ACKNOWLEDGEMENT

I am heartily thankful to my supervisor, Prof. Abd. Karim Alias, whose encouragement, guidance and support from the initial to the final level, enabled me to develop an understanding of the subject.

I would like to acknowledge and extend my heartfelt gratitude to my parents for their vital encouragement and support. Thank you to all of my friends Wahidah, Shariffa, Sapina, Mohamad, Sufha, Nizam, Ruri, Zura, Syiena for the help and inspiration they extended.

To all of staff, lecturers and lab assistant School of Industrial Technology, thank you for assisting in the collection of the topics for the chapters. I would like to express my special thanks to my current employer, for their understanding and assistance.

And to God, who made all things possible.

Nor Nadiha bt. Mohd. Zaki

July 2011

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**
DMSO	Dimethyl sulphoxide
DP	Degree of polymerization
DSC	Differential scanning calorimetry
RVA	Rapid Visco Analyzer
SEM	Scanning Electron Microscopy

LIST OF APPENDICES

Perbandingan Kerintangan Kanji Sagu, Jagung dan Ubi Kentang Terhadap Asid dan Alkali

Abstrak

Kesan perendaman kanji di dalam larutan 0.025 M natrium hidroksida dan asid hidroklorik selama 0, 15, dan 30 hari pada 30°C terhadap struktur granul dan sifat fiziko-kimia kanji sagu, jagung dan ubi telah dikaji. Perubahan morfologi pada granul kanji didapati bergantung kepada jenis dan sumber botani setiap kanji yang telah dikaji. Kajian sifat pes kanji menunjukkan 'kelikatan puncak', 'kelikatan terturun', 'kelikatan terencat' bagi kanji sagu dan ubi kentang telah menurun secara signifikan apabila direndam di dalam larutan alkali dalam masa yang lama. Namun, kanji jagung menunjukkan kesan sebaliknya iaitu ianya telah meningkat secara signifikan. Walau bagaimanapun, penurunan yang signifikan telah dipantau berlaku untuk 'kelikatan puncak' dan 'kelikatan terturun' bagi kesemua kanji yang direndam di dalam larutan berasid. Manakala, kelikatan terencat telah meningkat secara signifikan bagi kanji sagu yang direndam di dalam asid selama 30 hari. Kuasa pembengkakan bagi kanji ubi kentang dan jagung yang direndam di dalam larutan alkali telah meningkat secara signifikan, tetapi menurun secara signifikan untuk kanji sagu. Walau bagaimanapun, kuasa pembengkakan kanji sagu yang direndam di dalam larutan berasid telah meningkat secara signifikan, tetapi menurun bagi kanji jagung dan ubi apabila melalui proses yang sama. Kandungan amilosa di dalam semua kanji rendaman asid telah menunjukkan penurunan signifikan, tetapi tidak menunjukkan perubahan yang signifikan bagi kanji rendaman alkali jagung dan sago, malahan kandungan amilosanya meningkat untuk kanji ubi rendaman alkali. Suhu permulaan dan puncak untuk proses gelatinisasi (yang telah dianalisa dengan 'differential scanning calorimetry') telah meningkat secara signifikan, tetapi entalpinya telah menurun, bagi semua kanji yang direndam asid atau alkali. Kanji jagung rendaman asid menunjukkan sifat keterlarutan yang paling tinggi berbanding kanji yang lain. Keputusan

kajian menunjukkan peningkatan yang signifikan bagi kanji jagung dan ubi rendaman asid, manakala sedikit penurunan telah dilihat berlaku pada kanji sagu rendaman asid berbanding kanji sagu rendaman alkali. Analisa retrogradasi, suhu puncak menunjukkan penurunan yang signifikan bagi kesemua kanji yang direndam. Entalpi untuk kanji sagu dan ubi rendaman asid dan alkali menunjukkan penurunan yang signifikan, manakala kanji jagung rendaman asid dan alkali tidak menunjukkan perubahan yang ketara.. Justeru, hasil daripada kajian ini menunjukkan sifat fiziko-kima kanji yang terdiri daripada sumber botani yang berlainan akan mengalami kesan yang berbeza apabila direndam di dalam larutan alkali ataupun asid.

Comparative Susceptibility of Sago, Corn and Potato Starches towards Acid and Alkaline Treatments

Abstract

The effects of steeping starch (sago, corn, and potato) in 0.025 M of sodium hydroxide and hydrochloric acid for 0, 15, and 30 days at 30°C on its granular structure and other physicochemical properties were investigated. Changes in the morphology of starch granules indicated that the alkaline and acid solution affected the granular structure of the starch to different extent, depending on starch type and their botanical sources. Pasting studies showed that the peak viscosity, breakdown, and setback of alkali-treated sago and potato starch decreased significantly, whereas that of corn starch increased significantly, when steeping time was prolonged. Meanwhile, acid-treated sago, corn, and potato starch showed significant decreased in peak viscosity, and breakdown. Setback viscosity increased significantly for 30 days in acid-treated sago starch but decreased significantly for acid treated potato starch. Swelling power increased significantly for alkali-treated potato and corn starches, but decreased for sago starch. However, swelling power of sago starch showed an increase when treated with acid solution, whereas acid-treated corn and potato starch showed significant decrease. The amylose content of all acid-treated starches decreased significantly after treatment, but alkali treated sago and corn starch did not shows any significant difference, in fact shows the higher amylose content for alkali-treated potato starch. Onset and peak temperatures of gelatinization (from a differential scanning calorimeter measurement) increased significantly. Acid-treated corn starch shows the highest solubility compared to other treated starch. Results shows the significant increased in acid treated corn and potato starch, while there was a slight decreased of acid-treated sago starch compared to alkali-treated sago starch. For retrogradation, the peak temperature showed a significant decrease for all treated starches. The enthalpy of acid and alkali treated sago and potato shows a

significant decrease, meanwhile alkali and acid treated corn starch shows no significant changes. There are no significant changes of chain type when compared to their native starch. The results showed that the physicochemical properties of starch from various botanical origins were affected to variable degrees when treated with alkaline and acid solution.

