into the surrounding matrix (Stevens & Elton, 1981; Lelievre & Mitchell, 1975; Donovan, 1979; Hoover & Hadziyev, 1981), which leads to an increase in starch paste viscosity. Thus, starch gelatinization has been described as the dramatic changes which occur in the starch granule structure when it is heated in water (Fig. 2.12).

2.5.1 Mechanism

The mechanism by which gelatinization occurs is not clearly understood. However, it is widely accepted that gelatinization refers to the disruption of the starch granule structure (Zobel, 1984) followed by the collapse of the molecular order within the starch granule (Atwell *et al.*, 1988). Starch gelatinization may also be considered a non-equilibrium melting process of semicrystalline, kinetically metastable native starch in the presence of water (Atwell *et al.*, 1980).

Starch gelatinization is a two-step process which involves disruption of the weaker amorphous regions, followed by disruption of the more stable crystalline regions of the starch granule. When an aqueous suspension of starch granules is heated above its gelatinization

Fig. 2.12 Schematic illustration of the changes which occur in the starch granule during gelatinization and retrogradation. (Adapted from Aguilera & Stanley, 1990; Biliaderis, 1991).



temperature, hydrogen bonds between neighbouring starch molecules within the granule are weakened, thereby allowing water to be absorbed by the granules (Glicksman, 1969; Hoseney *et al.*, 1986). Hydration and swelling of the starch granule occurs first in the weaker amorphous regions (Hoseney *et al.*, 1986; Biliaderis *et al.*, 1980; Donovan, 1979) at the hilum of the granule (Glicksman, 1964; Hoseney *et al.*, 1986). The amorphous region hydrates first because of its lower order of crystallinity (Glicksman, 1964), which also makes it more susceptible to heating in the presence of water than the crystalline region. Swelling of starch granules and weakening of hydrogen bonds start at the hilum of the granule because crystallites between the hilum and the first growth ring are under more stress than those further from the centre (Hoseney *et al.*, 1986) (Fig. 2.13). Therefore, these crystallites are most likely to melt first.

Swelling of the amorphous region makes the crystalline region of the granule more susceptible to melting (Fig. 2.14) by imparting a stress on the crystalline regions, thereby allowing the granule to become disordered quickly (Biliaderis *et al.*, 1980; Donovan, 1979; Evans & Haisman, 1982). Melting of the starch crystallites occurs due to disordering of the double helices forming the crystalline clusters, followed by the dissociation of the double-helices to give loosely ordered semi-random external chains (Tester & Morrison, 1990a,b) (Fig. 2.15). Therefore, melting of starch crystallites may be considered a solvation assisted helix-coil transition. As the temperature of the starch-water suspension increases above the gelatinization temperature range, hydrogen bonds continue to be disrupted and hydrated. Consequently, the granules continue to swell. Eventually, the starch molecules become solubilized, and fully hydrated starch particles diffuse out of the granules into the surrounding aqueous medium. This leads to an increase in solution viscosity and clarity, (Oikku & Rha, 1978). At this point, the starch-water suspension is

Fig. 2.13 Schematic representation showing the susceptibility of different starch crystallites to swelling and melting during gelatinization (adapted from Hosoney *et al.*, 1986)

LINES REPRESENTING
ARRANGEMENT OF
STARCH CRYSTALLITES
WITHIN THE STARCH
GRANULE

CRYSTALLITES IN
"FAN-LIKE"
ARRANGEMENT ARE
LESS STABLE

MELT
FIRST!

HILUM

CRYSTALLITES IN
PARALLEL
ARRANGEMENT ARE
MORE STABLE

GROWTH
RINGS

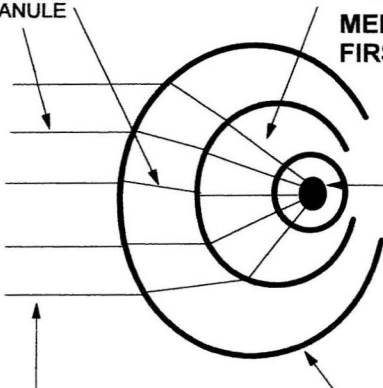


Fig. 2.14 Schematic representation of solvation-assisted melting of starch crystallites within the starch granule. (Adapted from Biliaderis *et al.*, 1980)

**STARCH
CRYSTALLITE**

**AMORPHOUS
REGION**

WATER-HEAT

**STARCH
CHAINS**

**DESTABILIZING
EFFECT**

SWELLING

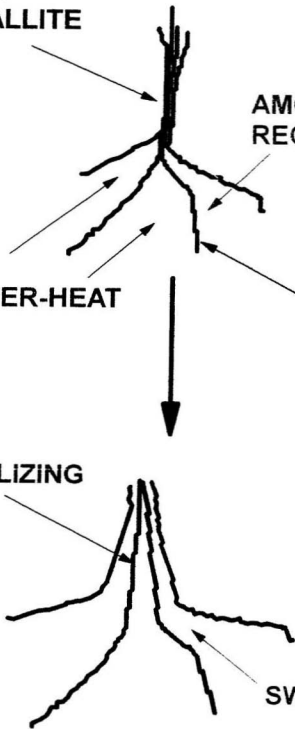


Fig. 2.15 Schematic representation showing melting of starch crystallites due to disordering and dissociation of amylopectin double helices forming the crystalline clusters.

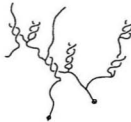
CRYSTALLITE



Cold water insoluble

1st Transition

**Double Helix &
Entanglements
Unravel**



**High swelling
Low solubilization**

2nd Transition

**Random Coil
Disordered**



High solubilization

referred to as a starch paste. The swollen starch granules become increasingly susceptible to shear, mechanical and thermal breakdown, and the bonding forces become very weak. Some starch solubles may diffuse back into the swollen granules. As the starch-thickened mixture cools, a gel-like mass is formed (called gel formation or gelation) which is held together by associative bonding (Olkku & Rha, 1978).

As the starch-thickened mixture cools after gelatinization is complete, bonds form between starch molecules in the mixture. Bonds that form between the branches of amylopectin molecules are very weak. However, bonds that form between the long-chain amylose molecules are relatively strong and form readily. This bonding produces a three-dimensional network which entraps water, thereby increasing the rigidity of the starch mixture. Thus, gelation (gel formation) is a different process from gelatinization. Gelation occurs on cooling the starch paste after the starch granules have been gelatinized. Gel formation is a slow process which proceeds over a period of several hours as the starch paste cools. Waxy varieties of starch (without amylose) do not form gels. It has been shown (Miles *et al.*, 1985) that amylose gelation is the result of a phase separation which produces regions that are rich (contain amylose) and deficient (contain amylopectin) in polymer. If the amylose content is sufficiently high, the region rich in polymer forms a three dimensional network .

2.5.2 Legume Starches

Many methods have been used to determine the gelatinization temperatures of starches including differential scanning calorimetry (Donovan, 1979), Kofler hot stage microscopy (Watson, 1964), viscoamylography (Sathe *et al.*, 1981), X-ray diffraction (Zobel *et al.*, 1988), pulsed nuclear magnetic resonance (Lelievre and Mitchell, 1975), and small-angle lightscattering

photometry (Marchant & Blanshard, 1980). However, only Kofler hot stage microscopy and differential scanning calorimetry have been used in studying the gelatinization parameters of legume starches.

The gelatinization temperatures (onset, T_o , midpoint, T_p , and end, T_c) and the enthalpy of gelatinization (ΔH) of legume and cereal starches are presented in Table 2.4. As shown (Table 2.4), legume starches exhibit wide variations in their gelatinization parameters. This suggests that structural differences exist within the crystalline (differences in the number of double helices within the crystalline clusters or the degree of crystalline perfection, or both), or the amorphous (degree of packing of amylose chains) regions of legume starches, or both. Furthermore, the gelatinization parameters of legume starches are generally higher than those of wheat and corn starches (Table 2.4). These differences are probably due to the longer average chain length of legume amylopectins (Table 2.3). As a result, the number of double helices that unravel and melt during gelatinization would be higher in legume starches than in corn and wheat starches.

2.6 Retrogradation

As stated previously, starch granules undergo irreversible swelling when heated in excess water above their gelatinization temperature, which results in amylose leaching into the surrounding aqueous medium. In the presence of a high starch concentration, when the starch thickened mixture cools, an elastic gel forms. The molecular interactions which occur after the gel has formed have been called "retrogradation". During storage of starch gels, additional bonds are formed between straight-chain amylose molecules, as well as between helical regions of amylose molecules. Some of the amylose molecules become aggregated in a particular area in an organized, crystalline manner. As these amylose molecules pull together, the gel network shrinks,

Table 2.4 Thermal characteristics of legume and cereal starchesGelatinization Transition Temperatures (°C)^a

Starch Source	To	Tp	Tc	ΔH ^b (cal/g)
Legumes				
DSC ^c	47 to 67	57 to 76	70 to 87	3.0 to 4.0
Kofler Hot Stage	58 to 71	59 to 76	61 to 85	-
Cereals				
DSC ^d	57 to 65	62 to 73	67 to 83	2.7 to 3.8
Kofler Hot Stage ^e	58 to 68	61 to 74	64 to 78	-

^a To, Tp and Tc indicates the temperature of the onset, mid point and end of gelatinization.

^b Enthalpy of gelatinization.

^c Differential scanning calorimetry.

^d For amylo maize, To = 63, Tp = 76, Tc = 82^o C , and ΔH = 1.5 cal/g.

^e For amylo maize, To = 67, Tp = 80, Tc = 92^oC.

(Hoover & Manuel, 1995, 1996; Hoover & Vasanthan, 1992, 1993; Hoover *et al.*, 1994; Hoover & Sosulski, 1985a; Biliaderis *et al.*, 1980; Schoch & Maywald, 1968; Sathe *et al.*, 1982; El Faki *et al.*, 1983a; Knight, 1969; Fleche, 1979; Whistler & Pashcall, 1965; Glicksman, 1969; Wurzburg, 1972; Leach *et al.*, 1959).

and water is pushed out of the gel in a process called "syneresis". Thus, syneresis occurs as a result of the increased association of starch molecules as a starch gel ages. The above interactions are both time and temperature dependent (Hoover & Sosulski, 1991).

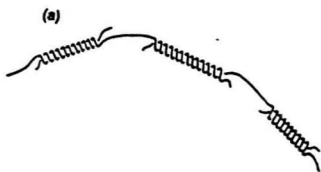
Recrystallization in amylose gels occurs in the polymer rich regions by extensive helix-helix aggregation (Fig. 2.16) (Miles *et al.*, 1985; Gidley, 1989). As the gel continues to age, recrystallization of amylopectin in the polymer deficient regions may also occur. (Cairns *et al.*, 1991). Therefore, retrogradation of starch gels may be viewed as the crystallization of the amylopectin skeletons of the gelatinized granules resulting in a stiffening of the granular structure and enhancement and reinforcement of the amylose matrix (Miles *et al.*, 1985) (Fig. 2.12).

Legume starches have been shown to exhibit high retrogradation rates (Hoover & Sosulski, 1985a; Tjahjadi & Breene, 1984). This makes native legume starches unsuitable for use in many commercial foods, which require low temperature storage (Hoover & Sosulski, 1991). However, modification by acetylation and hydroxypropylation has been found to greatly reduce the extent of retrogradation (measured by extent of syneresis) (Hoover & Sosulski, 1986; Hoover *et al.*, 1988).

2.7 Swelling Capacity and Amylose Leaching

Starch granule swelling is known to begin in the bulk, relatively mobile amorphous fraction, and in the more restrained amorphous regions immediately adjacent to the crystalline regions of the granule. Tester and Morrison (1990a,b) have shown, by comparative studies on normal and waxy barley starches, that swelling is primarily a property of amylopectin and that amylose is a diluent. These authors also showed that amylose and lipids in normal starches could inhibit granule swelling under conditions where amylose-lipid complexes are likely to be formed.

Fig. 2.16 Recrystallization in amylose gels by helix-helix aggregation. (a) Helix formation and chain elongation. (b) Lateral association of helical regions. (Adapted from Morris, 1990).



Many researchers have determined the swelling capacity of legume and cereal starches using the method of Leach and coworkers (1959), which does not distinguish between intragranular water and intergranular or interstitial water. Recently, Tester and Morrison (1990a) developed a method for measuring only intragranular water and, hence, the true swelling capacity (termed "swelling factor") at a given temperature. This method is based on the observation that blue dextran dye ($MW = 2 \times 10^6$) will dissolve in supernatant and interstitial water but not in the intragranular water.

The degree of swelling determined by both the above methods (Hoover & Sosulski, 1985a; Tester & Morrison, 1990a, b; Hoover *et al.*, 1994; Hoover & Vasanthan, 1993, 1994a, b; Hoover & Manuel, 1995, 1996) has shown that granular swelling occurs to a greater extent in cereal than in legume starches. The restricted swelling of legume starches is probably due to stronger associations between amylopectin chains and/or to a more compact arrangement of the amylose chains within the amorphous regions. It is likely that the above differences in swelling capacity could be due to differences in the magnitude of bonding forces between amylose chains of cereal and legume starches, since the extent of amylose leaching in legume starches is comparable to that of corn and is much higher than in wheat (Hoover & Vasanthan, 1992, 1993, 1994; Hoover *et al.*, 1994; Hoover & Manuel, 1995, 1996).

