ENZYMATIC HYDROLYSIS OF NATIVE GRANULAR AND MODIFIED STARCH BELOW GELATINIZATION TEMPERATURE

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS & ABBREVIATIONS

Symbols	**Caption**
T_c	Conclusion temperature
T_g	Glass temperature
T_o	Onset temperature
T_p	Peak temperature
ΔH	Gelatinization enthalpy

Abbreviations	**Caption**
AM	Amylose
AMP	Amylopectin
ECH	Epichlorohydrin
GSHU	Granular Starch Hydrolyzing Units
DMSO	Dimethyl sulphoxide
DNS	Dinitrosalycylic
DE	Dextrose equivalent
DP	Degree of polymerization
DSC	Differential scanning calorimetry
RDS	Rapidly digestible starch
RS	Resistant starch
RVA	Rapid Visco Analyzer
SDS	Slowly digestible starch
SEM	Scanning Electron Microscopy
SSF	Simultaneous saccharification and fermentation

LIST OF APPENDICES

HIDROLISIS BERENZIM KANJI ASLI BERGRANUL DAN TERUBAHSUAI DI BAWAH SUHU GELATINISASI

ABSTRAK

Kesan rawatan haba sederhana (di bawah suhu gelatinisasi), paut-silang dan 'annealing' terhadap tahap kerintangan kanji untuk dihidrolisis oleh enzim pada suhu pra-gelatinisasi (35ºC) telah dikaji. Rawatan haba sederhana telah dilakukan pada suhu 60ºC selama 30 minit. Rawatan paut-silang telah dilakukan dengan mencampurkan epichlorohydrin (ECH) dengan substrat kanji, manakala 'annealing' telah dilakukan pada suhu 50ºC selama 72 jam. Kanji ubi kayu dan ubi keledek telah dihidrolisis berenzim dengan campuran amylase dan glucoamylase daripada fungi pada suhu 35ºC selama 24 jam. Nilai dekstrosa (DE) bagi kanji terawat haba meningkat secara signifikan berbanding kanji asli, iaitu 36% kepada 50% bagi kanji ubi kayu dan 27% kepada 34% bagi kanji ubi keledek. Nilai DE bagi kanji ubi kayu terpaut-silang menurun dengan signifikan daripada 36% kepada 28%. Kedua-dua kanji ubi kayu dan ubi keledek yang telah dikenakan rawatan 'annealing' telah dihidrolisiskan kepada tahap yang lebih tinggi berbanding kanji asli. Analisis Scanning Electron Microscopy (SEM) pada kanji yang telah dihidrolisis menunjukkan hakisan enzim pada kanji berlaku terutamanya pada permukaan granul kanji. Kanji terawat haba sederhana yang telah dihidrolisis menunjukkan permukaan granul yang lebih kasar dan berliang-rongga berbanding kanji asli. Mikrograf SEM menunjukkan kanji terpaut-silang yang telah dihidrolisis mempunyai granul yang kurang berliang-rongga berbanding kanji asli. Kedua-dua kanji dengan rawatan 'annealing' mempunyai granul yang lebih berliang-rongga dan permukaan yang lebih kasar berbanding kanji asli, akibat daripada hakisan enzim yang berlaku terutamanya pada permukaan granul. Kuasa pembengkakan dan kelarutan kanji terawat haba,

paut-silang dan ‘annealing’ yang telah dihidrolisis menurun dengan signifikan berbanding kanji asli ubi kayu dan ubi keledek. Rawatan haba, paut-silang dan ‘annealing’ telah mempengaruhi sifat pempesan kanji dengan signifikan dan meningkatkan suhu gelatinisasi kanji. Kandungan amilosa kanji ubi kayu dan ubi keledek terawat haba dan ‘annealing’ yang telah dihidrolisis meningkat dengan signifikan. Analisis X-ray menunjukkan hakisan enzim berlaku terutamanya pada bahagian amorfus granul memandangkan tiada perubahan yang signifikan pada corak x-ray kanji asli dan terawat yang telah dihidrolisis. Pada asasnya, kanji ubi kayu dan ubi keledek terawat yang telah dihidrolisis menunjukkan taburan saiz partikel yang lebih kecil berbanding kanji aslinya. Rawatan haba sederhana di bawah suhu gelatinisasi adalah terbukti efektif bagi meningkatkan kadar hidrolisis kanji. Rawatan paut-silang didapati telah menurunkan kadar hidrolisis kanji ubi keledek dengan merendahkan kuasa pembengkakan dan kelarutan kanji. Kajian ini juga menunjukkan rawatan ‘annealing’ telah menyebabkan hidrolisis kanji oleh enzim berlaku dengan lebih efektif dengan kehadiran pori-pori kecil pada permukaan granul kanji yang terbentuk semasa rawatan ‘annealing’.

ENZYMATIC HYDROLYSIS OF NATIVE GRANULAR AND MODIFIED STARCH BELOW GELATINIZATION TEMPERATURE

ABSTRACT

The effects of mild heat treatment, cross-linking and annealing towards the susceptibility of granular starch to enzymatic hydrolysis at sub-gelatinization temperature (35ºC) were investigated. Mild heat-treatment was carried out at 60ºC for 30 minutes. Cross-linking was performed by re-acting the starches with 1% epichlorohydrin (ECH), while for annealing the starches were annealed at 50ºC for 72 hours. Tapioca and sweet potato starches were subjected to enzymatic hydrolysis with a mixture of fungal α-amylase and glucoamylase at 35°C for 24 hours. The dextrose equivalent (DE) value of heat-treated starch increased significantly compared to native starch i.e. 36% to 50% and 27% to 34% for tapioca and sweet potato starch, respectively. The DE of cross-linked tapioca starch decreased significantly from 36% to 28%. Both annealed tapioca and sweet potato starches were hydrolyzed to a greater degree compared to native starch. Scanning Electron Microscopy (SEM) examination on hydrolyzed starches showed that enzymatic erosion occurred mainly at the surface of starch granules. Hydrolyzed heat-treated starch exhibited rougher surface and porous granules compared to native starch. SEM micrograph revealed that hydrolyzed cross-linked starches had less porous granules as compared to their native counterparts. Both hydrolyzed annealed starches had more porous granules and rougher surface as compared to native starch due to enzymatic erosion. The swelling power and solubility of hydrolyzed heat-treated, hydrolyzed cross-linked and hydrolyzed annealed starches decreased significantly as compared to their native counterparts for both tapioca and sweet potato starch. Cross-linking and annealing had affected the pasting properties of the starches significantly