CHAPTER 1

INTRODUCTION

1.1 Background

Various types of chemical modifications have been applied to different starches to impart certain desirable properties when added to food. The chemical modifications include acid hydrolysis, alkali hydrolysis, oxidation, etherification, esterification, and cross-linking (Karim et al., 2000). Alkaline solution such as sodium hydroxide (NaOH), have been widely used in the production of many traditional food products such as tortillas (Campus-Baypoll et al., 1999), waxy rice dumplings, and yellow alkaline noodles (Lai et al., 2004). Acid is commonly used to produce 'soluble starch' for many decades by treating starch with acid for a certain period of time. The product of soluble starch is heterogeneous and has relatively high reducing value.

Alkalizing agents have been used to enhance desirable product quality characteristics such as color (Maher 1983; Miskelly & Moss, 1985; Moss et al., 1986; Bhattacharya & Corke, 1996), aroma, and flavor and develops a firm and elastic texture to the product (Lai et al., 2004). Alkali solution is also commonly used in the starch extraction (Chiou et al., 2002; Lim et al., 1999). Prolonged, gentle acid hydrolysis is commonly performed with either hydrochloric or sulfuric acid to yield Lintner or Naegeli amylodextrins, respectively. Lintner or Naegeli amylodextrins are the products of native starch granules under such conditions that the starch granules maintain much of their gross form and crystallinity (Watanabe, 1980). Acid-treated starch, which also

known as thin-boiling starch, is used extensively in food, textile and paper industries (Wurzburg, 1986).

Starch will undergo some changes in granular structure and physicochemical properties during alkaline and acid steeping (Gomez et al., 1990; 1992; Lin et al., 2005). The changes of properties on starch during alkaline treatment varied depending on the type of alkalizing agent, concentration used, duration of steeping and type of starch. Similarly, the changes in physicochemical properties of starch upon acid hydrolysis, especially in terms of viscosity and fluidity, also varied with the acid type and the condition used during modification (Lin et al., 2005).

1.2 Rationale

Relatively, very few studies have been conducted on alkali hydrolysis compared to the other starch modifications, such as enzyme and acid treatment. Reported studies on alkali hydrolysis mainly focused on corn starch (Mistry & Eckhoff, 1992; Bryant & Hamaker, 1997; Mondragón et al., 2004) because it is the most commonly used starch in the food industry. Several studies on alkali hydrolysis reported the changes that occurred due to this treatment such as the leaching out of protein from the granule surface during gelatinization of rice starch extracted using the alkaline method (Han & Hamaker, 2002), production of annealed starch molecules during nixtamalization due to the increase of gelatinization temperature and amylose content (Méndez-Montealvo et al., 2007), relatively higher peak viscosity in the pasting profile when treated with alkali (Lai et al., 2004) and the influences of the swelling and gelatinization of starch due to the ratio of starch/sodium hydroxide (NaOH) (Roberts & Camerons, 2002).

Differences in the rate and extent of acid hydrolysis between cereal, tuber, and legume starches exist and have been attributed to differences in granules size (Jane et al., 1994). The annealing process also allowed rapid hydrolysis towards A- and C-type starch compared to B-type starch (Nakazawa & Wang, 2003). Bertoft (2004) also suggested in his study on the effect of acid hydrolysis towards different crytalline type of starch that the amylose could retards the rate of lintnerization and decreases the amount of acid resistant to double helices.

To date, the comparison of alkali and acid steeping on various starches characteristics have not been studied extensively. Therefore, this study was designed to determine the comparative susceptibility of starches from different botanical origins to acid and alkali treatments. This would provide some insights on the effect of alkali and acid on physicochemical and functional properties of starch in relation to its granular structure of starch and its botanical origin. The results can be usefully applied to the development of novel products with improved textural attributes and extended shelf life.

1.3 Objectives

i) To study the effect of prolonged steeping of starch in alkali and acid on physicochemical and functional properties of starch.

ii) To compare the effect of botanical origin of starch on the extent of acid and alkali modification upon prolonged steeping in acid and alkali.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch

Starch is naturally found in all organs of higher plants that include leaves, stems, woody tissues, roots, tuber, bulbs, rhizomes, fruits, and flowers (Jane et al., 1994). Starch could be divided by different botanical sources such as from 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). Starch plays an important role as a predominant carbohydrate source in our daily meals such as rice, wheat, maize and potatoes.

There are a group of parameters that characterize a particular starch of same botanical origin, such as: granule size (1–100 mm in diameter), shape (round, lenticular, polygonal), size distribution (uni- or bi-modal), association as individual (simple) or granule clusters (compound) and composition (a-glucan, lipid, moisture, protein and mineral content) (Tester et al., 2004) (as shown in Table 2.1). The variation of starch granules and their molecular constituents can influence starch functional properties, (Copeland et al., 2009), and their physico-chemical properties (Svegmark & Hermansson, 1993). Starch contributes greatly to the textural properties of many foods and has many industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, water retention agent and adhesive.