2.8 Heat-Moisture Treatment (HMT)

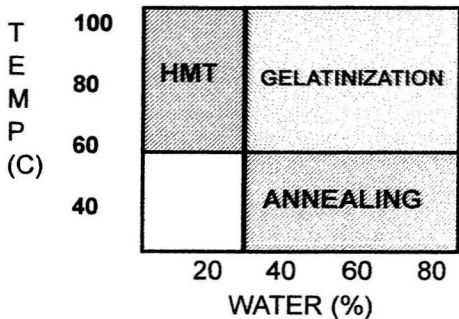
Heat-moisture treatment (HMT) is the term given to the process of heating starch at elevated temperatures (above the gelatinization temperature) under semi-dry conditions (water content is lower than that required for gelatinization) (Lorenz & Kulp., 1981, 1982; Kulp & Lorenz, 1981) (Fig. 2.17). Heat-moisture treatment at 18-30% moisture and 100°C has been

shown to alter the physicochemical properties (eg. amylose leaching, swelling capacity, X-ray diffraction intensities and pattern, susceptibility to attack by enzyme and acid) of normal maize, waxy maize, high amylose maize, wheat, oat, barley, potato, yam, pigeon pea and Laird lentil starches (Sair & Fetzer, 1944; Sair, 1967; Donovan *et al.*, 1983; Kulp & Lorenz, 1981; Lorenz & Kulp, 1982; Hagiwara *et al.*, 1991; Stute, 1992; Hoover & Vasanthan, 1994; Lorenz & Kulp, 1981; Hoover & Manuel, 1996; Franco *et al.*, 1995; Kawabata *et al.*, 1994; Maruta *et al.*, 1994; Hoover *et al.*, 1994). The magnitude of these changes was found to be dependent upon the moisture content during heat treatment as well as the starch source. The effects of heat-moisture treatment on the above properties are due to interplay between the following factors: (i) structural changes which occur in the crystalline and amorphous regions of the starch granule; and (ii) physical modifications to the surface of the starch granule, which occur during heat-moisture treatment.

Numerous structural changes have been shown to occur during heat-moisture treatment of starch granules, which include: (i) new crystallite formation and/or crystallite reorientation (Donovan *et al.*, 1983); (ii) conversion of B-type starches to an A + B type (Sair & Fetzer, 1944; Sair, 1967; Kulp & Lorenz, 1981; Lorenz *et al.*, 1982; Stute, 1992; Donovan *et al.*, 1983); (iii) increased associations between starch components (amylose-amylose, amylose-lipid, and/or amylose-amylopectin) (Hoover & Vasanthan, 1994; Hoover & Manuel, 1996); and (iv) conversion of amorphous amylose to a helical form (Banks & Greenwood, 1975).

The classic heat-moisture treatment studies by Sair (Sair & Fetzer 1944; Sair, 1967) showed that heating of starches at restricted moisture levels dramatically changes the properties of starches, especially tuber starches (eg. potato starch). In these studies, the properties of tuber

Fig. 2.17 Schematic representation of the temperature and moisture differences between gelatinization and heat-moisture treatment (HMT).



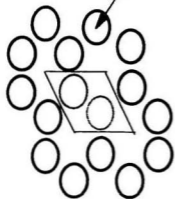
starches (gelatinization temperature ranges, swelling behaviour, and paste translucency) after heat-moisture treatment, approached those properties characteristic of the cereal starches. The X-ray diffraction patterns of potato starches were altered from a B-type to an A + B type pattern, as were those of corn and other cereal starches (Sair, 1964; Lorenz *et al.*, 1982). More recently, Lorenz and coworkers (1982) showed that cereal starches (barley, red millet and triticale) retained their A-type pattern after heat-moisture treatment, but exhibited a decrease in crystallinity.

The most obvious structural change which occurs during heat-moisture treatment of starch granules is the "B" to "A" transition, which occurs to varying extents depending on the severity of the treatment as well as the starch source (Stute, 1992). The "B" to "A" transition occurs as a result of dehydration of the B-type unit cell (36 water molecules) to form the A-type unit cell (8 water molecules) (Sarko & Wu, 1978a,b) (Fig. 2.18). During heat-moisture treatment, the double helices of the B-type unit cells rearrange in such a way that the central core of the unit cell, which was originally occupied by 36 water molecules (B-type), is now occupied by an amylosic helix (similar to A-type unit cells [Fig. 2.11A]). Therefore, as more and more B-type cells are converted to A-type cells, heat-moisture treated starches exhibit a C-type X-ray diffraction pattern, but never really become A-type starches (Stute, 1992). This explains the different sensitivities of different starches to heat-moisture treatment: (i) B-type starches are very sensitive; (ii) C-type (A + B unit cells) starches require more severe treatment conditions to induce the B to A transition; and (iii) A-type starches cannot be heat-moisture treated in this sense.

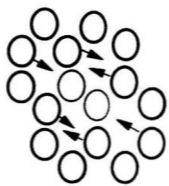
The changes in physicochemical properties of tuber starches in the direction of cereal starches has been attributed to two factors: (i) dehydration, which causes the crystallographic

- Fig. 2.18 Schematic representation of the B to A transition of B-type unit cells during heat-moisture treatment. (Adpated from Wu & Sarko, 1978a, b).
1. B-type unit cells.
 2. Rearrangement of double helices in the B-type unit cells.
 3. Formation of A-type unit cells with the central core of the unit cells occupied by a double helix.

PAIRS OF
AMYLOPECTIN
DOUBLE HELICES



1. B-TYPE



2. REARRANGEMENT



3. A-TYPE

pattern to change from the B-type to an A + B type (C-type) pattern; and (ii) conversion of a fraction of amorphous amylose to a helical form (Banks & Greenwood, 1975).

Dehydration is important since B-type (36 water molecules) starches are intragranularly bound by water bridges, which are converted, by heat-moisture treatment, to the direct hydrogen bonds that predominate in most cereal starches (Kulp & Lorenz, 1981). Conversion of some of the amorphous amylose to helical amylose during heat-moisture treatment is feasible for potato and other tuber starches since they are essentially free of lipid (Kulp & Lorenz, 1981). However, its effect is somewhat minimized in cereal starches because the amylose component occurs mainly in the form of amylose-lipid complexes (Kulp & Lorenz, 1981). This may also partly explain the low response of cereal starches to heat-moisture treatment. Conversion of amorphous amylose to the helical form decreases the solubility and swelling of starch granules, and consequently stabilizes the granules (Kulp & Lorenz, 1981). This increased stabilization probably occurs since the helical regions are thought to act as weak centres of crystallinity when the granule gelatinizes (Banks & Greenwood, 1975). Kulp and Lorenz (1981) showed that heat-moisture treatment of wheat starch caused only limited changes in the amylose fraction and to rearrangement of the polymers. They suggested that this low response was due to the presence of amylose-lipid complexes. The above authors also suggested that the effect was confined to the amorphous regions of the granules, since both solubility and enzyme susceptibility increased, but little change was observed in swelling power.

Donovan and coworkers (1983) used differential scanning calorimetry and X-ray diffraction to study the effects of heat-moisture treatment on wheat and potato starches. They also observed a B to A transition for tuber starches, and a decrease in crystallinity of cereal

starches during heat-moisture treatment (18-30% moisture, 100°C, 16 hours). They also noted that the physical properties of tuber starches improved, whereas those of cereal starches deteriorated, after heat-moisture treatment. Therefore, this suggests that physical changes occur within the starch granules during heat-moisture treatment. The above authors postulated that the effects of heat-moisture treatment are due either to new crystallization, or to recrystallization and perfection of the small crystalline regions of the granule. Starch chain reorientations in crystallites are accompanied by chain reorientation or movement in amorphous regions. Alterations in the amorphous regions have a pronounced effect on the ability of these regions to destabilize crystallites upon imbibition of water by the granule, and thus upon the gelatinization temperature, which has been shown to broaden in cereal (wheat) and tuber (potato) starches following heat-moisture treatment.

Recently, Hoover and Vasanthan (1994) and Hoover and Manuel (1996) reported that the extent of starch chain associations within amorphous regions, as well as the degree of crystallinity, are altered during heat-moisture treatment of wheat, oat, normal maize, waxy maize, high amylose maize, potato and Laird lentil starches. Furthermore, amylose-lipid interactions and crystallite reorientation has also been found to occur on heat-moisture treatment (Hoover & Manuel, 1996). The stability of the newly formed crystallites has been shown (by DSC analysis) to differ from that of the crystallites in the native starch (Hoover & Manuel, 1996). Lorenz and coworkers (1983) attributed the increased susceptibility of wheat and potato starches to amylases, following heat-moisture treatment, to a change in reorientation of the starch polymers in the granules, as well as a certain degree of degradation to the granules which rendered the starch granules more accessible to enzymolysis. Similarly, Hoover and coworkers (1993) attributed the increase in

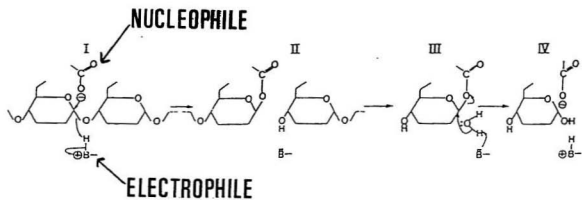
both acid and enzyme hydrolysis of pigeon pea starch following heat-moisture treatment to the interplay of the following factors: (i) changes in crystallite reorientation; (ii) modifications to the granular surface; and (iii) the degree of starch chain associations within the amorphous regions.

2.9 Digestibility of Starch by Porcine Pancreatic α -amylase

Starch digestion by porcine pancreatic α -amylase occurs by a multiple attack mechanism (Robyt & French, 1967), whereby the direction of attack starts at the reducing end of the starch molecule and moves towards the non-reducing end (Robyt & French, 1971). Alpha-amylase is an endoenzyme which cleaves α -(1-4) glucosidic bonds (Banks & Greenwood, 1975), but bypasses α -(1-6) glucosidic bonds without cleaving them. The products of α -amylase digestion assume the α configuration at the anomeric carbon, and include glucose and maltose (Banks & Greenwood, 1975). Porcine pancreatic α -amylase, which is widely used for studies on starch digestibility, has an active site which consists of five D-glucose sub-sites (Robyt & French, 1970), as well as a catalytic site consisting of imidazolium cations (which act as electrophiles) and carboxylate anions (which act as nucleophiles) (Fig. 2.19) (Hoover & Sosulski, 1985b).

Differences in *in vitro* digestibility of native starches both between and among species have been attributed to the interplay of several factors: (i) granule size (Snow & O'Dea, 1981; Ring *et al.*, 1988); (ii) presence of amylose-lipid complexes (Holm *et al.*, 1983), which tends to decrease digestibility; (iii) starch-protein interactions (Würsch *et al.*, 1986), which also tend to decrease digestibility; (iv) amylose/amylopectin ratio (Dreher *et al.*, 1984; Hoover & Sosulski, 1985a; Holm & Björk, 1988; Ring *et al.*, 1988); (v) percentage of retrograded starch (Ring *et al.*, 1988); (vi) extent of molecular associations between starch components (Dreher *et al.*, 1984; Hoover & Sosulski, 1985a; Holm & Björk, 1988); (vii) physical distribution of starch in relation

Fig. 2.19 Hydrolysis of $\alpha(1-4)$ glucosidic bonds via electrophilic and nucleophilic attack by porcine pancreatic α -amylase. (Adapted from Hoover & Sosulski, 1985b).



to dietary fibre components (Rao, 1969; Snow & O'Dea, 1981; Dreher *et al.*, 1984); (viii) degree of crystallinity (Dreher *et al.*, 1984; Hoover & Sosulski, 1985a; Ring *et al.*, 1988); (ix) amylose chain length (Jood *et al.*, 1988); (x) drying methods and storage conditions (Kayisu & Hood, 1979); and (xi) differences in α -amylase activity of enzyme preparations (Hoover & Sosulski, 1991). However, the mechanism by which enzyme digestion by α -amylase proceeds is not well understood. Thoma (1968) postulated that enzyme-catalyzed hydrolysis of the α -(1-4) glucosidic bonds of starch molecules involves enzyme-induced ring distortion of one of the D-glucosyl residues from the 4C_1 chair conformation to a "half chair" conformation (Fig. 2.20). Ring distortion decreases the enthalpy of activation (Fig. 2.21) and increases the susceptibility of the glucosyl residues to nucleophilic attack by functional groups on the enzyme, and water (Fig.2.20). László and coworkers (1978) have shown that ring distortion or the "half chair" conformation is involved in the transition state of α -amylase. Based on this theory, Hoover and Manuel (1995) postulated that conformational changes from the chair to the half chair, during α -amylase hydrolysis (of lentil starches) may be difficult for amylose chains that are complexed by native lipids, due to reduced chain flexibility. Therefore, it seems plausible that the same may be true for strongly associated starch chains and tightly packed starch crystallites in the amorphous and crystalline regions of the granule.

2.9.1 Legume Starches

Starch digestibility will vary depending on the starch source, and on processing and storage conditions (Dreher *et al.*, 1984). Legume starches in foods are more digestible than tuber and high amylose maize starches, but less digestible than cereal starches (Dreher *et al.*, 1984; Hoover & Sosulski, 1985a; Ring *et al.*, 1988). In a 24 hour period, wheat, maize, smooth pea

Fig. 2.20 Schematic representation of the chair (a) and half-chair (b) conformations of the D-glucosyl residues of starch molecules during hydrolysis by α -amylase.