and increased the gelatinization temperature. The amylose content increased significantly in hydrolyzed heat-treated and annealed tapioca and sweet potato starches. X-ray analysis suggested that enzymatic erosion preferentially occurred in amorphous areas of the granules for all starches as no significant changes were observed in the x-ray diffraction patterns of hydrolyzed native and pre-treated starches. The hydrolyzed pre-treated tapioca and sweet potato starches basically showed smaller value of particle size distribution in contrast to their native counterparts. Evidently, heating treatment below gelatinization temperature was effective in enhancing the degree of hydrolysis of granular starch. Cross-linking was found to reduce the extent of hydrolysis of tapioca starch by reducing the swelling power and solubility of the starch granules. This study also showed that annealing treatment had allowed the enzymes to hydrolyze the starch more extensively by the presence of pores on the starch granules created during the treatment.

1 INTRODUCTION

1.1 Background

Starch is a mixture of two polysaccharides, the linear molecule of amylose which consists of units of glucose, and amylopectin, a highly branched molecule. Starch can be sub-divided into cereal, legumes, palm and tuber or root starches. Tapioca (*Manihot esculenta* Crantz) and sweet potato (*Ipomea batatas* Lam) starches are examples of starch derived from roots or tubers. Granules of tuber and root starches are oval, although round, spherical, polygonal and irregular shaped also exist (Lideboom et al., 2004). Starch susceptibility to enzyme attack is influenced by several factors such as amylose and amylopectin content (Holm and Bjorck, 1988; Ring et al., 1988), particle size, crystalline structure and the presence of enzyme inhibitors. Among these factors, granular structure is believed to be the most important (Zhang and Oates, 1999). Among non-cereal starches, tapioca starch has relatively higher enzyme susceptibility than other starches such as potato and sweet potato (Rickard et al., 1991; Zhang and Oats, 1999). According to Hizukuri et al. (1988) and Kainuma (1988), sweet potato starch shows stronger resistance to α-amylase and glucoamylase attack.

Demand for fuel grade ethanol from agricultural raw materials continues to grow on a global basis. A variety of agricultural crops have the potential to be the primary substrate/starch source to liberate fermentable glucose in the production of a variety of biochemicals, including renewable transportation fuel, like ethanol. Industrial production of starch hydrolysis products such as glucose syrup is typically based on acid or enzyme hydrolysis on gelatinized starch. Conventional process for production of glucose syrups or bioethanol basically involved liquefaction and saccharification process of starch. Liquefaction and saccharification require the

starch granules to be extensively gelatinized at high temperature. This is an energy intensive process requiring the addition of heat energy to starch granule slurries until the gelatinization temperature of the starch is exceeded. The whole process requires a high-energy input, thus increasing the production cost of starch-based products. In view of energy costs, effective utilization of natural resources and viscosity problems, direct hydrolysis of starch below gelatinization temperature is desirable.

Typically, enzyme hydrolysis of granular starch yield low degree of conversion to fermentable sugars. Although starch macromolecules can be hydrolyzed in a granular state, however, attempts to hydrolyze native granules invariably result in a slowly and often poorly hydrolyzed product (Oates, 1997). Generally, the action of α- and β-amylases on native starch granules is not very effective because granules are very resistant to amylolytic digestion and a long hydrolysis period is required for degrading the starch (Sarikaya et al., 2000). With new technological advances in recent years, however, new generation of enzymes such as α-amylase from *Aspergillus kawachi* and glucoamylase from *Aspergillus niger* have been discovered. These enzymes work synergistically to hydrolyze granular starches that can directly hydrolyze the raw starch in a single step at moderate temperature well below the gelatinization temperature. These enzymes have the advantages of exo-activity of glucoamylase which is able to drill sharp and deep pinholes; as well as the endo-activity of α-amylase that enables widening of the pinholes. This combination enhances the release of fermentable glucose continuously from granular starches. Franco et al. (1988) reported that more hydrolysis can be obtained by hydrolyzing the starch with α-amylase together with glucoamylase. Further modification can be made in order to increase the efficiency of native starch hydrolysis. Oates (1997) suggested that better hydrolysis of native starch can be

achieved by increasing the incubation temperature approximately to 60°C. If the degree of conversion of native starch could be increased further, it would be very useful in the industrial process of fermentable sugars and bioethanol.

The present study was designed to study the action of amylolytic enzymes in hydrolyzing starch and the effects of mild heat-treatment (below gelatinization temperature), cross-linking and annealing treatment towards the susceptibility of granular starch to enzymatic hydrolysis.

1.2 Objectives

a) To investigate the feasibility of hydrolyzing starch (tapioca and sweet potato) at sub-gelatinization temperature using a novel amylolytic enzyme from fungal sources.

b) To study the action of amylolytic enzyme for its ability to hydrolyze starch in its native granular state, thus increase understanding of the factors contributing to the production of starch oligomers.

c) To study the effect of mild heat-treatment, cross-linking and annealing treatment of starch on the susceptibility of the starch to enzymatic hydrolysis.