Table 2.1: Characteristics of starch granules from different botanical sources

Starch	Type	Shape	Distribution	Size (μm)
Barley	Cereal	Lenticular (A-type) Spherical (B-type)	Bimodal	15-25, 2-5
Maize (waxy & normal)	Cereal	Spherical/polyhedral	Unimodal	2-30
Amylomaize	Cereal	Irregular	Unimodal	2-30
Millet	Cereal	Polyhedral	Unimodal	4-12
Oat	Cereal	Polyhedral	Unimodal	3-10 * 80 **
Pea	Legume	Rentiform (single)	Unimodal	5-10
Potato	Tuber	Lenticular	Unimodal	5-100
Rice	Cereal	Polyhedral	Unimodal	3-8 * 150 **
Rye	Cereal	Lenticular (A-type) Spherical (B-type)	Bimodal	10-40 5-10
Sorghum	Cereal	Spherical	Unimodal	5-20
Tapioca	Root	Spherical/ Lenticular	Unimodal	5-45
Triticale	Cereal	Spherical	Unimodal	1-30
Sago	Palm	Oval	Unimodal	20-40
Wheat	Cereal	Lenticular (A-type) Spherical (B-type)	Bimodal	2-10

* Single, ** compound. (Source: Tester & Karkalas, 2002)

2.2 Component of Starch

Starch is basically formed from homopolymers of six carbon sugars, glucose, that is known as building block in starch. D-glucopyranose units in starch has two

linkages known as α-1,4 and α-1,6 glycosidic bond (Figure 2.1). This linkages exist when carbon 1 react with carbon 4 that produce α-1,4 and carbon 6 that resulted α-1,6 linkages.

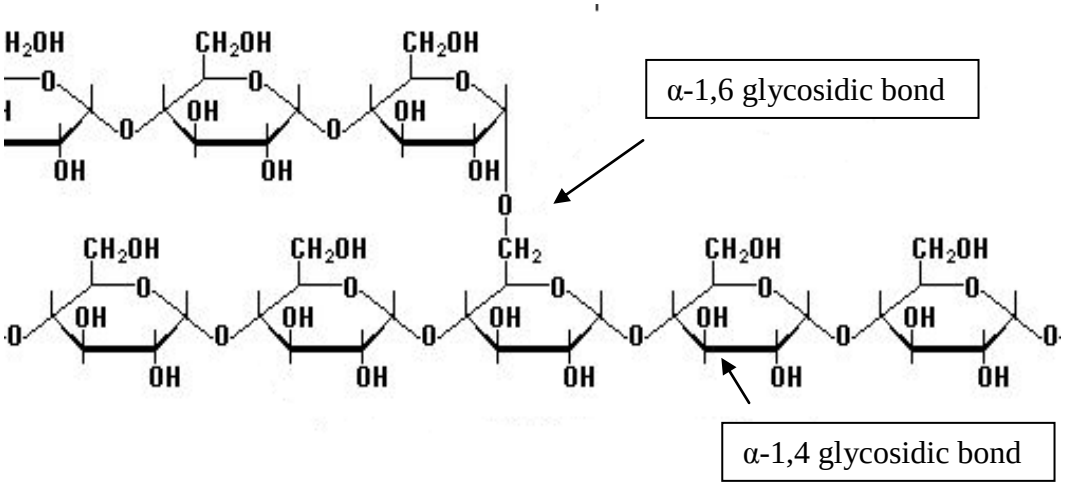


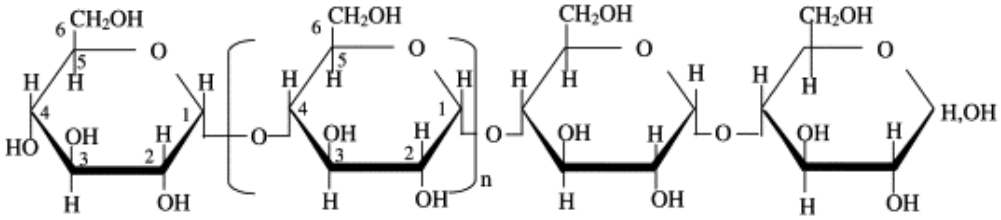
Figure 2.1: α-1,4 and α-1,6 glycosidic bonds of starch (Thomas & Atwell,1999)

Glucose polymerization of starch results in two types of polymers, amylose and amylopectin. Amylose and amylopectin represent approximately 98-99% (dry weight) of the starch granules (Tester et al., 2004), which amylose contributed about 15-30% of approximate weight (Jane et al., 1994). The ratio of the two polysaccharides varies among the starches according to their botanical origin (Tester et al., 2004). Amylopectin, the major component of most starches, consists of a large number of shorter chains that are bounded together at their reducing end side by a (1→6)-linkage, which makes this very large polysaccharide extensively branched (Manners, 1989). Amylose consists only of either a single or a few long chains, thus making the molecule linear or slightly branched (Takeda et al., 1992).

The variations between amylose and amylopectin contribute to the variation in behavior and properties. According to Zobel et al. (1988), the difference in properties between amylose and amylopectin can be observed in simple experiment such as gel forming. At 2% of concentration, amylose can forms a rigid and free standing gel, whereas amylopectin remain in solution form. Furthermore, amylose is known for its capacity to form coherent, free standing film that are reasonably strong and flexible when plasticized, but amylopectin film are very brittle and weak.