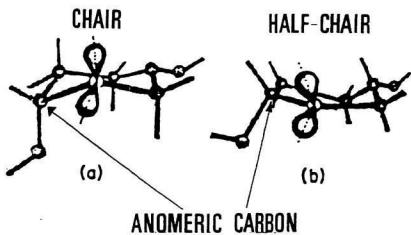
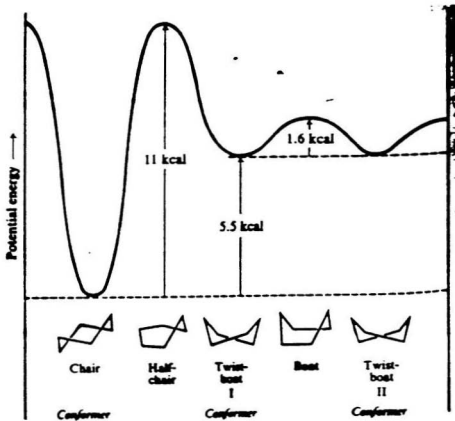


Fig. 2.21 Potential energy diagram of glucopyranose ring conformers (Adapted from László *et al.*, 1978). The half-chair conformer has a higher potential energy than the chair conformer. Thus, a lower activation energy is required for the hydrolysis reaction to occur when the D-glucosyl residues are in the half-chair conformation.



and potato starches were hydrolyzed to the extents of 100%, 95%, 67% and 15%, respectively, by porcine pancreatic α -amylase (Ring *et al.*, 1988). However, Hoover and Vasanthan (1994) showed that in a 72 hour period, wheat, oat, lentil, potato and yam starches were hydrolyzed to the extents of, 66.1%, 32.0%, 65.0%, 5.0% and 1.5%, respectively, by porcine pancreatic α -amylase. The differences observed between starches from the same botanical source (wheat and potato) in the two studies may be due to several factors (eg. amylose content, amylose-lipid complexes, extent of starch chain associations, enzyme preparation, α -amylase activity).

In addition to the factors mentioned previously, starches from various legume sources have been shown to differ in digestibility by α -amylase due to: (i) varying amylose contents (high amylose content decreases digestibility) (Rao, 1969); (ii) drying, isolation and fractionation methods used (Biliaderis *et al.*, 1981; Hellendoorn, 1969); (iii) degree of polymerization; (iv) microheterogeneity of the starch; (v) presence of non-starchy components such as proteins and lipids; (vi) presence of α -amylase inhibitors; and (vii) presence of other carbohydrate substances (eg. cellulose) (Reddy *et al.*, 1989). Dreher and coworkers (1984) have suggested that the digestibility of poorly digestible starches (legume and tuber starches) is improved by cooking, probably due to changes which occur in granular crystallinity, starch gelatinization, or inactivation of α -amylase inhibitors, which are naturally present in most legumes.

The mode of action of α -amylase on legume starches differs between and among species. Hoover and Sosulski (1985a), and Ramadas Bhat and coworkers (1983) observed (using scanning electron microscopy) that legume starches attacked by porcine pancreatic α -amylase exhibited highly roughened surfaces which is characteristic of surface erosion. Hoover and Manuel (1995), however, observed that while Laird lentil starch exhibited highly roughened surfaces and

numerous fissures after 72 hours hydrolysis by porcine pancreatic α -amylase, CDC Gold lentil starch exhibited fissures and craters of varying size and depth, indicating that the enzyme had entered the granule and preferentially hydrolyzed the interior portion. The mode of attack may also vary depending on the source of the enzyme. El Faki and coworkers (1983) observed that digestion of cowpea and horse gram starches by salivary α -amylase resulted in pitting and surface erosion of the granules. In comparison, cereal starches exhibited pinholes on the surface layer of the granule, with pores penetrating into the granule (Dronzek *et al.*, 1972; MacGregor & Ballance, 1980; Hoover & Sosulski, 1985a).

CHAPTER 3.

MATERIALS AND METHODS

3.1 Materials

The following legume seeds were obtained from the Department of Crop Science, University of Saskatchewan: Green Arrow pea (wrinkled pea) (*Pisum sativum* L.); Express field pea (smooth pea) (*Pisum sativum* L.); black bean (*Phaseolus vulgaris* L.); Othello pinto bean (*Phaseolus vulgaris* L.); and Eston lentil (*Lens culinaris* Medik). The legume seeds were divided into two lots representing whole samples. Each lot was further subdivided into two parts and starch was extracted from them using the procedure outlined below. Crystalline porcine pancreatic α -amylase (EC 3211), type 1A was obtained from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals and solvents were analytical grade.

3.2 Starch Isolation and Purification

Starch was isolated from legume seeds using a modification of the procedure of Schoch and Maywald (1968). Approximately 500 g of seeds were steeped overnight in 300 mL of tap water containing 0.01% sodium metabisulfite at room temperature. The swollen seeds were then washed and homogenized with distilled water (1 part soaked seed : 3 parts distilled water) in a Waring blender for three minutes at low speed, followed by another three minutes of blending at high speed. The resulting slurry was then filtered through a double layer of cheese cloth in a Büchner funnel using suction. The solid particles trapped by the cheese cloth were discarded and the slurry which passed through the cloth was allowed to stand overnight, with sodium metabisulfite, while the sediment settled. The supernatant was discarded and the remaining

sediment was washed with 0.02N sodium hydroxide and allowed to settle. This step was repeated until the standing supernatant was clear. Washing with sodium hydroxide removes the residual proteins. The final sediment was suspended in distilled water and filtered through a 70 micron polypropylene screen. The resulting filtrate was then filtered through a 10 micron polypropylene screen, neutralized to pH 7.0, and filtered through a Whatman #4 filter paper on a Büchner funnel with suction and thoroughly washed with distilled water. The filter cake was air dried overnight at room temperature followed by oven drying at 40°C for 24 hours.

3.3 Chemical Composition of Starch

3.3.1 Moisture Content

Moisture content was determined using the air oven method (AACC, 1984). A preweighed (3-5 g) amount of sample was dried in a forced air convection oven (Fisher Scientific, Iosotemp 614G, USA) at 130°C for one hour. The samples were removed from the oven and allowed to cool to room temperature in a desiccator for 25 minutes. The percentage weight loss of the sample was calculated and reported as the moisture content (AACC, 1984).

3.3.2 Ash Content

Samples (1.0-2.0 g) were transferred into clean, dry, preweighed porcelain crucibles, charred in a Bunsen burner flame and then transferred to a preheated (600°C) muffle furnace (Lab Heat, Blue M, Illinois, USA). The samples were kept in the muffle furnace overnight to obtain a grey ash and then placed in a desiccator (30 minutes) to cool. The samples were then weighed and the ash content was calculated as the percentage of the remaining material (AACC, 1984).

3.3.3 Nitrogen Content

The nitrogen content of the samples was determined using the Kjeldahl method. Each sample (100-300 mg) was weighed on a nitrogen-free paper and placed in a pre-labelled digestion tube of a Büchi 430 (Büchi Laboratoriums-Technik AG, Flawill/Schweiz) digester. Two Kjeltabs M pellets (catalysts) and 20 mL of concentrated sulphuric acid (H_2SO_4) were added to each digestion tube and the tubes were then placed in the Büchi 430 digester. The samples were heat digested at setting #3 for 20 minutes followed by another 30-40 minutes of digestion at setting #9, until a clear or light yellow solution was obtained. The digested samples were then cooled, diluted with 50 mL of distilled water and 100 mL of 25% (w/w) NaOH, and steam distilled, in a Büchi 321 distillation unit, until 150 mL of distillate were collected into flasks containing 50 mL of 4% (w/v) boric acid (H_3BO_3) and 12 drops of an end point indicator (methyl red/methylene blue indicator). The amount of ammonia in the distillate was determined by titrating it with 0.1N H_2SO_4 to a red end point (AACC, 1984).

Calculation of nitrogen content:

$$\%N = \frac{(\text{Volume titrant sample mL}) - (\text{Volume titrant blank mL}) \times \text{Acid N} \times 14.01}{\text{Weight of sample (mg)}} \times 100$$

3.3.4 Amylose Content

The apparent amylose contents of the native starches were determined using the method of Chrastil (1987) after the following procedure was performed. About 20 mg of starch (db) was placed in a 50 mL beaker and 10mL of 0.5N NaOH was added. The starch was completely dispersed in the NaOH using a magnetic stirrer and stir bar. The dispersed starch solution was then transferred to a 100 mL volumetric flask and then diluted to the mark with distilled water. A 10 mL aliquot of this solution was transferred to a 50 mL volumetric flask containing 5 mL of

0.1N HCl and then diluted to the mark with distilled water. The final solution was analyzed by the method of Chrastil (1987). The total amylose contents of the native starches were determined using the same procedure, except the samples were first defatted with hot n-propanol-water (3:1 v/v) for 7 hours, and dried to a uniform moisture content.

3.3.4.1 Chrastil's Method of Amylose Determination

A 0.1 mL aliquot of the final solution was transferred to a screw cap tube containing 5 mL of 0.5% trichloroacetic acid (TCA). Then, 0.05 mL of 0.1N I₂-KI solution was added and the solution was thoroughly mixed. The capped solution was allowed to stand at room temperature for 30 minutes and then its absorbance was measured at 620nm in a spectrophotometer (Novaspec Model 4049, LKB Biochrom, Cambridge, England). The absorbance of the reaction blanks with water was zero. The amylose content was then determined using the following formula (obtained through a calibration curve) and expressed as mg of amylose per 100 mg dry starch.

Calculation of amylose content:

$$\% \text{ Amylose} = (\text{Absorbance} \times 32.5 \text{ mg} \div 1000 \text{ mL}) \times (5.15 \text{ mL} \div 0.10 \text{ mL}) \times (100 \text{ mL} \div \text{starch dry wt in mg}) \times (50 \text{ mL} \div 10 \text{ mL}) \times 100$$

Where: Absorbance X 32.5 = mg of amylose/litre in cuvette

3.3.5 Lipid Content

Starch lipids were analyzed as follows. At ambient temperature (25-27°C), lipids (surface lipids) were extracted from legume starches (5 g db) with 100 mL of 2:1 chloroform-methanol (CM) under vigorous agitation in a wrist action shaker for one hour. Bound lipids from CM extraction were solvent extracted with 100 mL of 3:1 n-propanol water (PW) at 90-100°C for 7

hours in a Soxhlet apparatus. To determine the total starch lipids, starches (2.0g db) were hydrolysed with 25mL of 24% HCl at 70-80°C for 30 minutes, and the hydrolysate then extracted three times with 1-hexane (Goshima *et al.*, 1985).

3.3.6 Lipid Purification

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

3.4 Estimation of Starch Damage

The percentage starch damage of native starches was estimated using standard AACC methods (1984). Starch samples (1 g dry basis) were mixed with 45 mL warm (30°C) fungal α -amylase from *Aspergillus oryzae*. The α -amylase concentration was 277.7 units/mL and had a specific activity of 50-100 units/mg of protein. A uniform suspension was obtained and the reaction mixture was then incubated at 30°C for exactly 15 minutes. At the end of the incubation period, 3 mL of 3.68M sulfuric acid and 2 mL of 12% (w/v) sodium tungstate solution were added to the reaction mixture, which was mixed thoroughly and allowed to stand at room temperature for 2 minutes. The reaction mixture was filtered through a Whatman #4 filter paper, and the first 8-10 drops of the filtrate were discarded. The amount of reducing sugars was then determined, using 5 mL of the filtrate, by the method of Bruner (1964). A reagent blank was also determined by the same procedure without the starch sample.

Calculation of starch damage (%):

- 1) Determine the mg maltose per 10 g of sample

$$\text{mg maltose/10 g sample} = (1.64/100) \times 5(\text{mg maltose in filtrate} - \text{mg maltose in blank})$$

It has been empirically shown that under the conditions of this method (AACC, 1984), 61% of starch is converted to maltose. Therefore, in order to convert mg maltose to mg starch hydrolyzed per 10 g of sample, it is necessary to first multiply the amount of starch converted to maltose by 1.64, which is the reciprocal of 0.61. Division of this value by 100 converts the units to percent (%). The result of the reducing value method is multiplied by 5 since a 1:50 dilution is used.

- 2) % starch damage = (mg maltose/10 g) X 0.082

3.5 Heat-moisture Treatment (HMT)

The method of heat-moisture was that of Sair (1964). Starch samples (15g dry basis) were weighed into glass jars. The starch moisture content was brought to 30% by adding the appropriate amount of distilled water. The jars were sealed, kept for 24h at ambient temperature, and then placed in a forced air convection oven for 16h at 100°C. The samples were removed, the jars were opened, and the starch samples air dried to a uniform moisture content (~10%).

The conditions of heat-moisture treatment used in this study were determined by preliminary experiments. Amylose leaching and swelling factor were determined for samples of Green Arrow pea and black bean starches heat-moisture treated under various combinations of moisture, temperature and time conditions. These preliminary experiments revealed that structural changes occurred when legume starches were heat-moisture treated at 30% moisture for 16 hours at 100°C.