2 LITERATURE REVIEW

2.1 Starch

Starch is the main carbohydrate reserve in plants and is the major reserve polysaccharides of green plants. Starch is synthesized naturally in a variety of plants where the starch granules synthesized in amyloplasts and is deposited in the form of tiny granules in the major depots of seeds (e.g. corn, wheat, rice, sorghum), tubers (e.g. potato), roots (e.g. tapioca, sweet potato, arrowroot), legume seeds (e.g. peas, beans, lentils), fruits (e.g. green bananas, unripe apples, green tomatoes), trunks (e.g. sago palm) and leaves (e.g. tobacco) (Chen, 2003). Corn, potato, rice, wheat, sorghum and cassava plants are examples of plant that has high starch content.

Basically, starches for industrial use are extracted from the raw material and yield a wide range of products. According to Chen (2003), starch can be simply manufactured by the combination of grinding the starch-rich crop followed by wet separation techniques. The starch granules will sediment in water because of the higher density. Native starch is a white powder with bland taste and flavour, and insoluble in cold water. Improvements in the properties of starches for industrial use can be achieved by using chemical and physical modifications such as cross-linking and annealing treatment.

Starch varies greatly in form and functionality between and within botanical species, and even from the same plant cultivar grown under different conditions. Starches from different botanical sources, from different cultivars and from the same cultivars grown under different conditions are characterized by differing physicochemical properties. Starch granules from different botanical sources have different characteristic shapes, sizes and morphology. The sizes of starch granules vary from submicron to more than 100 microns in diameter. Starch granules varies in

shape include spherical, disk, oval, polygonal, dome-shape, elongated rod shape and compound starch (Jane, 2006). Huber and BeMiller (2001) reported that starches from various sources can be differ in terms of the granule morphology (size, shape, presence or lack of pores, channels and cavities), molecular structure (amylose and amylopectin fine structures) and composition (amylose-to-amylopectin ratio, content of non-starch components) lead to variations in starch properties (X-ray diffraction pattern, gelatinization temperature range, gel properties, retrogradation tendency, granule swelling power and pattern, etc.) which are indications of structural divergence.

Starch is classified as a complex carbohydrate and is a mixture of two polymers of glucose; amylose and amylopectin. The complexity is built around variations in the composition of *alpha*-glucan, moisture, lipid, protein, phosphorylated residues and structure of the components. Some granules contain almost entirely amylopectin (98%); others are high in amylose (45-80%), but granules generally are a mixture of amylose and amylopectin with amylose content of 15-30% (Whistler et al., 1984). According to Chen (2003), the amylose content and degree of polymerization (DP) of amylose are important for the physical, chemical and technological properties of starch. In addition to amylose and amylopectin, starch granules also contain small quantities of protein and lipid. Between species there is variation in the structure of amylopectin (Jane et al., 1999), the size and structure of amylose (Hizukuri et al., 1981) and the nature and amounts of proteins and lipids (Tester and Morrison, 1992). Generally, cereal starches such as corn, wheat and rice contain relatively high levels of lipids (0.2-0.8%) and protein (0.2-0.5%) resulting in lower paste transparency and a pronounced and persistent "raw cereal flavour" of the starch gels (Chen, 2003). According to Chen (2003)

again, tuber (e.g. potato) and root (e.g. tapioca) starches have lower levels of lipids (0.1-0.2%) and protein contents (0.1-0.2%). The moisture content of native starch granules is usually about 10% (Copeland et al., 2009). The chemical characteristics of starches obtained from various sources are presented in Table 2.1.

Table 2.1: Chemical characteristics of starches obtained from various sources

Starch	Amylose (%)	Lipids (%)	Proteins (%)	Phosphorus (%)
Corn[a]	28	0.80	0.35	0.00
Waxy corn[a]	>2	0.20	0.25	0.00
High-amylose corn[a]	50-70	nd	0.50	0.00
Wheat[a]	28	0.90	0.40	0.00
Potato[a]	21	0.10	0.10	0.08
Tapioca[b]	14-24	0.10	nd	0.01
Sweet potato[b]	20-25	0.01-0.26	nd	0.01-0.02
Mung bean[c]	39	0.30	0.30	nd

a: from BeMiller and Whistler, 1996; b: from Moorthy, 2002; c: from Hoover et al., 1997. nd: not determined.

It is well known that starch has different susceptibility to enzyme attack and some of the starch resists digestion by α-amylase. Englyst et al. (1992) proposed three classes of dietary starch: 1) rapidly digestible starch (RDS), which is likely to digested in the human intestine; 2) slowly digestible starch (SDS), which is likely to be slowly yet completely digested in the small intestine; and 3) resistant starch (RS), which is likely to resist digestion in the small intestine. According to Woo and Seib (2002), RS is defined as the sum of starch and starch degradation products not digested in the small intestine of healthy individuals.

2.2 Starch Chemistry

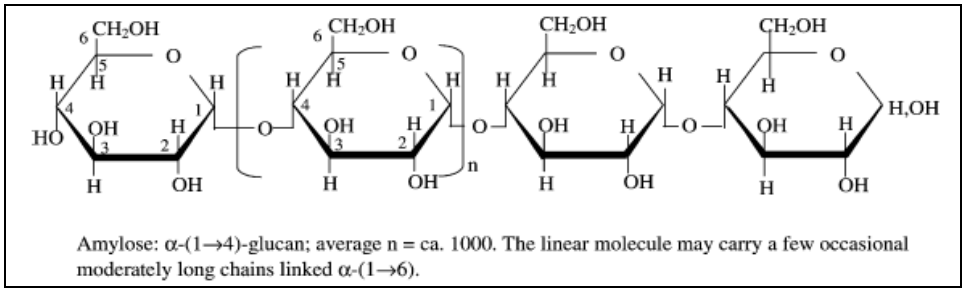
2.2.1 Amylose and Amylopectin

There are two types of glucose chains in starch, where the simple chain is called amylose and the complex branched is called amylopectin. The approximate weight amounts of amylose and amylopectin is in the range of 15-30% and 85-70%, respectively (Jane et al., 1994). Tester and Karkalas (2002) reported that both amylose and amylopectin represent approximately 98-99% of the dry weight. The ratio of the two polysaccharides varies among the starches according to their botanical origin (Tester and Karkalas, 2004). In starch granules, the amylose and amylopectin molecules are radially oriented with their single reducing end-groups towards the centre or hilum, and synthesis is by apposition at the outer non-reducing ends (Nikuni, 1978; French, 1984). Amylose which is a mostly unbranched component is interspersed among amylopectin clusters in both the amorphous and the crystalline regions (Jane et al., 1997). Amylose interspersed among amylopectin is more concentrated at the periphery than at the core of a starch granule. Amylopectin at the periphery of a starch granule consists of shorter branch chains than that at the inner part of the granule (Jane, 2006).