2.2.1 Amylose

Amylose is traditionally considered as a linear polymer that is linked through α-1,4 glycosidic linkages. Amylose consists mainly of α-1,4 linked D-glucopyranosyl residues (Figure 2.2). Study done by Curá et al. (1995) revealed that amylose is not linear but slightly branched by α-1,6 glycoside linkages. The side chain length can range from 4 to over 100 (Hizukuri et al., 1981) and the extent of chain length increases when the molecular size of amylose increases (Greenwood & Thompson, 1959). The molecular weight of amylose has been reported to vary between 10^5 and 10^6 Dalton (Morrison & Karkalas, 1990) and DP ranging from 500 to 5000 (Galliard & Bowler, 1987). DP is the total number of anhydroglucose residues present divided by the number of reducing ends. According to McGrance et al. (1998), the formation of a helical complex between amylose and iodine is responsible for the intensity of the typical deep blue color of starch dispersions stained with iodine and such principle forms the basis of quantitative determination of amylose content. Amylose is present in the amorphous structure.



Amylose: α-1,4-glucan; ave n=ca 1000. The linear molecule may carry a few occasional moderately long chain linked α-1,6.

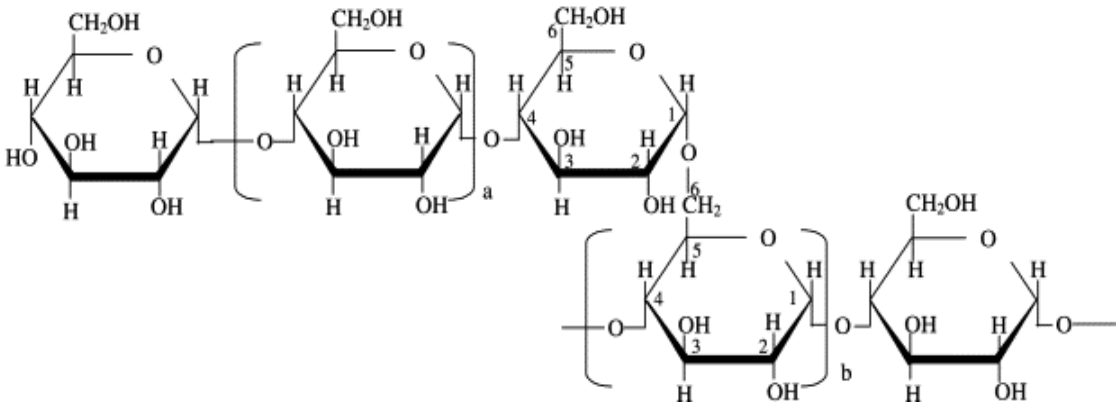
Figure 2.2: Amylose molecule (Tester & Karkalas, 2002).

Amylose molecules are thought to occur in the granule as individual molecules, randomly interspersed among the amylopectin molecules and in close proximity with one another. The location of amylose can be in the amorphous and/or crystalline region depending on the botanical source of the starch (Oates, 1997). The amylose fraction can be isolated by few methods such as aqueous leaching procedures (Hizukuri, 1996), dispersion and precipitation (Adkins & Greenwood, 1969; & Banks et al., 1971) and ultracentrifugation methods (Montgomery et al., 1961; Majzoobi et al., 2003).

2.2.2 Amylopectin

Figure 2.3 and 2.4 shows structure and chain distribution of amylopectin. In contrast to amylose, amylopectin is a much larger molecule compared to amylose. The molecular weight of amylopectin is about 10^7-10^9 Dalton (Buléon et al., 1998) and the degree of polymerization is typically within the range of 9600-15900 (Takeda et al., 2003). Amylopectin is highly branched polysaccharide that consists of approximately 95% α-1,4 and 5% of α-1,6 linkages. Figure 2.4 shows the cluster model of amylopectin

adopted from Tester et al. (2004). Chen (2003) suggested that amylopectin structure consists of three types of chains (Figure 2.4) 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.



Amylopectin: α-1,6 branching point. For exterior chains a=ca. 12-23. For interior chains b=ca 20-30. Both a & b vary according to the botanical origin.

Figure 2.3: Amylopectin molecule (Tester & Karkalas, 2002)

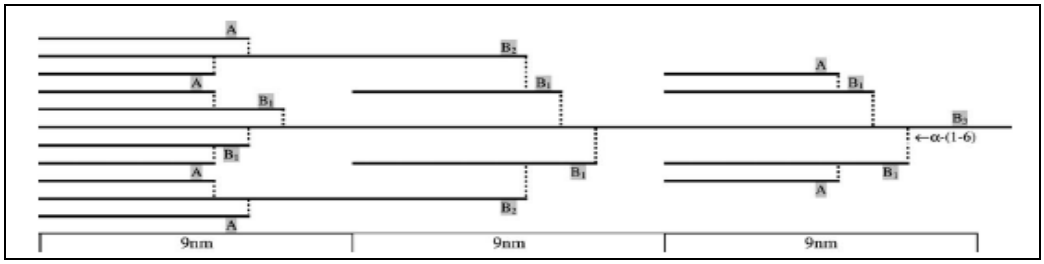


Figure 2.4: Chain distribution of amylopectin (Tester et al., 2004).

Some of the physicochemical properties of amylose and amylopectin are summarized in Table 2.2.

Table 2.2: Summary of the physicochemical properties of amylose and amylopectin.