3.6 Extent of Amylose Leaching

Approximately 20 mg (db) of the various starches in distilled water (10 mL) were heated (70-95°C) in screw capped tubes for 30 minutes. The tubes were then cooled to ambient temperature and centrifuged at 3600 rpm for 10 minutes. The method of Chrastil (1987) was then used to determine the amount of amylose that leached out of the granules. A 0.1 mL aliquot was withdrawn from the supernatant, added to a screw cap tube containing 5 mL of distilled water, and the contents thoroughly mixed. Then, 0.05 mL of 0.01N I₂-KI solution was added to the contents of the tube, and the contents thoroughly mixed. The tubes were sealed and allowed to stand at room temperature for 30 minutes. The absorbance of the blue colour was read at $\lambda=620\text{nm}$, using a spectrophotometer (Novospec Model 4049, LKB Biochron, Cambridge, England). The absorbance of the reaction blanks with water was zero. The amount of leached amylose was calculated using the following equation (obtained through a calibration curve):

% Amylose Leaching

$$= (\text{Abs} \times 32.5 \text{mg} \times 5.15 \text{mL} \times 10 \text{mL}) \div (1000 \text{mL} \times 0.01 \text{mL} \times \text{starch dry weight mg}) \times 100$$

Where: Absorbance $\times 32.5$ = mg of amylose/litre in cuvette

The percentage amylose leaching represents the mg of amylose leached per 100mg dry starch.

Results used for calculation were means of three determinations.

3.7 Swelling Factor

The swelling factors (SF) of the starches when heated at 60-95°C in excess water were measured according to the method of Tester and Morrison (1990a). Starch samples (50mg db) were weighed accurately into screw cap tubes. Distilled water (5mL) was added to the tubes, which were then sealed and incubated, with constant agitation, in a water bath at the desired

temperature for 30 minutes. The sample mixtures were then cooled to room temperature, 0.5mL of blue dextran ($M_r = 2 \times 10^6$, 5mg/mL) was added to each tube, and the contents were mixed by inverting the sealed tubes several times. The tubes containing the sample mixtures were centrifuged at 1,500 X g for 5 minutes and the absorbance of the supernatant (A_s) was measured at 620nm. The absorbance of a reference (A_r) sample containing no starch was also measured at 620nm using a spectrophotometer (Novospec Model 4049, LKB Biochron, Cambridge, England).

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

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

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

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

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

$$V_o = W/1400$$

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

$$V_1 = 5.0 - FW$$

Hence the volume of the swollen starch granules (V_2) is

$$V_2 = V_o + V_1$$

$$\text{and SF} = V_2/V_o$$

This can also be expressed by the single equation

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

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

This method measures only intragranular water and, hence, the true SF at a particular

temperature. The SF is reported as the ratio of the volume of swollen starch granules to the volume of the dry starch. Results used for the calculation were means of three determinations.

3.8 X-Ray Diffraction

X-ray diffractograms of legume starches were obtained with a Rigaku RU 200R X-ray diffractometer with a chart speed of 20 mm/min. The starch powder (~10.0% moisture) was scanned through the 2θ range of 3-35°. Traces were obtained using a Cu-K α 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, 0.30mm receiving slit, 1s time constant and scanning rate of 3°/min.

3.9 Differential Scanning Calorimetry (DSC)

Gelatinization temperatures of native, heat-moisture treated, and 72h enzyme treated residues of native starches were measured and recorded on a Perkin-Elmer DSC-2 (Norwalk, CT) differential scanning calorimeter, equipped with a thermal analysis data station. Water (11 μ L) was added with a microsyringe to starch (3.0 mg db) in the DSC pans, which were then sealed, weighed, kept overnight at room temperature, and then reweighed to ensure no moisture was lost from the system. The scanning temperature range and the heating rate were 20-120°C and 10° min⁻¹, respectively. The thermogram was recorded with water as a reference. Indium was used for calibration. The thermal transitions of starch were defined in terms of temperature at To (onset), Tp (peak) and Tc (conclusion) of gelatinization. The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and a base line under the peak, and was expressed in terms of joules per unit weight of dry starch (cal/g). The experiments were replicated at least twice.

3.10 Digestibility by Porcine Pancreatic α -amylase

Digestion of native and heat-moisture treated legume starches by porcine pancreatic α -amylase was determined according to the procedure of Knutson *et al.* (1982). However, a higher concentration of enzyme was used in this study. A crystalline suspension of porcine pancreatic α -amylase in 0.5M saturated sodium chloride containing 3mM calcium chloride were used, in which the concentration of α -amylase was 23.9 mg/mL and the specific activity was 1240 units per milligram of protein. One unit was defined as the α -amylase activity which liberated 1 mg maltose in 3 min at 20°C at pH 6.9. Starch (100 mg) was suspended in distilled water (25mL) and 5mL aliquots were placed in a constant temperature shaking water bath at 37°C. Then, 4.0 mL of 0.1M phosphate buffer (pH 6.9) containing 0.006M NaCl were added to the starch slurry. The mixture was gently stirred for 15 minutes before adding 4 μ L α -amylase suspension. The reaction mixtures were shaken by hand twice daily (once in the morning and once in the afternoon) to resuspend the deposited granules. Then, 1.0 mL aliquots were removed at specified time intervals, pipetted into 0.2 mL of 95% ethanol, and centrifuged at 1,500 X g for 10 minutes. Aliquots of the supernatant were analyzed for soluble carbohydrate (Bruner, 1964). Percentage hydrolysis was calculated as the amount (mg) of maltose released per 100 mg of dry starch. Controls without enzymes but subjected to the above experimental conditions were run concurrently. The above experiment was replicated at least twice.

3.11 Acid Hydrolysis

Legume starches, native and heat-moisture treated, were hydrolyzed with 2.2N HCl at 35°C (1.0 g starch/40 mL acid) for periods ranging from 1 to 15 days. The starch slurries were shaken by hand daily to resuspend the deposited granules. At 24h intervals, 1.0 mL aliquots of

the reaction mixtures were neutralized with 1.0mL of 2.2N NaOH and centrifuged (1,500 X g, for 10 min) and the supernatant liquid was assayed for total carbohydrate (Bruner, 1964). Controls without acid but subjected to the above experimental conditions were run concurrently. The percentage hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial starch. The above experiment was replicated at least twice.

3.12 Determination of Reducing Sugars

The amount of reducing sugars present in various starch samples was determined using the method of Bruner (1964). A 1.0 mL aliquot of the starch sample was mixed with 2.0 mL of 3,5-dinitrosalicylic acid (DNS) [20 g 3,5-DNS in 700 mL of 1.00N NaOH] and diluted to 4.0 mL with distilled water. The reaction mixture was then heated in a boiling water bath for exactly 5 minutes, and then chilled in an ice bath for ~ 5 minutes. The reaction mixture was allowed to stand until it reached room temperature (10-15 minutes) and was then diluted to 12.0 mL with distilled water. The absorbance of the reaction mixture was read at 540/590 nm using a spectrophotometer (Novospec Model 4049, LKB Bichrom, Cambridge, England). A reagent blank was determined using the above reagents in the absence of a starch sample. Calibration curves using both glucose and maltose were determined using the above procedure, and the linear regression analysis performed. The calibration curves were then used to calculate the amount of reducing sugars present per starch sample.

Calculation of reducing sugars:

Reducing sugar (μmol) = [(absorbance - y-intercept)/x-coefficient]. (see Appendix I).

3.13 Scanning Electron Microscopy (SEM)

Granule morphology of native and heat-moisture treated starches, and the mode of action of α -amylase were examined by SEM. Starch samples were mounted on circular aluminum stubs with double sided sticky tape, coated with a thin layer (20 nm) of gold and then examined and photographed with a Hitachi (S 570) scanning electron microscope at an accelerating potential of 5 KV. Enzyme digested granules were prepared for SEM by rapidly freezing in liquid nitrogen and freeze drying at -55°C . The dried samples were prepared for viewing as described above.

3.14 Statistical Analysis

Analysis of variance was performed using the Minitab statistical package (Minitab, Inc., 1991). Duncan's New Multiple Range Test was utilized for multiple comparison among means at the 5% level of probability.

CHAPTER 4.

RESULTS AND DISCUSSION

4.1 Chemical Composition and Granule Morphology

The yield of legume starches was in the range 22.7-32.9% on a total seed basis (Table 4.1). The chemical composition (Table 4.1) showed that the starches contained 0.02-0.13% nitrogen and 0.06-0.18% ash. These low values indicated high purity and the absence of non-starch lipids. Therefore, the total lipids (0.12-0.22%) obtained by acid hydrolysis (Table 4.1) of the legume starches mainly represented free and bound lipids. The total amylose content ranged from 37.8-86.5%. A comparison of the apparent and total amylose content showed that the amount of lipids present within the amylose helix (bound lipids) in these starches ranged from 5.8-21.1%. The low level of surface lipids (<0.07%) and nitrogen (<0.13%) suggested that they are not significant factors in restricting the absorption of α -amylase at the site of fixation on the granule surface. The extent of starch damage was higher in Green Arrow pea (2.4%) than in the other starches (0.2-1.8%).

The starch granule sizes are presented in Table 4.1, with their physical forms illustrated in Figures 4.1 and 4.2. Most of the legume starch granules showed a wide distribution range which included a mixture of large (elliptical to oval), intermediate (oval) and small (oval to elliptical) granules. Some of the large granules were irregularly shaped. Green Arrow pea starch, which is a wrinkled pea starch (high amylose), consisted of a mixture of simple and compound granules (Fig. 2C), with the latter being composed of 2-6 individual subunits joined together (Fig. 2D). Some of the granules were fragmented into pieces of varying sizes. It is highly likely, that

TABLE 4.1. Chemical composition of native legume starches*.

Characteristic	Composition (% dry weight)				
	Green Arrow Pea	Black Bean	Othello Pinto Bean	Express Field Pea	Eston Lentil
Yield	22.7	24.4	24	32.9	27
Moisture	9.75 ¹ ± 0.05	9.6 ¹ ± 0.6	10.9 ¹ ± 0.0	8.8 ¹ ± 0.1	9.5 ¹ ± 0.6
Ash	0.09 ¹ ± 0.01	0.18 ¹ ± 0.03	0.06 ¹ ± 0.03	0.06 ¹ ± 0.02	0.11 ¹ ± 0.02
Nitrogen	0.13 ¹ ± 0.01	0.02 ² ± 0.01	0.05 ² ± 0.01	0.06 ¹ ± 0.01	0.06 ² ± 0.01
Lipid					
Solvent Extracted:					
CHCl ₃ -MeOH ^b	0.07 ¹ ± 0.01	0.04 ² ± 0.01	0.02 ² ± 0.01	0.02 ² ± 0.01	0.04 ² ± 0.01
n-Propanol-Water ^c	0.15 ¹ ± 0.02	0.16 ¹ ± 0.03	0.16 ¹ ± 0.02	0.10 ¹ ± 0.01	0.09 ¹ ± 0.01
Acid Hydrolyzed ^d	0.22 ¹ ± 0.03	0.20 ¹ ± 0.02	0.18 ¹ ± 0.03	0.12 ¹ ± 0.02	0.13 ¹ ± 0.02
Amylose Content					
Apparent ^e	67.5 ¹ ± 0.7	35.7 ¹ ± 1.2	37.9 ^{2,1} ± 0.1	39.8 ² ± 0.8	35.6 ² ± 1.1
Total ^f	85.6 ¹ ± 0.9	41.2 ² ± 1.1	44.7 ² ± 2.0	43.2 ^{2,1} ± 0.5	37.8 ⁴ ± 0.6
Amylose complexed with native lipid ^g	21.1	13.3	15.2	7.8	5.8
Starch Damage	2.4 ¹ ± 0.2	0.7 ² ± 0.1	0.2 ⁴ ± 0.1	0.4 ^{2,4} ± 0.1	1.8 ² ± 0.5
Granule Size (µm)	10 to 15	6.4 to 21.8	8 to 18	5.5 to 28.8	3.8 to 19.2

*All data reported on dry basis and represent the mean of 3 determinations ± standard deviation.

^bLipids extracted from native starch by chloroform-methanol 2:1 (v/v) at 25°C (mainly unbound lipids).

^cLipids extracted by hot n-propanol-water 3:1 (v/v) from the residue left after chloroform-methanol extraction (mainly bound lipids).

^dLipids obtained by acid hydrolysis (24% HCl) of the native starch (total lipids).

^eApparent amylose was determined by iodine binding without removal of free and bound lipids.

^fTotal amylose was determined by iodine binding after removal of free and bound lipids.

^g[(Total amylose-Apparent amylose) + Total amylose] X 100.

^hMean values in each row not followed by the same superscript are significantly different (p < 0.05).

Fig. 4.1 Scanning electron micrographs of native starches: Black bean (A & B), Pinto bean (C & D), and Express field pea (E & F).

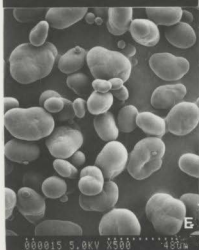
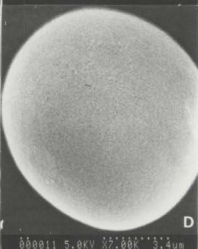
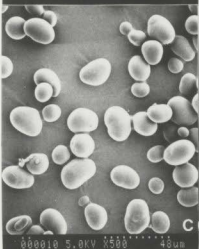
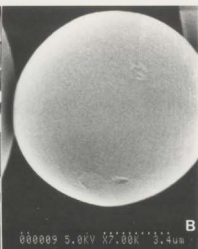


Fig. 4.2 Scanning electron micrographs of native starches: Eston lentil (A & B) and Green Arrow pea (C & D).