Although amylose and amylopectin are both composed of D-glucopyranose molecules, dissimilarities between these two polymers result in major differences in functional properties. The presence of amylose lowers the melting point of crystalline regions and the energy for starting gelatinization (Flipse et al., 1996).

2.2.1 (a) Amylose

The structure of amylose has been extensively analyzed and is shown to have multiple branches; the number of branches of amylose molecules depends on the molecular size and botanical source of the starch. Amylose is considered to be an essentially linear polymer composed almost entirely of α-1,4-linked D-glucopyranose and a very few α-1,6 linked D-glucopyranose (Figure 2.1). Amylose can be made of several thousand glucose units and the number of repeated glucose subunits (n) can be many thousands. Amylose is found with molecular weights ranging from 10^5-10^6 and with the number of glucose residues per molecule, (DP) ranging from 500 to 5000 (Galliard and Bowler, 1987). DP is the total number of anhydroglucose residues present divided by the number of reducing ends. Amylose is present in the amorphous structure.



Amylose: α-(1→4)-glucan; average n = ca. 1000. The linear molecule may carry a few occasional moderately long chains linked α-(1→6).

Figure: 2.1 Structure of amylose (Tester et al., 2004).

According to Chen (2003), amylose can form complexes with iodine and various organic compounds such as butanol and fatty acids. The α-1,4 bonds promote the formation of a helix structure. The interior of the helix contains hydrogen atoms and is therefore hydrophobic, allowing amylose to form a type of and clathrate (an inclusion complex wherein a "host" molecule entraps a second molecular species as

the “guest”) complex with free fatty acids, fatty acid components of glycerides, some alcohols and iodine. Iodine complexation is an important diagnostic tool for the characterization of starch. Iodine molecules fit neatly inside the helical structure of amylose, binding with the starch polymer that absorbs certain known wavelengths of light. In the amylose-iodine reaction, the yellow solution of iodine will turn to blue-black colour in the presence of amylose. The intensity of the colour can be used as a reference to characterize the type of the starch.

The amylose fraction can be isolated by few methods such as aqueous leaching procedures (Hizukuri, 1996), dispersion and precipitation (Adkins and Greenwood, 1969; Banks et al., 1971) and ultracentrifugation methods (Montgomery et al., 1961; Majzoobi et al., 2003). Amylose in water is unstable, quickly precipitating to initiate gelation, which is the terminology to describe the process of “setback” or rigidity development that occurs as starch gels are cooled. It is known that enzyme digestibility of granular starch is inversely related to the amylose content of the starch. For instance, waxy rice and waxy maize starches are more easily digestible than normal rice and normal maize starch counterparts (Jane, 2006).

2.2.1 (b) Amylopectin

Amylopectin is one of the largest molecules in nature. It is a branched polysaccharides composed of hundreds of short α-1,4-glucan chains, which are interlinked by α-1,6-linkages (Figure 2.2). Amylopectin has molecular weights ranging from 10^7-10^9, depending upon the source. The amylopectin branches may be classified according to their pattern of substitution: A-chains are defined as unsubstituted, B-chains are substituted by other chains and there is a single C-chain that carries the reducing glucose. Chen (2003) suggested that amylopectin structure

consists of three type chains (Figure 2.3) i.e A-chain, B-chain and C-chain. The C-chain carries the sole reducing group in the molecule to which the B-chains are attached, while the terminal A-chain is attached to B-chain (Manners, 1989). The characterization of amylopectin basically depends on the ratio of A-chain to B-chain. Hizukuri (1996) reported that a proportion of A-chain gives a low tendency to retrogradation of amylopectin.

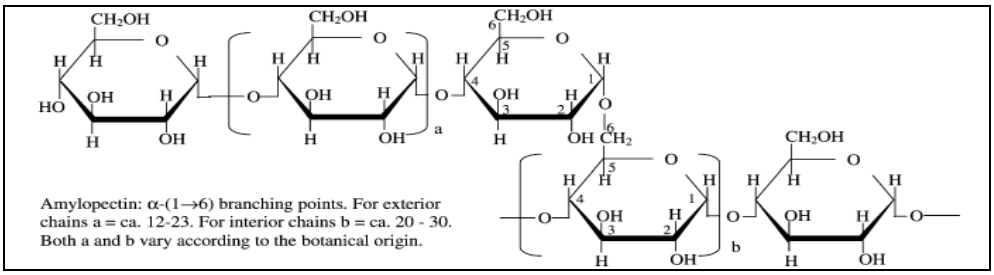


Figure 2.2: Structure of amylopectin (Tester et al., 2004).

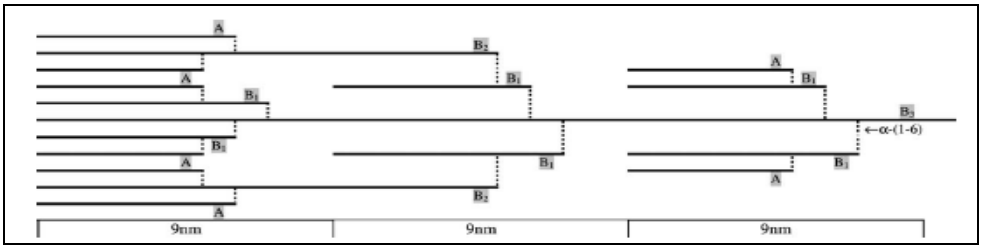


Figure 2.3: Chain distribution of amylopectin (Tester et al., 2004).