Properties	Amylose	Amylopectin
Molecular structure	Essentially linear	Highly branched
Molecular weight	150,000-400,000	10-15 million
Gels	Firm, irreversible	Soft, reversible
Films	Coherent	Not readily form
Iodine colors	Blue	Reddish-purple
Digestibility by amylase	100%	60%

(Source: Eliasson, 2004)

Botanical origin and starch type are the most important factors that determine the ratio of amylose and amylopectin in starch granules. High amylomaize has a high amylose content that is as high as 80%, while waxy starch may contain about 97% amylopectin and normal starch might have 20-50% amylose, depending on starch type. The ratio of amylose and amylopectin within a particular type of starch is an important criteria to consider with respect to starch functionality in food applications.

2.2.3 Minor Component of Starch

Despite the presence of the major components, the existence of minor components such as lipids, proteins and minerals play a significant importance in the properties of starches. 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). The contents of minor constituents of starch differ with different botanical sources of starch. Table 2.3 below shows the differences in chemical characteristics from different botanical sources of starch.

Table 2.3: Chemical characteristics of starch granules from different botanical sources.

Starch	Amylose (%)	Lipids (%)	Protein (%)	Phosphorus (%)
Corn[a]	28	0.8	0.35	0.00
Waxy corn[a]	<2	0.2	0.25	0.00
High amylose corn[a]	50-70	0.2	0.5	0.00
Wheat[a]	28	0.9	0.4	0.00
Potato[a]	21	0.1	0.1	0.08
Mung bean[b]	40-50	0.3	0.3	0.00

(Source: a: BeMiller & Whistler, 1996; b: Hoover et al., 1997)

2.3 Granular Structure of Starch

2.3.1 Starch Morphology

Native starch granules show a dark birefringence cross (‘Maltese cross’) centred at the hilum when viewed under polarised light. This characteristic shows the crystalline substances in the starch granular structure as shown in Figure 2.5. The intensity of birefringence strongly depends on the shape and on the orientation of the granules with regard to the light beam Buleon et al. (1998).

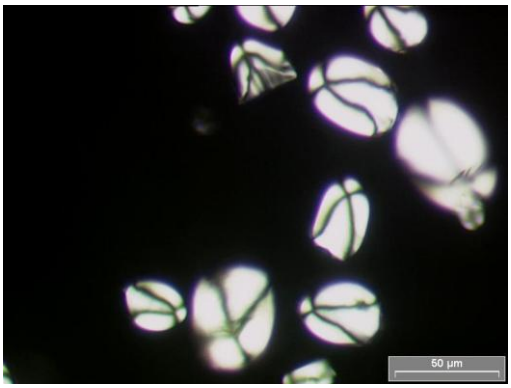


Figure 2.5: Polarized light micrograph for sago starch

The degree of crystallinity, the orientation of the crystallites and the thickness of the granules are the main factors that influence the apparent intensity of birefringence shown in polarized light. In starch granules, the branches of amylopectin form double helices, which are arranged in crystalline domains. Contrarily, amylose largely makes up the amorphous regions, which are randomly distributed between the amylopectin clusters (Blanshard, 1987).

Gallant & Sterling (1976) approached the treatment of acid hydrolysis to observe the distribution of crystalline and amorphous areas of starch. Under this condition, granules weaken crack and show lamellar organization. Layered concentric shell structure of starch granules has been observed by scanning electron microscopy (SEM) and transmission electron microscope (TEM) after acid hydrolysis (Figure 2.6). It is suggested that the crystalline part of the granules is represented by resistant lamellar remained in starch granules. Starch granules are proposed to consist of 120-400 nm thick amorphous and semi-crystalline layers (French, 1984). Gallant et al. (1997) observed

that lamellar are organized in spherical blocklets with a diameter ranges from 20 to 500 nm, varies on their location within the granules and botanical origin.

The shape and size of starch granules can be identified by Scanning Electron Microscopy. It is 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. The characteristics of some starch granules are listed in Table 2.4.

Table 2.4: 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

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

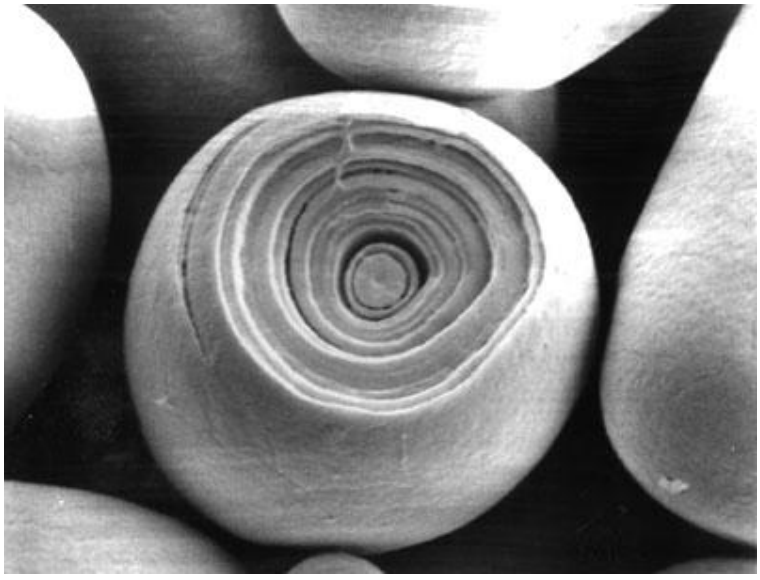


Figure 2.6: The presence of amorphous growth ring in starch granules.