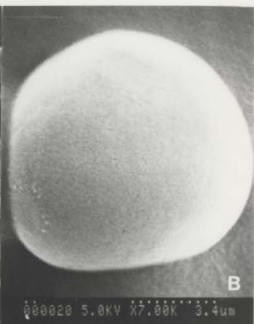
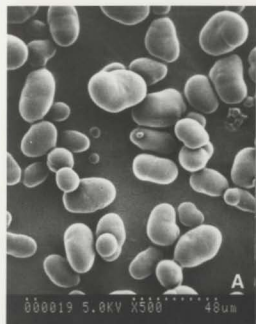
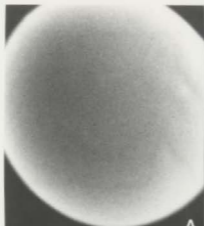
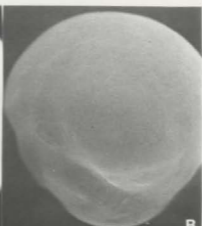


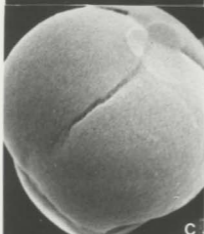
Fig. 4.3 Scanning electron micrographs of heat-moisture treated (16h, 100°C, 30% moisture) starch granules: Black bean (A), Pinto bean (B), Eston lentil (C), Express field pea (D), and Green Arrow pea (E).

**A**

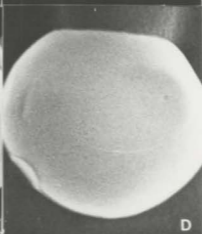
000024 5.0KV X6,000K 4.0um

**B**

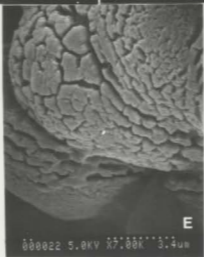
000026 5.0KV X7,000K 3.4um

**C**

000027 5.0KV X11,000K 2.1um

**D**

000029 5.0KV X7,000K 3.4um

**E**

000022 5.0KV X7,000K 3.4um

fragmentation may have occurred during starch isolation. The surfaces of all legume starch granules appeared to be smooth when viewed under the scanning electron microscope (Fig. 1B, D, F and Fig. 2B, D). These results on granular size and shape were in agreement with previous reports (Hoover & Sosulski, 1985a; Naivikul & D'Appolonia, 1979; Sathe & Salunkhe, 1981; Bertoft *et al.*, 1993; Colonna *et al.*, 1982).

Heat-moisture treatment did not alter the size or shape of the granules used in this study. Similar observations have also been made on wheat (Kulp & Lorenz, 1981; Hoover & Vasanthan, 1994), potato (Kulp & Lorenz, 1981; Hoover & Vasanthan, 1994), yam (Hoover & Vasanthan, 1994) and Laird lentil (Hoover & Vasanthan, 1994) starches. The surface of the native granules of black bean, pinto bean and Express field pea appeared smooth even after heat-moisture treatment (Fig. 4.3A, B, D). However, the surface of some granules of Eston lentil starch exhibited deep cracks (Fig. 4.3C), and the surface of many granules of Green Arrow pea starch appeared to be broken up into a large number of separate pieces of varying size (Fig. 4.3E) following heat-moisture treatment.

4.2 Amylose Leaching (AML) and Swelling Factor (SF)

The extent of amylose leaching (AML) and swelling factor (SF) of native and heat-moisture treated starches are presented in Tables 4.2 and 4.3, respectively. The SF and AML were within the ranges reported for legume starches (Hoover & Vasanthan, 1994; Hoover *et al.*, 1993). The extent of AML in native starches followed the order: Eston lentil > Express field > pinto bean > black bean > Green Arrow pea (Table 4.2). The corresponding order for SF was: Express field pea ~ Eston lentil > black bean > pinto bean > Green Arrow pea (Table 4.3). Heat-moisture treatment decreased AML (Express field pea > Eston lentil > Green Arrow pea > black

TABLE 4.2. Amylose leaching of legume starches^a.

Starch source & treatment	Amylose Leaching (% dry weight)				
	Temperature (°C)				
	70	80	85	90	95
Green Arrow Pea					
Native	4.9 ¹ ± 0.2	7.4 ¹ ± 0.5	8.8 ¹ ± 0.8	10.4 ¹ ± 0.5	14.7 ¹ ± 0.2
HMT ^b	0.6 ² ± 0.1	2.1 ² ± 0.2	3.2 ² ± 0.1	3.5 ² ± 0.1	7.4 ² ± 0.4
Black Bean					
Native	7.3 ¹ ± 0.4	7.6 ¹ ± 0.4	9.2 ¹ ± 0.1	11.1 ¹ ± 0.1	17.1 ¹ ± 0.4
HMT	3.7 ² ± 0.4	5.5 ² ± 0.2	7.1 ² ± 0.2	7.7 ² ± 0.1	12.1 ² ± 0.2
Othello Pinto Bean					
Native	7.9 ¹ ± 0.1	8.2 ¹ ± 0.1	10.3 ¹ ± 0.7	11.3 ¹ ± 0.2	18.2 ¹ ± 0.4
HMT	4.9 ² ± 0.7	6.2 ² ± 0.4	8.5 ² ± 0.3	8.7 ² ± 0.4	13.1 ² ± 0.4
Express Field Pea					
Native	8.6 ¹ ± 0.3	10.6 ¹ ± 0.5	14.6 ¹ ± 0.3	19.3 ¹ ± 0.3	25.2 ¹ ± 0.2
HMT	1.7 ² ± 0.4	3.2 ² ± 0.1	7.7 ² ± 0.5	9.0 ² ± 0.3	15.8 ² ± 0.1
Eston Lentil					
Native	9.2 ¹ ± 0.2	11.8 ¹ ± 0.6	15.9 ¹ ± 0.1	20.2 ¹ ± 0.1	25.7 ¹ ± 0.4
HMT	2.9 ² ± 0.4	3.5 ² ± 0.1	9.0 ² ± 0.2	9.2 ² ± 0.2	16.8 ² ± 0.3

^aData represent the mean of 3 determinations ± standard deviation.

^bHeat - moisture treatment at 100°C for 16 hours.

^cMean values in each column (native vs HMT) not followed by the same superscript are significantly different (p<0.05).

TABLE 4.3. Swelling factor of legume starches*

Starch source & treatment	Swelling Factor			
	Temperature ($^{\circ}\text{C}$)			
	60	70	85	95
Green Arrow Pea				
Native	2.1 ¹ ± 0.8	6.0 ¹ ± 0.5	7.4 ¹ ± 0.1	11.4 ¹ ± 0.1
HMT ^b	1.6 ² ± 0.1	3.6 ² ± 0.1	4.2 ² ± 0.1	6.1 ² ± 0.1
Black Bean				
Native	8.9 ¹ ± 0.6	9.6 ¹ ± 1.0	17.7 ¹ ± 0.5	30.1 ¹ ± 0.7
HMT	6.6 ¹ ± 0.3	6.8 ² ± 0.2	13.2 ² ± 0.5	25.1 ² ± 1.0
Othello Pinto Bean				
Native	4.9 ¹ ± 0.5	6.6 ¹ ± 0.1	11.1 ¹ ± 0.5	23.4 ¹ ± 0.5
HMT	3.3 ² ± 0.3	5.5 ² ± 0.3	9.3 ¹ ± 0.2	18.9 ² ± 0.2
Express Field Pea				
Native	9.2 ¹ ± 0.2	17.5 ¹ ± 0.5	24.1 ¹ ± 0.5	27.2 ¹ ± 1.0
HMT	4.1 ² ± 0.5	7.8 ² ± 0.4	9.9 ² ± 0.7	12.9 ² ± 0.5
Eston Lentil				
Native	8.6 ¹ ± 0.1	17.4 ¹ ± 0.3	25.5 ¹ ± 0.5	28.7 ¹ ± 0.6
HMT	4.1 ² ± 0.2	8.1 ² ± 0.3	10.4 ² ± 0.4	14.7 ² ± 0.7

*Data represent the mean of 3 determinations ± standard deviation.

^bHeat - moisture treatment at 100 $^{\circ}\text{C}$ for 16 hours.

*Mean values in each column (native vs HMT) not followed by the same superscript are significantly different ($p < 0.05$).

bean ~ pinto bean) (Table 4.2) and the SF (Eston lentil ~ Express field pea > Green Arrow pea > black bean ~ pinto bean) (Table 4.3). The results showed that the extent of AML is not influenced by the total amylose content of these starches since, if it were, the extent of AML would be expected to have followed the order: Green Arrow pea > pinto bean > Express field pea > black bean > Eston lentil. Several researchers (Vasanthan & Hoover, 1992; Hoover & Hadziyev, 1981; Maningat & Julian, 1980; Biliaderis *et al.*, 1979) have shown that bound lipids (lipids present within the hydrophobic core of the amylose helix) restrict AML. The extent of AML (Table 4.2) in native green arrow pea, express field pea and eston lentil starches closely followed the level of bound lipids (Green Arrow pea>Express field pea>Eston lentil). It is likely that the extent of AML is also influenced by the magnitude of interactions between amylose and amylopectin chains in the amorphous regions of the native granules. This seems plausible, since AML in pinto bean starch (15.2% bound lipid) is higher than in black bean starch (13.3% bound lipid), implying that amylose-amylose (AM-AM) and amylose-amylopectin (AM-AMP) interactions within native granules of black bean are slightly stronger than in pinto bean starch. Thus, AML in native legume starch granules is influenced by the interplay of bound lipid content and the extent of associations between starch chains.

Starch granule swelling is known to begin in the bulk, relatively mobile amorphous fraction and in the more restrained amorphous regions immediately adjacent to the crystalline region. It has also been shown (Maruta *et al.*, 1994; Tester & Morrison, 1990a; Hoover & Hadziyev, 1981; Maningat & Julian, 1980) that amylose-lipid complexes inhibit granule swelling. The results showed that the SF (Table 4.3) of native starches closely paralleled the bound lipid content (Table 4.1). However, it is highly unlikely that the bound lipid content alone could be

responsible for the very low SF exhibited by Green Arrow pea starch. Therefore, it can be postulated that amylose chains within native granules of Green Arrow pea starch are packed more compactly (due to its higher amylose content [Table 4.1]) than in the other legume starches. This would decrease the accessibility of water molecules to the binding sites on the amylose chains of green arrow pea starch. Therefore, it seems that an interplay of bound lipid content and the arrangement of amylose chains in the amorphous regions of the granule are responsible for the net swelling factor of native legume starches.

The decrease in AML after heat-moisture treatment (Table 4.2) has been attributed (Hoover & Vasanthan, 1994) to an interplay of two factors: (i) interaction between free starch lipids and the amylose helix, and (ii) interaction between starch chains. In this study, the difference in free lipid content among the legume starches was marginal (Table 4.1). Therefore, differences in the extent of AML on heat-moisture treatment would reflect mainly AM-AM and AM-AMP interactions. Express field pea and Eston lentil starches differed marginally in amylose content (Table 4.1). This implies that amylose chains in the above starches are probably packed in a similar manner within the amorphous regions of the granule. Thus, on heat-moisture treatment, the extent of AM-AM and AM-AMP interactions would most likely be of the same order of magnitude in both starches. This would then explain the similar decrease in AML on heat-moisture treatment of Express field pea and Eston lentil starches (Table 4.2). The extent of AM-AM and AM-AMP interactions on heat-moisture treatment of Green Arrow pea, Express field pea and Eston lentil starches cannot be explained on the basis of chain length, since the degree of polymerization (DP) of amylose chains (1100-1400) (Biliaderis *et al.*, 1979) and the average chain length (CL) of amylopectin (20-22) (Biliaderis *et al.*, 1979) of these starches do not differ widely.

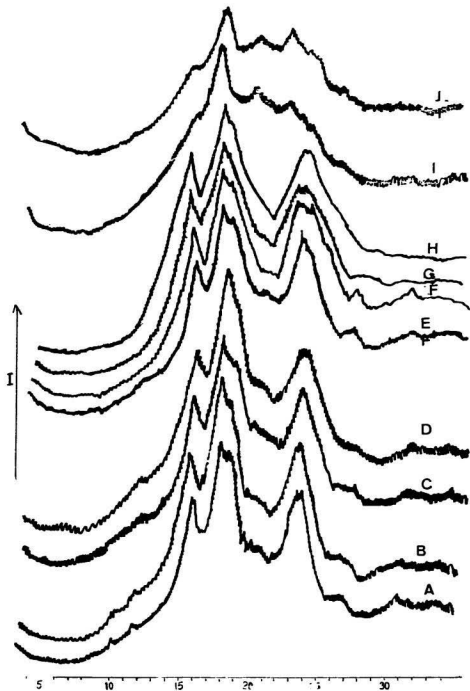
However, DP of amylose and CL of amylopectin chains may have been a factor influencing the small decrease in AML on heat-moisture treatment of black bean and pinto bean starches (Table 4.2). This seems plausible, since the amylose contents of these starches (41.2-44.7%) are fairly close to those of Express field pea and Eston lentil (37.8-43.2%). It is likely, that the DP of amylose and the CL of amylopectin chains of black bean and pinto bean starches are probably similar, but are longer than those of Express field pea and Eston lentil (published data not available). Long chains of amylose and amylopectin would facilitate strong interchain associations within the native granule. Therefore, additional AM-AM and AM-AMP interactions during heat-moisture treatment would be minimal in black bean and pinto bean starches, and the decrease in AML of heat-moisture treated black bean and pinto bean starches would be smaller than in Express field pea and Eston lentil.