Amylopectin is the major component of starch. The content of amylopectin varies from 91-100% in waxy starch, 70-80% in normal starches and 30-50% in high amylose starches (Song and Jane, 2000). Being a major component in starch, its crystalline structure nature and its swelling power, amylopectin plays an important

and dominating role in the starch properties such as gelatinization and pasting properties.

Amylopectin in the granule is present in the semi-crystalline structure (Jane, 2006). Amylopectin is highly crystalline and it is responsible for the crystallinity of starch granules and results in insolubility of starch granules in cold water. According to Zobel (1988), amylopectin is responsible for the crystalline structure of starch granules where native starch granules display 15-45% crystallinity. Starch granules need to be heated to a temperature above its gelatinization temperature in the presence of water in order to disperse and develop a paste. Amylopectin has short branches on about 4% of the D-glucosyl residues. Amylopectin molecules are very large, ranging from 50 million to over a 100 million in molecular weight (Whistler et al., 1984). Some important physicochemical properties of amylose and amylopectin are listed in Table 2.2.

Table 2.2: Some important physicochemical properties of amylose and amylopectin

Property	Amylose	Amylopectin
Molecular structure[a]	Linear (α-1,4)	Branched (α-1,4; α-1,6)
Molecular weight[b]	~10^6 Daltons	~10^8 Daltons
Degree of polymerization[a]	1500-6000	$3x10^5$-$3x10^6$
Helical complex[b]	Strong	Weak
Iodine colour[a]	Blue	Red-purple
Dilute solutions[a]	Unstable	Stable
Retrogradation[b]	Rapidly	Slowly
Gel property[a]	Stiff, irreversible	Soft, reversible
Film property[a]	Strong	Weak and brittle

a: from Jane (2000); b: from Zobel (1988).

2.2.2 Minor Constituents of Starch

According to Moorthy (2002), the extracted starch is usually accompanied by various other components such as fibre, lipid, proteins, and minerals, depending on the number of factors, for instance, method of extraction, age of the crop, environmental conditions etc. These small amounts of non-carbohydrate components, particularly lipids, proteins, phosphate and ash, would affect the behaviour of starch in various applications. Some of these impart desirable qualities to the starch, while others affect the quality (Moorthy, 2002). Proteins, lipids, moisture and ash (minerals and salts) are present in starch granules in very small quantities. Typically, moisture content of starch powder equilibrates to about 12% whereas ash content is typically less than 0.5%. The contents of minor constituents of starch differ with different botanical sources of starch. Tuber and root starches contain less lipid and protein as compared to cereal starches, for instances.

Starch granule proteins have been divided into two types on the basis of their ability to be extracted from the granules which is “surface” and “integral” protein. Theoretically, the surface proteins are loosely associated with the exterior part of the starch granule while the integral proteins are imbedded and possibly covalently bound in the amylose-amylopectin structure of the granule. Surface associated proteins can be removed with salt solutions whereas integral proteins need a more intense treatment, for example with sodium dodecyl sulphate. Integral proteins have a higher molecular weight than the surface proteins (~50-150 and ~15-30 kDa, respectively) and this includes the residues of enzymes involved in starch synthesis, especially starch synthase. All protein analysis is reported as percent nitrogen and the protein content varies based on the source of the starch (Eliasson, 2004). Generally,

commercial starches sold within the food industry contain less than 1% protein (Eliasson, 2004).

Starch lipids are contained within the starch granules. It may be present in the form of integral lipids which comprise of lysophospholipids (LPL) and free fatty acid (FFA) (Tester and Karkalas, 2002). It has a strong effect on the starch properties (Krog, 1973; Fredriksson et al., 1998; Eliasson and Gudmundsson, 1996) where the formation of the starch-lipid or starch-surfactant complexes would improve the textural properties of various foods (Hoover et al., 1998; Biliaderis et al., 1993). Tuber starches contain much lower quantities of lipids as compared to cereal starches, therefore, the effect on tuber starches is not so pronounced as in cereal starches. According to Moorthy et al. (1996), the lipid content in five cultivars of tapioca varied between 0.11-0.22% in starch and 0.27-0.45% in flour. Commercial starches supplied to the food industry usually contain less than 1% lipid (Eliasson, 2004).

Phosphorus is another component that present in starch which is associated in the synthesis of starch in the chloroplasts. Phosphorus is found in three major forms; phosphate monoesters, phospholipids and inorganic phosphates (Blennow et al., 1998). The root (tuber) and legume sources of starch contain esterified phosphorus (phosphate mono-esters) where the phosphate linked to the C-6 and C-3 hydroxyl groups of the glucose units (Eliasson, 2004). The phosphorus content in sweet potato starch is nearly similar to tapioca starch (Tian et al., 1991; Madamaba et al., 1975), but much less than that of potato starch.

Starches also contain trace amounts of mineral elements and inorganic salts. Basically, these minerals and salts are referred to as ash. Native starches have been

reported to have different ash content. The variance in ash content is primarily dependent upon the source or origin of the starch. Eliasson (2004) reported that the ash content for most commercial starches is reported to be less than 0.5% based on a dry starch basis.

Water or moisture content of starch varies significantly in its native state. According to Eliasson (2004), starch as prepared commercially contains on the average approximately 12% of moisture. Generally, the moisture content of dry starch ranged between 6-16%, depending on the drying process of the starch (Moorthy, 2002).