The spherical blocklets of starch granules as observed by TEM (Figure 2.6) are also known as ‘growth rings’ (Gallant et al., 1997; Smith, 1999; Baker et al., 2001; Donald et al., 2001; Ridout et al., 2002; Li et al., 2003; & Pilling & Smith, 2003). Figure 2.7 represents the proposed granular structure in starch granules. Layered granular structure between amorphous and crystalline region in growth ring are sugested as a general granular structure in starch granules.

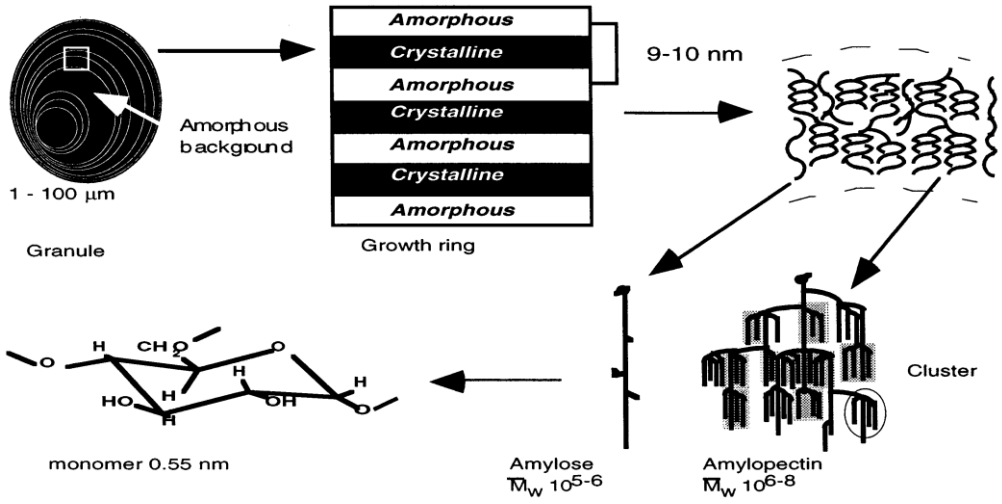


Figure 2.7: Diagrammatic representation of the lamellar structure of a starch granule according to Donald et al. (1997).

According to Cameron & Donald (1992), starch granules contain relatively broad radial growth rings comprising semi-crystalline shells (about 140 nm thick) separated by broad amorphous zones of at least the same thickness. In this model, the semi-crystalline growth rings themselves (104 nm) contain 16 radiating clusters of amylopectin exterior chains (A_1 and B_2) with the actual length of the registered double helices about 6.65 nm (equivalent to the crystalline lamellae) interspersed within amorphous lamellae of about 2.2 nm (amylopectin α-1,6 branch points).

The structure of amylopectin molecules, those branches from the hilum (centre of growth) towards the periphery of the granule, is also known as ‘hair liked’ structure (Tester et al., 2004). Meanwhile, amylose does not appear to have any significant effect on crystallinity in normal and waxy starches (which may be virtually are amylose free)

both of which display strong birefringence). However, the amylose may contribute significantly to the crystallinity in high amylose starches (Tester et al., 2000).

The location of amylose and amylopectin in starch granule is uncertain and still a subject of debate by many researchers. According to Zobel (1988), location of the amylose and amylopectin vary depending on their starch type. The location of amylose molecules in normal maize starch are suggested to be separated from amylopectin, while in potato starch the amylose molecules are interspersed among amylopectin. Jane et al. (1986) suggested that amylose and amylopectin are interspersed and intertwined with each other, which consequently can preserve the integrity of starch granule during thermal transition by holding amylose and amylopectin together. This statement is in agreement with Jane (2006) that also suggested that amylose is interspersed among amylopectin. Olkkhu & Rha (1978) suggested that the structure of the starch granule is usually taken to be amorphous near the surface of the granule and the amylopectin and amylose macromolecules are thought to be of a crystalline nature inside the granule. The granule is then totally or partially covered with protein, which explains why starch always contains some protein when manufactured industrially.

2.3.2 Starch Crystallinity

When viewed under X-ray diffraction, native starch granules exhibit two different type of crytallinity. Generally, the A-type starches are mainly from cereal starches and the B-type is from tuber amylose-rich starches. Meanwhile, C-type starches, which are mostly found in legumes, stems, and, cereals are from the mixture of A- and B- type diffraction diagrams. Other than that, V-type starches are reported to be found where starches conjugate with fatty acids and monoglycerides, which appear upon gelatinization of starch, which is rarely detected in native starches Buléon et al. (1998).

Starches contain about 10% of moisture, which is not distributed evenly in the granules. The concentric pattern of semi-crystalline layers as shown in Figure 2.8 is also responsible for the birefringence of native starch granules when viewed under polarizing light microscopy (Copeland, 2006).

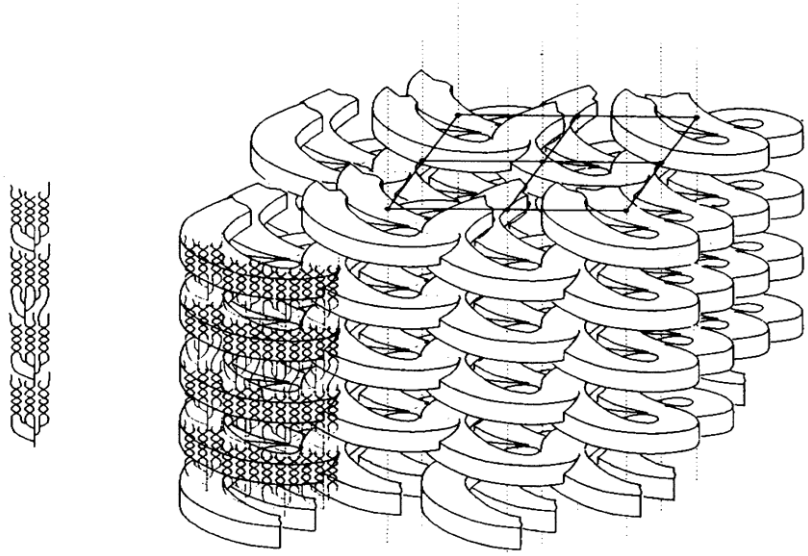


Figure 2.8: Schematically polymorphic structure of starch granules.