The ranking of starches with respect to the decrease in SF (Table 4.3) after heat-moisture treatment paralleled the ranking with respect to the decrease in AML (Table 4.2). This indicates that the factors influencing the decrease in AML also influence the decrease in SF. Among the legume starches, Green Arrow pea may have exhibited the highest decrease in SF due to stronger interactions between amylose chains, if its surface had not cracked when heat-moisture treated as entry of water through the cracks into the granule interior would increase SF. Therefore, it seems that the SF of heat-moisture treated Green Arrow pea starch is influenced by the interplay of AM-AM interactions and modifications to its granule surface.

4.3 X-ray Diffraction

The X-ray spectrum of native Green Arrow pea starch was of the "B" type (Fig. 4.4) characteristic of tuber starches with three main reflections at 5.2, 4.0 and 3.9Å. However, the

Fig. 4.4 X-ray diffraction patterns of native and heat-moisture treated (HMT) legume starches: HMT black bean (A), Native black bean (B), HMT Express field (C), Native Express field pea (D), HMT pinto bean (E), Native pinto bean (F), HMT Eston lentil (G), Native Eston lentil (H), HMT Green Arrow pea (I), and Native Green Arrow pea (J).



peak at 16.6\AA , which is typical of potato starch, ("B" type) was not present. Furthermore, the intensities of the major peaks were weaker than those in "B" type starches. The other four starches showed the characteristic "C" pattern of legume starches (Fig. 4.4), with a strong intensity line at 5.13\AA and two medium intensity lines at 5.9 and 3.8\AA . The intensity of the peak at 5.13\AA followed the order: black bean > Express field pea > Eston lentil > pinto bean (Table 4.4). While heat-moisture treatment increased the X-ray intensities of Green Arrow pea starch (Fig. 4.4, Table 4.4), the X-ray intensities of the other starches decreased after HMT (Express field pea > black bean > pinto bean > Eston lentil). The X-ray pattern of all starches, however, remained unchanged after heat-moisture treatment (Fig. 4.4).

Starch crystallites are due to sequential packing of double helices (Wu & Sarko, 1978a, b) that are formed between the flexible A chains of amylopectin (French, 1972). Therefore, the low X-ray intensity pattern ("B" type) of native Green Arrow pea starch is probably due to its low amylopectin content (14.4%) and/or to the presence of small crystallites.

The difference in X-ray intensities among the native "C" type starches cannot be due to differences in crystallite size since all four legume starches exhibited sharp X-ray patterns (Fig. 4.4), or in amylopectin content since the starches differ only marginally in their amylopectin contents (Table 4.1). Therefore, the differences in X-ray intensities (Fig. 4.4, Table 4.4) among the "C" type starches was probably due to the manner in which the double helices are arranged within the crystalline domains of the granule. The results indicate that the double helices of black bean starch are more closely packed and/or are better arranged to diffract X-rays than those of the other "C" type starches.

The X-ray pattern of tuber starches has been shown to change from "B" to one half "A"

Table 4.4. X-ray diffraction intensities of the major peak of native and heat-moisture treated legume starches.

Starch source and treatment	Moisture Content (%)	Interplanar spacing (d) in Å with intensities (CPS) ^a
Green Arrow Pea		
Native	10.2	5.20 (1414)
HMT ^b	10.1	5.24 (1707)
Black Bean		
Native	10.3	5.12 (2959)
HMT	10.2	5.12 (2426)
Pinto Bean		
Native	10.0	5.12 (2315)
HMT	10.2	5.11 (2108)
Express Field Pea		
Native	10.2	5.13 (2900)
HMT	10.0	5.15 (2317)
Eston Lentil		
Native	10.0	5.18 (2402)
HMT	10.2	5.13 (2219)

^aCounts per second.

^bHeat-moisture treated.

(typical pattern for cereal starches) and one half "B", and to decrease in intensity after heat-moisture treatment (Hoover & Vasanthan, 1994). The change in X-ray pattern has been attributed to rearrangement of double helices into a crystalline array that contains an amylosic helix (characteristic of "A" type unit cells) in the central cavity of the unit cell (Imberty *et al.*, 1991), whereas the decrease in intensity on heat-moisture treatment has been attributed to a loss of crystalline order (Hoover & Vasanthan, 1994). The X-ray pattern ("B" type) of Green Arrow pea starch does not change after heat-moisture treatment, since AM-AM interactions which occur during heat-moisture treatment (this occurs to a greater extent in Green Arrow pea than in the other "B" type starches due to the higher amylose content of the former [85.6% vs 20-24% amylose]) would prevent the rearrangement of double helices by decreasing their mobility during heat-moisture treatment. The increase in X-ray intensities after heat-moisture treatment of Green Arrow pea starch indicates that additional crystallites may have formed as a result of interaction between amylose chains. These crystallites are probably better arrayed to diffract X-rays than those present within the native granule.

The decrease in X-ray intensities after heat-moisture treatment of the "C" type starches (Fig. 4.4, Table 4.4) implies that crystallite reorientation may have occurred during heat-moisture treatment, resulting in a crystalline array that did not diffract X-rays as strongly as the crystallites within the native granules. As discussed earlier, AM-AM interactions also occur during heat-moisture treatment of "C" type starches. However, the extent of this interaction is weaker (due to their lower amylose content [Table 4.1]) than in Green Arrow pea starch. Therefore, the number of crystallites resulting from this interaction may not have been high enough to have any significant impact on X-ray intensities. Thus, crystallite reorientation is probably the only factor

influencing changes in X-ray intensities in the "C" type starches.

Previous studies have shown that X-ray intensities of all cereal starches ("A" type) (Hoover & Vasanthan, 1994) increase after heat-moisture treatment, whereas decreases occur for all tuber starches (Hoover & Vasanthan, 1994). However, a similar trend does not occur in legume ("C" type) starches. For instance, X-ray intensities of pigeon pea starch (Hoover *et al.*, 1993) remained unaltered after heat-moisture treatment, whereas they increased in Laird lentil starch (Hoover & Vasanthan, 1994). Gernat *et al.* (1990) have shown that the legume starch "C" crystalline polymorph is a mixture of "A" and "B"-type unit cells, and that these starches contain pure "A" and pure "B" polymorphs in varying proportions. For instance, pea starch is composed of 38.6% type "B" and 61.4% type "A", whereas broad bean starch is composed of 17.0% type "B" and 83.0% type "A". This suggests that the unit cell ratio (A/B) may differ widely among legume starches. This variation could then influence the direction and magnitude of the change in X-ray diffraction intensities during heat-moisture treatment. The high proportion of "A"-type unit cells in legume starches, may also explain the unaltered X-ray pattern (Fig. 4.4) after heat-moisture treatment, as the X-ray pattern of "A" type starches are not altered after heat-moisture treatment (Hoover & Vasanthan, 1994; Hoover & Manuel, 1996).

4.4 Differential Scanning Calorimetry (DSC)

The native legume starches displayed differences in gelatinization temperatures (eg. onset [To], peak [Tp], and end [Tc]) and enthalpy (ΔH) changes, as indicated by DSC analysis (Table 4.5). To, Tp, Tc and ΔH of the native starches followed the order: black bean ~ pinto bean > Express field pea ~ Eston lentil (the values for the thermal properties of Green Arrow pea were too high to be recorded). The gelatinization temperature ranges (Tc-To) of the starches were:

62.5–82°C (black bean), 59–81°C (pinto bean), 55–69°C (Express field pea) and 56–68°C (Eston lentil). T_o , T_p , T_c and ΔH of black bean and pinto bean starches were higher than the corresponding values for wheat, maize and potato starches (Hoover & Vasanthan, 1994), whereas T_o , T_p , T_c and ΔH of Eston lentil and Express field pea starches were comparable to those reported for cereal and tuber starches (Hoover & Vasanthan, 1994). Heat-moisture treatment increased T_o , T_p , T_c for all legume starches (Table 4.5). The extent of the increase followed the order: Eston lentil ~ Express field pea > black bean ~ pinto bean. Similar increases have been reported on heat-moisture treatment of cereal starches (Hoover & Vasanthan, 1994; Lorenz & Kulp, 1982; Hoover & Manuel, 1996) and tuber starches (Hoover & Vasanthan, 1994).

Gelatinization involves the uncoiling and melting of external chains of amylopectin that are packed together as double helices in clusters. Cooke and Gidley (1992) have shown through studies of starches isolated at various steps of the gelatinization process, that the relative decrease in double helix content parallels the relative decrease in both crystallinity and residual gelatinization enthalpy, but occurs at higher temperatures than the relative decrease in granular birefringence. The above authors have also shown by studies on granular starches and model crystallites, that ΔH is due mainly to the disruption of the double helices rather than the longer range disruption of crystallinity. It has been shown that the enthalpy and melting temperatures of the double helices are influenced by the length of the starch chain forming the double helix (Hoover & Vasanthan, 1994).

The higher gelatinization parameters for native black bean and pinto bean starches, suggest that the chains forming the double helices in these starches are longer (greater interchain associations) than those of Express field pea and Eston lentil starches. The higher proportion of

TABLE 4.5. Thermal characteristics of legume starches^a.

Starch source & treatment	Transition ^b		Temperatures (°C)		
	To	Tp	Tc	ΔT (Tc-To)	ΔH ^c (cal/g)
Green Arrow Pea					
Native	d	d	d	-	-
HMT ^f	d	d	d	-	-
Black Bean					
Native	62.5 ² ± 0.5	71.0 ² ± 0.5	82.0 ² ± 0.1	19.5	3.0 ¹ ± 0.3
HMT	71.5 ¹ ± 0.1	81.0 ¹ ± 0.4	91.0 ¹ ± 0.2	19.5	2.9 ¹ ± 0.3
Othello Pinto Bean					
Native	59.0 ² ± 0.1	68.0 ² ± 0.5	82.0 ² ± 0.1	23.0	2.9 ¹ ± 0.1
HMT	68.3 ¹ ± 0.1	79.5 ¹ ± 0.2	92.5 ¹ ± 0.5	24.2	2.8 ¹ ± 0.1
Express Field Pea					
Native	54.0 ² ± 0.5	62.6 ² ± 0.1	69.0 ² ± 0.5	15.0	2.3 ¹ ± 0.1
HMT	64.5 ¹ ± 0.1	74.0 ¹ ± 0.1	82.0 ¹ ± 0.2	17.5	2.3 ¹ ± 0.0
Eston Lentil					
Native	56.0 ² ± 0.1	62.4 ² ± 0.1	69.0 ² ± 0.3	13.0	2.4 ¹ ± 0.2
HMT	64.1 ¹ ± 0.4	76.0 ¹ ± 0.1	80.0 ¹ ± 0.5	15.9	2.5 ¹ ± 0.1

^aData represent the mean of 3 determinations.

^bTo, Tp and Tc indicate the temperature of the onset, midpoint and conclusion of gelatinization.

^cEnthalpy of gelatinization in calories per gram of starch.

^dToo high to be recorded.

^eHeat-moisture treatment at 100°C for 16 hours.

^fMean values in each column (native vs HMT) not followed by the same superscript are significantly different ($p \leq 0.05$).

long chains in black bean and pinto bean starches could also result in double helices varying in size and perfection. The result could then be a large but broad endothermic peak observed upon heating in the DSC. This would then explain the differences in the gelatinization temperature range (T_c - T_o) among the legume starches (pinto bean > black bean > Express field pea > Eston lentil>).

Hoover and Vasanthan (1994a) have shown that changes in crystalline stability on heat-moisture treatment is not a factor influencing increases in T_o , T_p and T_c . Furthermore, comparative studies on heat-moisture treated maize starches (normal, high amylose and waxy) (Hoover & Manuel, 1996) have shown that increases in T_o , T_p and T_c are not due to interaction between amylopectin chains, but reflect mainly AM-AM and amylose-lipid interactions. In this study, the influence of amylose-lipid interactions on increases in T_o , T_p and T_c would be marginal, since only a small amount of free lipids (Table 4.1) was available to interact with the amylose helix during heat-moisture treatment.

Donovan (1979) reported that crystalline and double helical melting during gelatinization are assisted by hydration and swelling of the starch granule amorphous regions. The swelling of amorphous regions imparts a stress on the crystalline regions and, thereby, strips polymer chains from the surface of the starch crystallites. AM-AM interactions on heat-moisture treatment (higher in Express field pea and Eston lentil than in black bean and pinto bean) would suppress the mobility of the amorphous regions. Consequently, the amorphous regions would require a higher temperature to incur swelling that could contribute to the disruption of the crystalline regions (co-operative melting). This would then explain the increase in T_o , T_p and T_c after heat-moisture treatment (Table 4.5).

The lack of influence of heat-moisture treatment on ΔH of legume starches (Table 4.5) suggests that double helices present within the native starch granules do not disrupt under the conditions prevailing during heat-moisture treatment. This implies that identical amounts of double helices unravel and melt during gelatinization of native and heat-moisture treated legume starches.