2.3 Granular Structure

2.3.1 Morphology of Starch

Scanning electron microscopy (SEM) has been used widely to study the granule morphology. According to Jane et al. (1994), SEM has two major advantages over light microscopy: firstly, it has hundreds of times greater depth of focus than the light microscope, and, secondly, it has a much higher order of resolution and magnification. Starch granules naturally exist in different ranges of shapes, size distribution and dimensions which are affected by the botanical source, growing and harvest conditions. The granule size varies from the tiny granule in rice and oat starches (1.5 - 9 μm) to the large ones in potato starch (up to 100 μm) (Chen, 2003). The size, shape and structure of starch granules vary substantially among botanical sources. SEM of undamaged and native starch granules shows that the granule surface is relatively smooth and free from pores, cracks or fissures. SEM study by Mishra and Rai (2006) revealed that the starches differed in granule shapes and sizes

ranging from large (potato) to small (corn and tapioca) and oval (potato) to polyhedral (corn) or spherical with some truncated (tapioca) granules. Small grooves were also observed by Mishra and Rai (2006) in some granules of tapioca. Jane et al. (1994) discovered that tuber and root starches have smooth, oval and round morphologies of relatively large size with axes of 10-100μm. There are a number of types of sizes and shapes of starch granules that can be correlated with the various types of biological sources from which the starch are isolated. Some of starches have unusual morphologies, occurring as relatively flat plates or lenticularly shaped disks (Jane et al., 1994). The variation in size and shape of starch granules may be due to biological origin (Svegmark and Hermansson, 1993). Singh and Singh (2001) reported that the morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast and physiology of the plant. The characteristics of some starch granules are listed in Table 2.3.

Table 2.3: Characteristics of some starch granules.

Starch	Granule shape	Diameter range (μm)
Corn[a]	Round, polygonal	2-30
Wheat[a]	Round, polygonal	1-45
Potato[a]	Round, lenticular	5-100
Tapioca[b]	Round, truncated, cylindrical, oval, spherical, compound	4-43
Sweet potato[b]	Round, polygonal, oval, bell, polygonal	2-72
Arrowroot[b]	Round, polygonal	5-50
Mung bean[c]	Oval, round	7-26

a: from Chen (2003); b: from Moorthy (2002); c: from Hoover et al. (1997).

When a starch granule is damaged or eroded, either by acidic or enzymatic treatment, the starch granules are greatly weakened so that many granules cracked open and expose a pronounce layer structure. Figure 2.4 shows the SEM micrograph of hydrolyzed sweet potato starch granules.

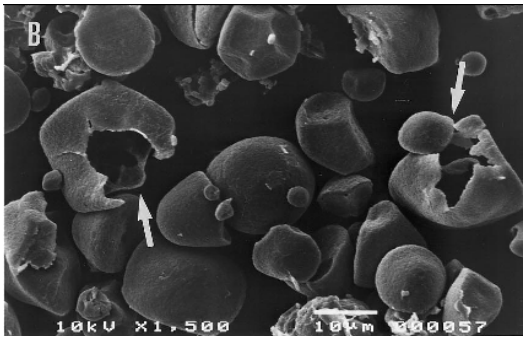


Figure 2.4: SEM micrograph of hydrolyzed sweet potato starch (Zhang and Oates, 1999).

SEM reveals that different starches are attacked in patterns that are related to the surface and internal texture of starch granules. For instance, corn starch attacked by amylases exhibits many surface pits which lead to the interior part of the granule. The granule interior may be highly eroded or solubilised, yet the exterior surface or parts of it are resistant (Figure 2.5).

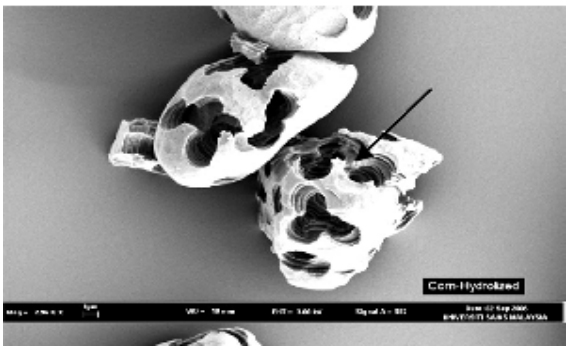


Figure 2.5: SEM micrograph of corn starch hydrolyzed by amylases (Uthumporn et al., 2010).

2.3.2 Ordered Structure of Starch Granules

2.3.2 (a) Amorphous Structure of Starch Granules

Crystalline regions have been known to be formed in ordered manner while the remaining regions of unordered starch can be referred as amorphous regions (Eliasson, 2004). Amorphous can also be considered as the non-crystalline material in starch granule. It has been reported that some amylose molecules are located in the amorphous region with some interaction with the branch chain of amylopectin. According to Zobel (1992), amorphous starch can be defined as material not exhibiting an x-ray diffraction pattern. The amorphous regions of starch are susceptible to chemical reaction and the main swellable structural elements of the native granule. During the early stage of acid hydrolysis, a rapid initial attack occurs on the amorphous regions of starch that contain branching points with α-1,6-linkages. Then, a slower hydrolysis takes place on the more crystalline areas during the second stage.

2.3.2 (b) X-Ray Diffraction Pattern

The most useful information about the ordered arrangement of alpha-glucan molecules is provided by X-ray diffraction and related techniques. Wide angle X-ray scattering and small angle X-ray scattering are used in parallel to reveal the complex ultrastructure of the granule and quantification of crystallinity and polymorphic forms or crystalline laminates, respectively (Tester, 1997). The X-ray diffraction patterns of the starch granules prove that there are areas of crystallinity in the starch granules. In addition to providing information about the crystal structure of the crystallites of starch granules, x-ray diffraction can give information about the relative amounts of crystalline and amorphous phases.