The forms of double helical structure between A- and B- type crystalline forms are essentially the same. Buléon et al. (1998) proposed that the arrangement of amylopectin chains in starch granules plays an important role in determining the differences in starch types. However, the packing of the helices in the A-type crystalline structure is more compact than in B- type crystallites, which have more open structure with a hydrated core. It is also suggested that A-type starch has a shorter chain of amylopectin than B-type starch (Jane, 2006; Copeland, 2009). The differentiation of A-type and B-type starch granules has been shown in Figure 2.9.

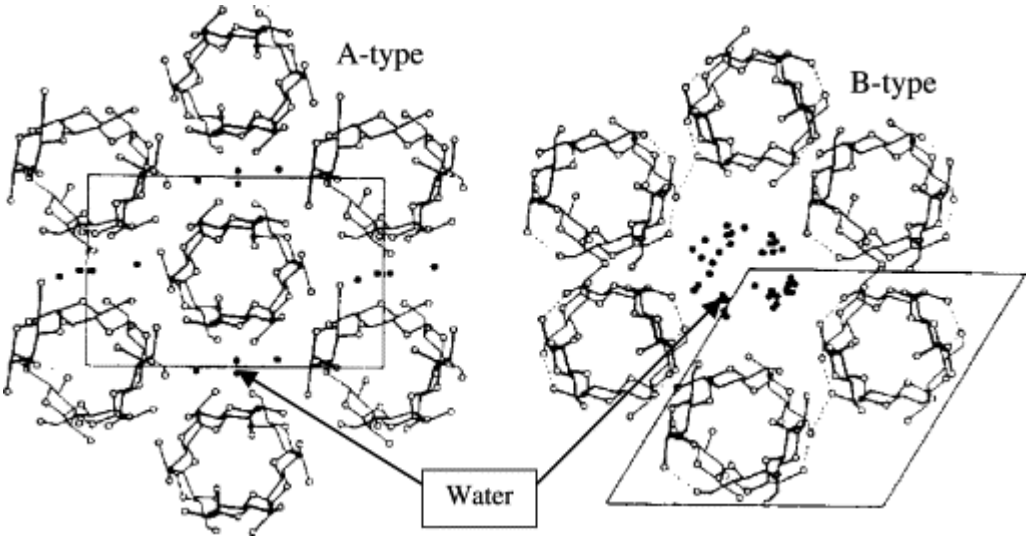


Figure 2.9: Schematically A- and B-type starch structure.

2.4 Functional Properties of Starch

2.4.1 Swelling and Solubility Properties

Swelling of starch granules is resulted from the heating of the starch granules in the presence of excess water. Donovan (1979) suggested that the swelling is the result from the expansion of the amorphous regions in the granules, causing the disruption of

crystalline region, thereby enhancing the possible interaction of the granules with water. There are several important factors that influence the swelling behavior of the starch granules namely as amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and conformation (Eliasson, 2004) and formation of lipid-starch complex (Swinkles, 1985). According to the study done by Singh et al. (2003) on the swelling power and solubility of starches from different botanical sources, they suggested that the swelling power and solubility of the starches differed significantly.

The existence of hydroxyl group in the starch granule accelerates their tendency to absorb water. However, the hydrogen bonds, which are pre-treated, that bind the granules together begin to weaken, permitting the granule to swell uniformly to many times its original size when the starch is heated in water beyond a critical temperature (which ranges from 56°C upwards depending on the starch). The swelling is reversible and the granules shrink on drying (Morrison & Karkalas, 1990).

Swelling power is the method to quantify the granule swelling ability. It is calculated by measuring the weight sedimented of 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 & 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).

Meanwhile, solubility of the starch depends on several factors including starch source, inter-associative forces, swelling power and the presence of other components (Moorthy, 2002). However, there are no correlation between swelling and solubility Moorthy (2001). Eliasson and Gudmundsson (1996) also suggested 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.

2.4.2 Pasting Properties

According to Whistler et al. (1984), pasting is the phenomenon following gelatinization in the dissolution of starch. Thomas & Atwell (1999) reported that pasting process is not a separate process from gelatinization but rather an overlapping occurrence, perhaps best described as a continuation of gelatinization. Pasting involves granular swelling, exudation of molecular components from the granules and finally, total disruption of the granules. Although there is no definitive point at which gelatinization ends and pasting begins, pasting is usually related to the development of viscosity.

Pasting properties of starch was measured by Rapid Visco Analyzer. Viscosity and pasting profile of starch can be quantified and deliberated when starch slurry is subjected to certain shear strength with excess of water at a controlled temperature. It involves starch-swelling, gelatinization, becoming a paste, and then retogradation. According to Atwell et al. (1988), pasting is the phenomenon following gelatinization; it involves granular swelling, exudation of molecular components from granule, and lastly total disruption of granules. Sigh et al. (2003) claimed that gelatinization occurs

primarily in the amorphous regions, because of the weak hydrogen bonding in this region opposed to the crystalline region. Starches from different botanical sources represent different pasting profiles due to their own characteristics such as rigidity of the granules that affects the swelling potential (Karim et al., 2000) and amylose leaching of the granules (Morris, 1990).