4.5 Digestibility by Porcine Pancreatic α -Amylase

The extent of hydrolysis of native legume starches by porcine pancreatic α -amylase is presented in Fig. 4.5. From the results, it is apparent that Green Arrow pea and Eston lentil starches are better substrates than the other three legume starches, undergoing 73 and 70% hydrolysis, respectively, in 4 days. The corresponding values for black bean, pinto bean and Express field pea were 35.4, 36.0, and 58.5%, respectively. After 24 hours, the increase in hydrolysis was only marginal in Green Arrow pea starch. For instance, after 4 days, hydrolysis had increased by only 5.5% in Green Arrow pea starch, whereas the corresponding values for the other starches were: 17.3% (black bean), 15.3% (pinto bean), 11.1% (Express field pea) and 18.3% (Eston lentil). It is interesting to observe that, initially, Eston lentil starch is hydrolysed to a lower extent than Green Arrow pea starch (Fig. 4.5). However, on the fourth day, the difference in hydrolysis between the two starches is negligible.

The mode of attack by α -amylase on granules of native legume starches was investigated by SEM (Figs. 4.6 & 4.7). After 72 hours hydrolysis, the attack of α -amylase on native black and pinto bean granules was less conspicuous than on Eston lentil, Express field pea and Green Arrow pea starches. The enzyme attack on black bean and pinto bean granules manifested itself in only mild superficial surface erosion (Fig. 4.6A,C). In contrast, the surfaces of Express field pea (Fig.

Fig. 4.5 Hydrolysis of native and heat-moisture treated (HMT) (16h, 100°C, 30% moisture) starches by porcine pancreatic α -amylase.
* The error bars were too small to see.

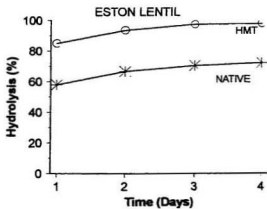
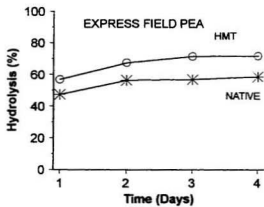
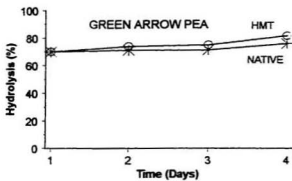
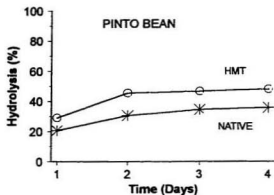
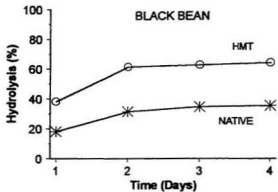


Fig. 4.6 Scanning electron micrographs of native starches after attack (72h) by porcine pancreatic α -amylase: Black bean (A), Pinto bean (B & C), Express field pea (D & E).

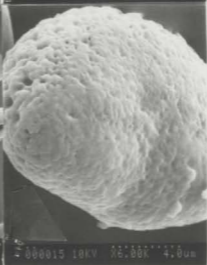
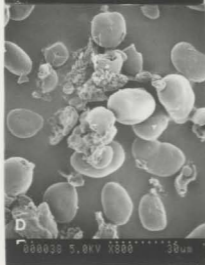
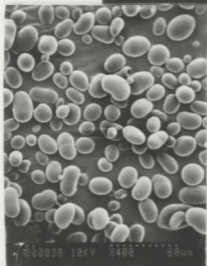
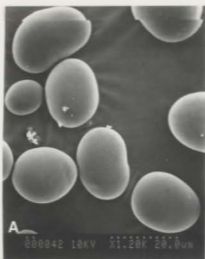
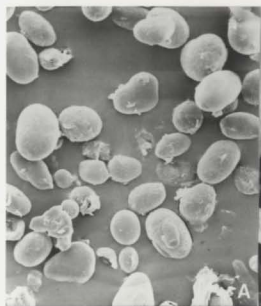


Fig. 4.7 Scanning electron micrographs of native starches after attack (72h) by porcine pancreatic α -amylase: Eston lentil (A & B), Green Arrow pea (C & D).



000028 10KV X800 30um



000030 10KV X5.00K 4.8um



000008 10KV X2.50K 9.6um



000009 10KV X10.0K 2.40um

4.6D, E) and Eston lentil (Fig. 4.7A,B) granules were extensively eroded with numerous fissures present on the granule surface. A small proportion of the granules were deformed. Granule splitting was not visible in either of the above two starches. During the same time interval, many granules of Green Arrow pea starch were deformed (Fig. 4.7C) and some were split open (Fig. 4.7C). Fissures were not present on the surface as in Express field pea and Eston lentil starches. However, the surface of Green Arrow pea starch was covered with craters of varying size and depth, as if the α -amylase had entered the granule and preferentially hydrolysed the interior portion (Fig. 4.7D).

Heat-moisture treatment increased the susceptibility of all legume starches towards hydrolysis by α -amylase (Fig. 4.5). The extent of this increase (after 24h hydrolysis) followed the order: Eston lentil > black bean > Express field pea > pinto bean > Green Arrow pea. Thereafter, the difference in hydrolysis between native and heat-moisture treated starches followed the order: black bean > Eston lentil > Express field pea > pinto bean > Green Arrow pea.

Increased susceptibility towards α -amylase hydrolysis following heat-moisture treatment has also been observed in pigeon pea (Hoover *et al.*, 1993), and potato and yam (Hoover & Vasanthan, 1994) starches. A comparison of Figs. 4.6 & 4.7 with Fig. 4.8 clearly shows the increased susceptibility towards α -amylase hydrolysis following heat-moisture treatment. The difference in hydrolysis among the native legume starches reflects the interplay of the following factors: (i) amylose content; (ii) percentage of lipid complexed amylose chains; (iii) extent of starch damage during isolation.

The enzyme catalyzed hydrolysis of α -D-(1-4) glucosidic linkages has been shown

Table 4.6. Differential scanning calorimetry characteristics of native starches and enzyme treated granular residues (following hydrolysis of native starches with α -amylase).

Starch source and treatment	T _p ^b (°C)	ΔH^c (cal/g)
Black Bean		
Native	69.0 ² ± 0.5	3.0 ¹ ± 0.1
Enzyme treated ^a	72.5 ¹ ± 0.1	2.9 ¹ ± 0.1
Pinto Bean		
Native	68.0 ² ± 0.5	2.9 ¹ ± 0.1
Enzyme treated	71.0 ¹ ± 0.1	2.8 ¹ ± 0.1
Express Field Pea		
Native	62.6 ² ± 0.1	2.3 ¹ ± 0.1
Enzyme treated	65.5 ¹ ± 0.5	1.7 ² ± 0.1
Eston Lentil		
Native	62.4 ² ± 0.1	2.4 ¹ ± 0.1
Enzyme treated	65.0 ¹ ± 0.5	1.4 ² ± 0.1

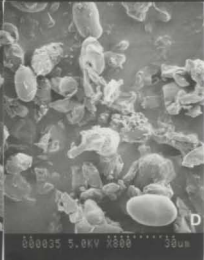
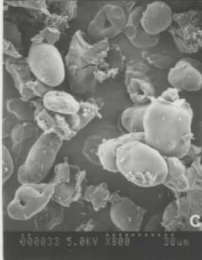
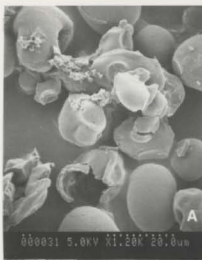
^aObtained after 72h hydrolysis with porcine pancreatic α -amylase.

^bMid-point of the gelatinization endotherm.

^cEnthalpy of gelatinization.

^dMean values in each column (native vs enzyme treated) not followed by the superscript are significantly different ($p \leq 0.05$).

Fig. 4.8 Scanning electron micrographs of heat-moisture treated (16h, 100°C, 30% moisture) starches after attack (72h) by porcine pancreatic α -amylase: Black bean (A), Pinto bean (B), Express field pea (C), Eston lentil (D), and Green Arrow pea (E).



(Thoma, 1968) to involve enzyme-induced ring distortion of one of the D-glucosyl residues from the 4C_1 chair conformation to a "half chair" conformation. This ring distortion decreases the enthalpy of activation and increases the susceptibility of the glucosyl residues to nucleophilic attack by functional groups on α -amylase and water. László *et al* (1978) have shown that ring distortions or a "half-chair" conformation is involved in the transition state of α -amylase. It is therefore, plausible that a conformational change (chair \rightarrow half-chair) during α -amylase hydrolysis may be difficult for those amylose chains that are complexed by native lipids. Several researchers (László *et al.*, 1978; Larsson & Miezis, 1979; Seneviratne & Biliaderis, 1991) have shown that amylose complexed lipids exhibit reduced susceptibility to α -amylase hydrolysis. Thus, in this study, Green Arrow pea starch should have been hydrolysed to a lesser extent (due to its higher content of lipid complexed amylose chains [Table 4.1]) than the other legume starches. The results suggest that the rapid hydrolysis of native Green Arrow pea starch (Fig. 4.5) is more likely due to the presence of split granules (Fig. 4.2C) and to its higher amylose content (Table 4.1). The above two factors probably negate the influence of lipid complexed amylose chains on enzyme hydrolysis. The differences in the rate and extent of hydrolysis between the other native legume starches (Eston lentil > Express field pea > pinto bean > black bean) can be attributed to their level of lipid complexed amylose chains (black bean > pinto bean > Express field pea > Eston lentil). This seems plausible, since the starches differed only marginally in their amylose contents (Table 4.1).

A summary of the DSC analysis of native and enzyme hydrolysed (72 hours) legume starch granules is presented in Table 4.6. Hydrolysis of Express field pea and Eston lentil resulted in a large decrease in ΔH . The extent of this decrease was 34.7 and 42%, respectively. However,

the decrease was only marginal in black bean (6.6%) and pinto bean (3.4%) starches.

As discussed earlier, ΔH values reflect the unravelling and melting of double helices. The large decreases in ΔH on hydrolysis (after 72h) of Express field pea and Eston lentil starches (Table 4.6) suggest that α -amylase attacks the double helices in the amorphous and crystalline regions of the granule. The marginal decreases in ΔH after enzyme hydrolysis of native black bean and pinto bean starches can be attributed to strong bonding forces (due to their long amylopectin chain lengths) between the starch chains joining the double helices.

The results for amylose leaching (Table 4.3) and DSC analysis (Table 4.5) showed that AM-AM and AM-AMP interactions occur during heat-moisture treatment. On this basis, the extent of hydrolysis of all starches should have decreased after heat-moisture treatment, since α -amylase preferentially hydrolyses the amorphous regions (Marsden & Gray, 1986) of the granule. Therefore, the observed increase in enzyme hydrolysis following heat-moisture treatment of Green Arrow pea starch (Fig. 4.5) suggests that the concentration of α -amylase within the granule interior may have been higher in heat-moisture treated granules, perhaps due to rapid diffusion of α -amylase through the cracks on the granule surface (Fig. 4.3) than in native granules. The results suggest that at the concentration of α -amylase prevailing within the granules of heat-moisture treated Green Arrow pea starch, even the strongly associated amylose chains are readily hydrolysed.

In black bean starch, changes in crystallite orientation following heat-moisture treatment (Fig. 4.4, Table 4.4) render the starch crystallites accessible to attack by α -amylase. This would explain the increase in hydrolysis after heat-moisture treatment (Fig. 4.5). The increase in hydrolysis after heat-moisture treatment is lower in Express field pea (in spite of crystallite

reorientation [Table 4.4, Fig. 4.4]) than in black bean starch, due to stronger interactions that occur between AM-AM and AM-AMP chains during heat-moisture treatment of Express field pea starch (this reduces the accessibility of α -amylase to the glucose units of the aggregated chains).

X-ray diffraction data showed that crystallite reorientation occurred only to a limited extent in pinto bean and Eston lentil starches (Table 4.4, Fig. 4.4). Furthermore, on heat-moisture treatment, AM-AM and AM-AMP interactions occurred to a greater extent in Eston lentil than in pinto bean starch (Table 4.2). Thus, the increase in hydrolysis after heat-moisture treatment should have been greater in pinto bean than in Eston lentil starch. The unexpected increase in hydrolysis in the latter was likely due to the presence of cracks on the granular surface (Fig. 4.3), which permitted ready access of α -amylase into the granular interior.

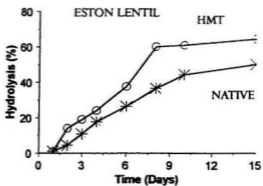
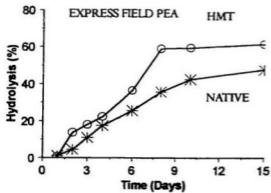
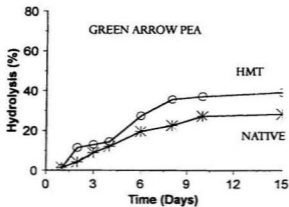
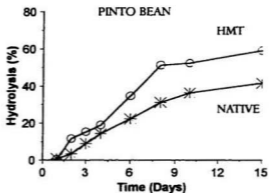
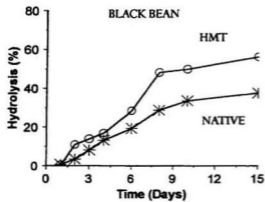
4.6 Acid Hydrolysis

The hydrolysis of the legume starches by 2.2N HCl is depicted in Fig. 4.9. All starches exhibited a two stage solubilization pattern. A relatively higher rate was observed during the first 8 days, followed by a lower rate between 8 and 15 days. At the end of the 8th day of hydrolysis (corresponding to the degradation of the amorphous regions of the granule), native starches were hydrolysed to the extent of 36.7% (Eston lentil), 35.6% (Express field pea), 31.2% (pinto bean), 30.2% (black bean) and 22.5% (Green Arrow pea).