Native starches are known to exhibit three different types of X-ray diffraction patterns which is A-, B- and C-types, which correspond to different crystalline structures. The difference in crystalline structure of starch granules relates to the branch chain lengths of amylopectin. Starch that consists shorter branches (such as rice, maize, wheat, taro and tapioca, with average chain length of 19-28 anhydro-glucose units, also known as degree of polymerization [DP]) displays the A-type X-ray pattern. Most A-type starches have branch chain length between DP 21 and 25 except for waxy rice (DP19) and tapioca (DP28). Starch that consists of longer branch-chain amylopectin (such as potato, high-amylose maize, and canna, with average DP 29-31) exhibits B-type X-ray pattern. Starch consisting of amylopectin with both very long and very short branch-chains (such as green banana and water chestnut, with average DP 25-27) exhibits the C-type pattern (Jane et al., 1999; McPherson and Jane, 1999; Hizukuri, 1985). C-type pattern actually is a mixture of A- and B-type pattern. Some tapioca starch samples have been reported to exhibit the C-type pattern. For A-type starches, α-1,6-linkages are present in both amorphous and crystalline regions (Robertson et al., 2006). For B-type starches, the α-1,6-branches are concentrated in the amorphous lamellae at the root of the chain clusters and the parallel glucans make up the crystalline lamellae. A fourth pattern, the V pattern, arises from complexes formed by amylose with a variety of polar organic molecules (Zobel, 1988). The crystalline V-form characteristic of amylose complex with fatty acids and monoglycerides, which appears upon gelatinization of starch, is rarely detected in native starches (Buleon et al., 1998).

2.3.2 (c) Characteristics of Starch Crystallites

According to Parker and Ring (2001), the starch granule is partially crystalline with crystallinity in the region of 30% being reported. It is the crystalline regions that give the granule its structure and facilitate identification of a raw (uncooked) starch (Eliasson, 2004). A number of crystalline forms are known the A form which is found in most cereal starches, including wheat, consists of starch double helices packed into a monoclinic array. The B form, which is found in some tuber starches and high amylose cereal starches, consists of double helices packed in a hexagonal array (Figure 2.6). The structure of the starch granule is shown in Figure 2.7.

Crystallinity occurs within the ordered arrays of amylopectin and is created by the intertwining of chains with a linear length of more than 10 glucose units to form double helices. The crystalline regions are predominantly located in the hard layers of the granule and are composed of stacks of crystalline lamellae which form the backbone of the starch granule. Crystallinity is derived from the structure of amylopectin, with its radial arrangement in the granule being at right angles to the granule surface (Davis, 1994). Crystallization or double helix formation can occur either between adjacent branches in the same amylopectin branch cluster or between adjacent clusters in three dimensions (Oates, 1997). The crystallinity of starch granules can be determined using the separation and integration of the areas under the crystalline peak and amorphous region in x-ray diffraction pattern (Zobel, 1988).

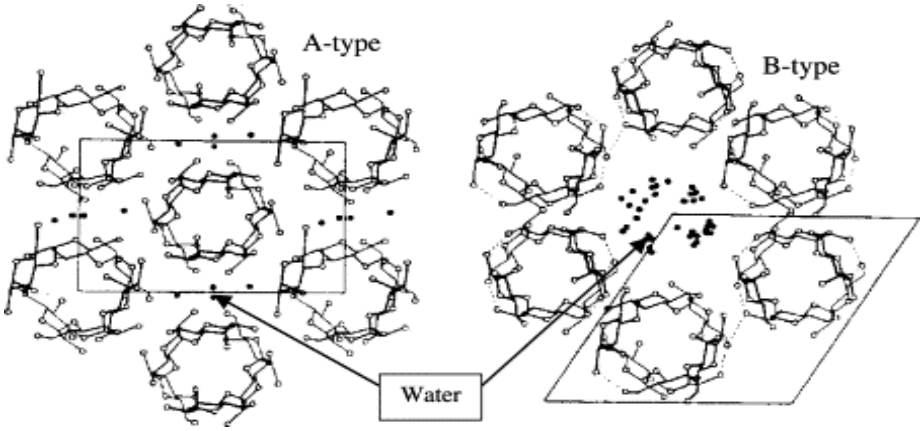


Figure 2.6: Diagrammatic structure of A- and B- type polymorphs of amylose (Wu and Sarko, 1978).

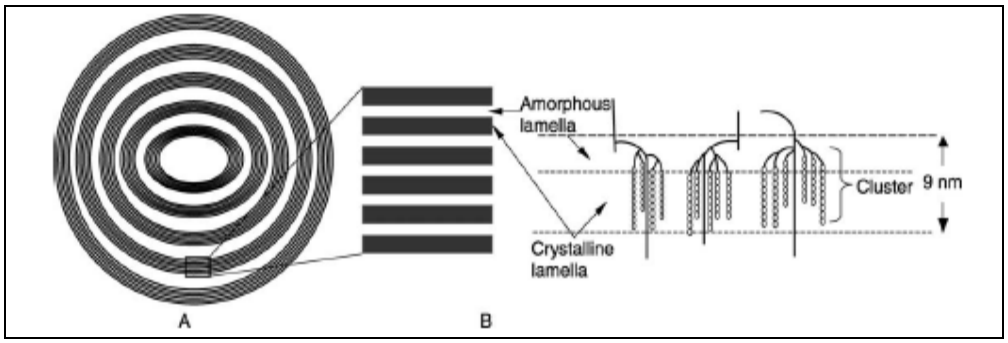


Figure 2.7: Structure of the starch granule (Tester et al., 2004).