The interaction of starch and water can be observed by the disappearance of polarising crosses under polarised microscope. The absence of polarising crosses in starch granules indicates that the crystalline structure of starch has been destroyed. There are several phases in the breakdown of starch granular structures. Kerr (1950) suggested the first phase of starch breakdown occurs before the onset of gelatinisation. Water is slowly and reversibly taken up. This has been confirmed by Jaska (1971) in his study using Proton Magnetic Resonance (PMR). The study observed that the mobility of water decreased as the temperature increased right before the gelatinization occurs. This suggests that the water is being reversibly complexed with the starch molecules in the granule. Although this change is reversible at temperatures below the onset of gelatinisation, continued exposure of the starch to water can cause changes in the granule itself (Gough & Pybus, 1971).

The changes in the starch granules behavior and tendency of starch to retrograde can be studied from its pasting behaviour, by monitoring the changes in starch viscosity during programmed heating and cooling of a starch suspension by Brabender Amyloviscograph or Rapid ViscoAnalyser (RVA). These techniques allow researchers to determine the parameters that are important when measuring the pasting behaviour of

starch. Those parameters are (i) the peak viscosity (*P*), which is the highest apparent viscosity obtained during pasting; (ii) peak viscosity temperature (*PT*), the ease of cooking; (iii) resistance to breakdown(*H*), it illustrates the stability of paste during cooking; (iv) setback (*C*), indicated by the apparent viscosity of the paste after cooling, (v) stability of the cooked paste, indicated by the apparent viscosity after stirring at certain temperature for certain periods of time. It is crucial to observe the pasting behaviour of starch because it reflects the integrity of starch granules, as well as the quality and acceptability of different starches in industrial application.

2.4.3 Gelatinization Properties

Practically, gelatinization refers to the loss of order of birefringence and X-ray crystallinity as well as to the swelling of the granule, which in part accompanies loss of order (Blanshard, 1987). Detail explanation regarding starch gelatinization phenomena has been made by Olkku & Rha, (1978), Glicksman (1969) and Donovan (1979). Gelatinization is primarily a swelling driven process. Due to the existence of excess water and heat, the amorphous growth ring uptake the water causing this region to swell. According to Glicksman (1969), the molecules in starch granules are held together by hydrogen bond force; during heating this force is weakened by heat, allowing water to be absorbed by the granules. This denotes the 'initial gelatinisation temperature'. After that, granules swell tangentially and simultaneously lose their birefringence. These phenomena start at the hilum or centre of the granules and spread rapidly to the periphery. Gelatinization begins in the intercellular areas where the hydrogen bondings are the weakest. It occurs in different temperature ranges for different starches. Consequently, swelling in the amorphous regions built a stress upon the amylopectin

crystallites, and finally this stress will cause the amylopectin double helices within the crystallites to dissociate, hence leading to the breakdown of granule integrity (Donovan, 1979).

However, other researchers also tried to define starch gelatinization in their own perspective. Lelievre (1974) suggested that the gelatinization of starch is related to the melting of homogeneous polymers. Marchant & Blanshard (1978) postulated at least three processes occur in starch gelatinization, that are diffusion of water into the unswollen starch granule, disappearance of the birefringence due to hydration-facilitated melting, and swelling of granules. Atwell et al. (1988) defined gelatinization as a disordering of the crystalline structure in the starch granule. Hermansson and Svegmark (1996) described starch granules swell and form gel particles during gelatinization. They suggested that amylopectin is embedded in swollen starch granules while amylose diffuses out of the swollen granules and makes up the continuous phase outside the granules. Ratnayake and Jakcson (2006) proposed that gelatinization process can be initiated at low temperatures and continues until the granules are completely disrupted. They also suggested that amylose molecules located in amorphous regions play an important role in gelatinization process.

In summary, the gelatinisation of starch involved the following sequence of events (Olkku & Rha, 1978):

(1) Granules hydrate and swell to several times their original size.

(2) Granules lose their birefringence.

(3) Clarity of the mixture increases.

(4) Marked, rapid increase in consistency occurs and reaches a peak.

(5) Linear molecules dissolve and diffuse from ruptured granules.

(6) Mixture retrogrades to a paste-like mass or gel.

Along with the development of technologies and enhanced knowledge of the structure of granule, there are many new equipments and techniques to observe starch gelatinization process. These include light microscopy, electron microscopy, light transmission, viscometer, swelling and solubility determinations, nuclear magnetic resonance, laser light scattering, X-ray diffraction (Blanshard, 1987) differential scanning calorimetry and, high performance size exclusion chromatography (HPSEC) (Ratnayake & Jackson, 2007).

Differential scanning calorimetry (DSC) has been used to study the thermal behavior of starch including gelatinization (Stevens & Eltons, 1971; Donovan, 1979; Hari et al., 1989; Fredriksson et al., 1998; & Ratnayake & Jackson, 2006) and retrogradation (Mclver et al., 1968; Rusell, 1987; Jacobson and Bemiller, 1998; Ratnayake and Jakcson, 2007).

Data resulted from DSC are T_o which denotes the initial temperature at which starch granules start to swell; T_p represents peak temperature of the starch granule and T_c shows the completion of gelatinization process and ΔH represents the exothermic/endothermic energy during the measurement.