Heat-moisture treatment increased the susceptibility of the starches towards acid (H_2O^+) hydrolysis. In all starches, the extent of this increase was marginal for the first 6 days of hydrolysis (Fig. 4.9). Thereafter, increases were more pronounced (black bean > Express field pea > Eston lentil ~ pinto bean > Green Arrow pea). However, previous studies (Hoover & Vasanthan, 1994; Hoover & Manuel, 1996; Hoover *et al.*, 1993) have shown that acid hydrolysis

Fig. 4.9 Acid hydrolysis of native and heat-moisture treated (HMT) starches.

* The error bars were too small to see.



decreases slightly after heat-moisture treatment of normal maize, waxy maize, high amylose maize, Laird lentil and pigeon pea starches, but increases in oat starch (Hoover & Vasanthan, 1994).

The results (Fig. 4.5 & Fig. 4.9) showed that legume starches are more susceptible to enzyme than acid hydrolysis. Similar findings have been reported for cereal and tuber (Hoover & Vasanthan, 1994) starches. The ranking of legume starches (with the exception of Green Arrow pea starch) towards acid hydrolysis (Eston lentil – Express field pea > pinto bean > black bean) was the same as for enzyme hydrolysis. It is interesting to observe that although both Green Arrow pea and Eston lentil starches were hydrolysed to nearly the same (~ 70%) extent (Fig. 4.5) by α -amylase, they were hydrolysed to different extents by H_3O^+ . For instance, on the 15th day, the extent of hydrolysis for Green Arrow pea and Eston lentil starches was 28 and 50%, respectively.

As in enzyme hydrolysis, a change in conformation of D-glucopyranose units (chair – half chair) is a prerequisite for hydrolysis of glucosidic bonds by H_3O^+ . Therefore, the extent of hydrolysis will depend on the accessibility of the glucosidic linkage to the hydrolysing agent. The degree of accessibility in turn would depend on the extent of packing of the starch chains within the amorphous and crystalline regions of the granule. Thus, extensive interactions between starch chains within native granules would hinder hydrolysis by H_3O^+ . The data from amylose leaching (Table 4.2) and swelling factor (Table 4.3) showed that starch chains in Green Arrow pea starch were highly associated. Furthermore, these chains were readily hydrolysed by α -amylase (Fig. 4.5), but were fairly resistant to hydrolysis by H_3O^+ (Fig. 4.9). This suggests that ring distortion (chair – half-chair) during hydrolysis occurs more readily in the presence of α -amylase.

Evidently, H_3O^+ is unable to access the glucosidic linkages which are buried within the associated starch chains. Furthermore, the susceptibility of the starches towards H_3O^+ (Eston lentil ~ Express field pea > pinto bean ~ black bean > Green Arrow pea) closely paralleled the extent of interaction of the starch chains within the native granule (Green Arrow pea > black bean ~ pinto > Express field pea > Eston lentil). Therefore, it seems that the major factor influencing H_3O^+ hydrolysis is the degree of starch chain associations within the native granule.

It has generally been accepted (Kainuma & French, 1971; Ring *et al.*, 1988; Cairns *et al.*, 1990) that heterogenous acid hydrolysis preferentially attacks the more accessible amorphous regions of the granule, whether they are at the surface or interior. In contrast, crystalline regions are less accessible to hydrated protons and are attacked only after a period of 8-12 days. The results show that the major factor influencing the increase in hydrolysis on heat-moisture treatment is the change that occurs within the starch crystallites (crystallite reorientation). This seems plausible since the difference in hydrolysis between native and heat-moisture treated starches were more pronounced only after the 8th day (Fig. 4.9) of hydrolysis. The increase in hydrolysis after heat-moisture treatment was higher in black bean and Express field pea starches, due to chain reorientation in crystallites (Fig. 4.4) being more pronounced in these two starches than in the other starches. The extent of increase in hydrolysis was practically the same for pinto bean and Eston lentil starches, since chain orientation in crystallites occurred to the same extent in both starches (Fig. 4.4, Table 4.4). The increase in hydrolysis was only marginal for Green Arrow pea starch due to the interplay of two factors: (i) formation of new crystallites (tends to decrease hydrolysis), and (ii) the formation of cracks on the granule surface on heat-moisture treatment (tends to increase hydrolysis).

CHAPTER 5.

CONCLUSION

Heat-moisture treatment did not alter the size or shape of the starch granules used in this study. The surface of the native granules of black bean, pinto bean and Express field pea appeared smooth even after heat-moisture treatment. However, the surface of some granules of Eston lentil exhibited deep cracks, whereas the surface of many granules of Green Arrow pea were broken up into numerous pieces of varying size.

The results showed that amylose leaching in native legume starches were influenced by the interplay of the bound lipid content (restricts amylose leaching) and the extent of associations between the starch chains (AM-AM and AM-AMP). The swelling factor of the native starches closely followed the bound lipid content. However, the net swelling factors of the native starches were influenced by the interplay of the bound lipid content, and the arrangement of amylose chains within the amorphous regions of the granules (close packing of amylose chains in native Green Arrow pea contributed to its very low swelling factor). Following heat-moisture treatment, all starches exhibited a decrease in amylose leaching and swelling factor. The decreases were attributed to increased associations between starch chains (AM-AM and AM-AMP) during heat-moisture treatment. Black bean and pinto bean starches exhibited smaller decreases in amylose leaching and swelling factor after heat-moisture treatment than did the other starches. This indicates that the starch chains of black bean and pinto bean starches may be longer than those of the other starches (long chains facilitate strong interchain associations within the native granule). Therefore, the formation of additional AM-AM and AM-AMP interactions during heat-moisture treatment of black bean and pinto bean starches would be inhibited. The swelling factor of heat-

moisture treated Green Arrow pea was attributed to the interplay of AM-AM interactions (inhibit swelling), and modifications to the granules (facilitate swelling by allowing more water to enter the granules).

Native starches exhibited a "C" type X-ray diffraction pattern, except Green Arrow pea starch which exhibited a "B" type pattern. The differences in intensities among the "C" type starches were attributed to differences in double helical packing arrangements in the crystalline regions of the native starches. The low X-ray intensities of native Green Arrow pea starch was attributed to its low amylopectin content, and perhaps to the presence of small crystallites. The X-ray diffraction patterns of the native starches were not altered by heat-moisture treatment. It was therefore suggested that strong starch chain associations within the native granules may have prevented the movement of double helices during heat-moisture treatment. This prevents the rearrangement from the "B" type to the "A" type unit cell. Since the "C" type legume starches are a mixture of "A + B" unit cells it is also likely that native "C" type starches may have contained a high proportion of "A" type unit cells and consequently, no "B to A" transition was observed. The "C" type starches all exhibited a decrease in intensity after heat-moisture due to reorientation of the starch crystallites which resulted in a crystalline array that did not diffract X-rays as strongly as the crystallites within the native granules. Green Arrow pea starch, however, did exhibit an increase in X-ray intensities following heat-moisture treatment perhaps due to formation of new crystallites which were better arrayed to diffract X-rays than those present within the native granules.

Native black bean and pinto bean starches exhibited higher gelatinization parameters than those of Express field pea and Eston lentil starches which suggests that the chains forming the

double helices in the former are longer than those of the latter. The broad gelatinization temperature range of black bean and pinto bean starches also indicate that these starches may contain a higher proportion of long chains (which could result in double helices varying in size and perfection) than Express field pea and Eston lentil. The gelatinization temperatures of the native starches increased following heat-moisture treatment. This increase was attributed to the increased starch chain associations which occurred during heat-moisture treatment. It was postulated that the increase in starch chain associations would suppress the amorphous regions of the granule which, as a result, required a higher temperature to incur swelling that could contribute to the disruption of the crystalline regions. Heat-moisture treatment did not affect ΔH of legume starches which indicates that identical amounts of double helices unravel and melt during gelatinization of native and heat-moisture treated legume starches.

Digestibility studies by porcine pancreatic α -amylase indicate that Green Arrow pea and Eston lentil starches were better substrates than the other legume starches. The differences observed in the rate and extent of hydrolysis among the native legume starches were influenced by their bound lipid contents. The rapid hydrolysis of Green Arrow pea starch was influenced by the presence of split granules, as well as its high amylose content. These two factors together probably cancelled the effect of the high bound lipid content (reduces susceptibility to enzymatic attack) of Green Arrow pea starch. After 72h hydrolysis, the ΔH values of Express field pea and Eston lentil exhibited a large decrease which suggests that α -amylase attacked the double helices in the amorphous and crystalline regions of the granules. Black bean and pinto bean starches exhibited only marginal changes in ΔH values, after 72h hydrolysis, due to strong bonding forces between the starch chains joining the double helices. The results for amylose leaching and DSC

measurements indicate that AM-AM and AM-AMP interactions occur during heat-moisture treatment. Therefore, the extent of α -amylase hydrolysis of all starches should have decreased after heat-moisture treatment. However, an increase in hydrolysis was observed following heat-moisture treatment. The increase in enzyme hydrolysis was attributed to: (i) the rapid diffusion of α -amylase through the cracks on the granule surface which increased the concentration of α -amylase within the granule interior (Green Arrow pea, Eston lentil); and (ii) changes in crystallite orientation following heat-moisture treatment which rendered the starch crystallites more accessible to attack by α -amylase (Black bean>Express field pea>Eston lentil>pinto bean).

The results of hydrolysis of legume starches by 2.2N HCl indicate that the starches are more susceptible to attack by enzyme than acid. This suggests that ring distortion (chair \rightarrow half chair) during hydrolysis occurred more readily in the presence of α -amylase than H_3O^+ . It appears that the H_3O^+ was unable to access the glucosidic linkages of the starch chains. Therefore, the major factor affecting hydrolysis by H_3O^+ was the degree of starch chain associations within the native starch granules which in turn influences the accessibility of the glucosidic linkage to the hydrolysing agent. The rate and extent of acid hydrolysis increased in all starches following heat-moisture treatment, with the increase being more pronounced after the 8th day of hydrolysis (corresponds to degradation of the crystalline regions). The increases in hydrolysis was attributed to crystallite reorientation which rendered the crystalline regions of the granules more accessible to H_3O^+ . Green Arrow pea exhibited only a marginal increase in hydrolysis after heat-moisture treatment due to the formation of new crystallites (tends to decrease hydrolysis) and, the formation of cracks on the granule surface (tends to increase hydrolysis).

The results show that starch chain interactions (amylose-amylose and amylose-amylopectin) within native legume starches during heat-moisture treatment are influenced by the interplay of several factors including: (i) amylose content; (ii) arrangement of amylose chains within the amorphous regions of the granule; and (iii) amylose and amylopectin chain lengths. The physicochemical properties of heat-moisture treated legume starches are influenced by the magnitude of the above interactions, orientation of starch crystallites and changes to the granular surface (in Green Arrow pea and Eston lentil).

The results of this study indicate that additional study is needed to provide more insights into the effect of heat-moisture treatment (under different conditions of temperature, time and moisture) on the thermal, rheological and retrogradation properties of legume starches. However, before such a study is initiated, it is necessary to investigate the molecular structure and the proportion of A/B unit cells in various legume starches. This would provide a better understanding of the structural changes that occur within the amorphous and crystalline regions of legume starches during heat-moisture treatment. Only then can the functional properties of heat-moisture treated legume starches be investigated.

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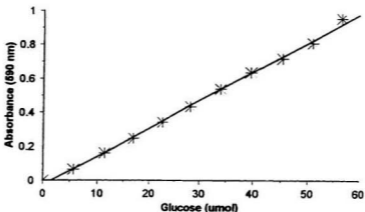
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APPENDIX I

3,5-DNS Glucose Calibration



Linear Regression Analysis

Constant	0
Std Err of Y Est	0.023047
R Squared	0.993707
No. of Observations	10
Degrees of Freedom	9
X-Coefficient	0.015976
Std Err of Coef.	0.000207

Therefore: $Y = 0.015976X + 0$

Where: $Y = \text{Absorbance at } 590 \text{ nm}$
 $X = \text{Glucose (umol)}$

And: $X = Y/0.015976$

Sample Calculation:

GIVEN:

Sample solution of unknown glucose concentration has absorbance of 0.354 at 590nm (Bruner's Method).

DETERMINE:

The amount of glucose present in the sample:

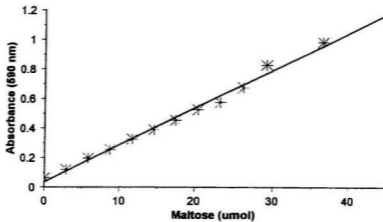
SOLUTION:

$$X = Y/0.015976$$

$$X = 0.354/0.015976$$

$$X = 22.15 \text{ umol glucose}$$

3,5-DNS Maltose Calibration



Linear Regression Analysis

Constant	0
Std Err of Y Est	0.008886
R Squared	0.999007
No of Observations	12
Degrees of Freedom	11
X-Coefficient	0.022416
Std Err of Coef.	0.000109

Therefore: $Y = 0.022416X + 0$

Where: $Y = \text{Absorbance at 690 nm}$
 $X = \text{Maltose (umol)}$

And: $X = Y/0.022416$