2.3.2 (d) Relationship between the Crystalline Structures and Enzyme Digestibility

The branch structure of amylopectin is very closely related to the enzymatic hydrolysis of starch granules (Fuwa, 1980). Starches of different polymorphisms are known to display different starch digestibility (Jane, 2006). A-type starches are highly susceptible to hydrolytic amylases while B-type starches are very resistant to

enzymatic hydrolysis. C-type starches are consists of A-type and B-type structures. In A-type starches, there are short-branch chains with scattered arrangements in the crystalline region. The scattered branch-linkages carrying short double helices located in the crystalline region are likely to create weak points that are more susceptible to enzyme attack. The longer branch chains and clustered branch linkages in B-type starches contribute into highly ordered crystalline structures with greater length. The forms of this structure are major factor in the high resistance properties of B-type starches to enzymatic hydrolysis. The A-type starch granules also show pinholes on the surface and serpentine-like channels inside of the granule, but the B-type starch granules do not show these features. The A-type polymorphic starch granules have loosely packed internal structures, which are easily hydrolyzed by amylases, whereas the B-type polymorphic starch granules have solid internal structures and are resistant to enzyme hydrolysis. A-type starches were always more susceptible to degradation than B-type, with the suggestion that the resistance to amylolysis was related to the distribution of B-type crystallites rather than just their number (Gerard et al., 2001). Consequently, the A-type starches are more digestible to enzymatic hydrolysis as compared to B-type starches (Jane, 2001).

2.3.3 Particle Size and Distribution

Particle size distribution of starch is an important characteristic that can influence the starch chemical composition, which in turn may affect its functionality. Some of researchers reported that starch composition (Soulaka and Morrison, 1985), gelatinization and pasting properties (Kulp, 1973), enzyme susceptibility (Kulp, 1973), crystallinity and swelling properties were all affected by granule size. According to Ellis et al. (1988), two methods of determining granule size distribution are based on the response of an instrument to particle volume.

In terms of starch digestibility, Franco et al. (1988) noticed that there is a relationship between the enzymatic susceptibility and the size of the starch granules. The larger tapioca or corn granules are attacked easier by α-amylase and amyloglucosidase (Franco et al., 1988). Valetudie et al. (1993) claimed that the small granules are more rapidly hydrolyzed than bigger granules in relation to more important surface area. When the granules are small and round shaped, hydrolysis is more difficult than when they are polyhedric. Clearly if granules are large they have a smaller surface area to volume ratio and one would predict that amylase binding and potential hydrolysis would be less for smaller granules (Tester et al., 2004). The presences of truncatures are so weakness points of the granule structure leading to better susceptibility (Valetudie et al., 1993). It has been postulated that a smaller size of the starch granules resulted in a higher percentage of hydrolysis for corn and tapioca starches (Franco et al., 1992). Kasemwong et al. (2008) reported that the difference in the reactivity of small and large granules could be presumably attributed to the organization of glucan chains in ordered and/or less ordered structure of these two fractions.

2.4 Starch Functionality

2.4.1 Starch Swelling Power and Solubility

The swelling power and solubility of starch provide evidence of non-covalent bonding between starch molecules. It can be influenced by a number of factors like amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and conformation (Eliasson, 2004). The swelling volumes also depend on the presence of various chemicals and treatments carried out on

starch (Moorthy, 2002). Moorthy (2002) who studied the physicochemical and functional properties of tropical tuber starches claimed that there was no significant correlation between amylose content and swelling volumes. Besides that, the formation of lipid-starch complex can also affect the swelling volumes (Swinkles, 1985).

The granule swelling ability is usually quantified by swelling power (the weight of sedimented swollen granules per gram of dry starch) or swelling volume (the volume of sedimented swollen granules per gram of dry starch) at the corresponding temperature (Konik et al., 1993; Pinnavaia and Pizzirani, 1998; Konik et al., 2001). Swelling power is a measure of hydration capacity, since the test is a weight measure of swollen granules and their occluded water (Whistler et al., 1984). Singh et al. (2003) have studied the swelling power and solubility of starches from different botanical sources and found that the swelling power and solubility of the starches differ significantly. Starch swelling behaviour not only depends on starch origin but also depends on amylose content (Chen, 2003). When starch molecules are heated in excess water, the crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin, which causes an increase in granule swelling and solubility. The magnitude of granule swelling is an important factor in starch reactions (Kweon et al., 1996). Swelling power and amylose leaching of native and heat-moisture treated starches increased with increase in temperature (Gunaratne and Hoover, 2002).

Solubility of starch depends on a number of factors such as source of the starch, inter-associative forces, swelling power, presence of other components, etc. (Moorthy, 2002). Moorthy (2001) observed no direct correlation between swelling and solubility. According to Eliasson and Gudmundsson (1996), the low solubility of

starches could be attributed to the semi-crystalline structure of the starch granule and the hydrogen bonds formed between hydroxyl groups in the starch molecules. Mishra and Rai (2006) discovered an increase in solubility at high temperature (70ºC) for potato and tapioca starch. This observation was supported by the fact that when the temperature of an aqueous starch suspension is raised above the gelatinization range, hydrogen bonds continue to disrupt followed by the attachment of water molecules to liberated hydroxyl groups and resulting in the continuous swelling of the granule (Mishra and Rai, 2006).

2.4.2 Starch Pasting and Gelatinization

Pasting is defined as the state following gelatinization of starch. Generally, a starch paste can be described as a two-phase system composed of a dispersed phase of swollen granules and a continues phase of leached amylose. When starch is cooked, the flow behaviour of a granule slurry changes markedly as the suspension becomes a dispersion of swollen granules, partially disintegrated granules, then molecularly dispersed granules. The cooked product is called a starch paste. The pasting properties of a particular sample of starch are governed by the concentration, rate of heating and the presence of other food components (Eliasson, 2004).

Gelatinization behaviour and the pasting profiles of flour-water and starch-water mixtures are commonly monitored using a Rapid Visco Analyzer (RVA), which is a heating and cooling viscometer that measures the resistance of a sample to controlled shear. The RVA provides information on starch characteristics similar to the Brabender Visco Amylograph. However, the RVA has the advantages of using a small sample size, short testing time and the ability to modify testing conditions.