EFFECTS OF ULTRAVIOLET TREATMENT ON THE MECHANICAL AND PHYSICAL PROPERTIES OF EDIBLE STARCH BLEND FILMS

UMMI SHAFIQAH BINTI MAT SALLEH

UNIVERSITI SAINS MALAYSIA

2011

EFFECTS OF ULTRAVIOLET TREATMENT ON THE MECHANICAL AND PHYSICAL PROPERTIES OF EDIBLE STARCH BLEND FILMS

by

UMMI SHAFIQAH BINTI MAT SALLEH

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

2011

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS and ABBREVIATIONS

Symbols	Caption
a_w	Water activity
kGy	Kilo Gray
T_g	Glass transition temperature
W	Watt

Abbreviation	Caption
%E	Elongation at break
CFR	Code of Federal Regulations
CL	Chain lengths
DMSO	Dimethyl sulphoxide
DP	Degree of polymerization
DSC	Differential scanning calorimetry
ESR	Electron spin resonance
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FFA	Free fatty acid
FTIR	Fourier Transform Infrared
GRAS	Generally Recognized as Safe
IAEA	International Atomic Energy Agency
IR	Infra red
LBG	Locust bean gum
LDPE	Low density polyethylene
LPL	Lysophospholipids
MDSC	Modulated Differential Scanning Calorimetry
OPS	Oxidized potato strch
pI	Isoelectric point
PVA	Polyvinyl alcohol
RDP	Radiation degradation products
RH	Relative humidity
RVA	Rapid Visco Analyzer
RVP	Relative vapour pressure

SANS	Small angle neutron scattering
TL	Thermoluminescence
TS	Tensile strength
UV	Ultraviolet
WHO	World Health Organization
WVP	Water vapour permeability
WVTR	Water vapour transmission rate

LIST OF APPENDICES

KESAN RAWATAN ULTRALEMBAYUNG TERHADAP SIFAT-SIFAT MEKANIKAL DAN FIZIKAL FILEM BOLEH MAKAN DARI GABUNGAN KANJI

ABSTRAK

Filem-filem boleh makan telah dihasilkan dari kanji sagu, kacang hijau dan jagung dengan menggunakan gliserol sebagai bahan pemplastikan dan dirawat dengan sinaran ultra lembayung selama 1, 2 dan 3 jam. Gabungan kanji yang berbeza bagi kanji sagu: kacang hijau dan sagu: jagung telah dihasilkan dengan pelbagai nisbah seperti 100:0, 75:25, 50:50 dan 25:75. Sifat pempesan dan kandungan amilosa bagi setiap kanji telah dikaji. Filem kawalan dan dirawat akan dinilai ketebalan, ciri-ciri mekanikal, ketelapan wap air, kelarutan air, corak pembelauan X-ray, suhu peralihan kaca dan isoterma sorpsi. Analisis spektroskopi FTIR dijalankan untuk menentukan kemungkinan berlakunya interaksi atau perubahan terhadap sampel yang disebabkan oleh rawatan ultra lembayung. Umumnya, projek ini dijalankan untuk memastikan hipotesis yang mengatakan bahawa rawatan ultra lembayung dapat silang paut kan kanji, meningkatkan sifat mekanikal dan memberi kesan kepada sifat fizikal filem-filem. Keputusan yang diperolehi menunjukkan bahawa sifat pempesan setiap kanji dan gabungan kanji telah mempengaruhi sifat-sifat filem. Selain daripada itu, penambahan gliserol dapat memberikan filem yang lebih mudah diuruskan. Kandungan amilosa bagi kanji kacang hijau (44.00 %) adalah lebih tinggi daripada kanji sagu (25.40 %). Filem sagu: kacang hijau 25:75 yang dirawat dengan ultra lembayung selama 2 jam memberikan nilai kekuatan tensil yang paling tinggi. Bagi analisis ketelapan wap air, rawatan ultra lembayung tidak memberi kesan terhadap filem-filem. Namun begitu, ketelapan wap air bagi filem kawalan dengan nisbah yang berbeza telah menunjukkan perubahan di mana filem SM 7525 memberikan nilai

kekuatan tensil yang paling tinggi. Analisis X-ray menunjukkan bentuk jenis B bagi filem-filem kawalan dan yang dirawat. Manakala, filem-filem yang dirawat telah mempamerkan ciri-ciri yang lebih kristal berbanding dengan filem-filem kawalan. Hal ini mungkin disebabkan oleh kemusnahan bahagian amorfus pada granul kanji. Kajian termal bagi filem-filem kanji menunjukkan amaun bahan pemplastik turut mempengaruhi sifat-sifat filem di mana kandungan bahan pemplastik yang kurang daripada 27% memberikan kesan antipemplastikan. Isoterma sorpsi bagi semua filem mempamerkan bentuk sigmoid. Filem-filem kawalan menyerap air dan memberikan lengkungan lebih tinggi daripada filem-filem yang dirawat.

EFFECTS OF ULTRAVIOLET TREATMENT ON THE MECHANICAL AND PHYSICAL PROPERTIES OF EDIBLE STARCH BLEND FILMS

ABSTRACT

Edible films made from sago, mung bean and corn starches were prepared using glycerol as the plasticizer, and then they were treated under ultraviolet (UV) radiation for 1, 2 and 3 h exposure time. Different blends were made by varying the ratios of sago: mung bean and sago: corn starches as follows: 100:0, 75:25, 50:50, 25:75, and 0:100. The pasting properties and amylose content of the starches then were measured. Control (untreated) and ultraviolet (UV) treated films were evaluated for thickness, mechanical properties, water vapor permeability (WVP), water solubility, X-ray diffraction, glass transition temperature and sorption isotherms. Fourier transform infrared (FTIR) spectroscopy was conducted to determine possible interactions or changes induced on UV treatments of sample. The general aim of this work was to confirm the hypothesis that UV treatment can crosslink starch, enhance the mechanical properties and affect the physical properties of starch films. The results indicated that pasting properties of each starch and the combinations affect the properties of films whereby corn starch (C100) had the lowest setback value and therefore could not develop edible film. The incorporation of a plasticizer (glycerol) resulted in more manageable films. The amylose content of mung bean starch (44.00 %) was higher than that of sago starch (25.40 %) which gave higher Young modulus (YM) and tensile strength (TS) values. All films (for YM and TS values) showed slight changes after treated under UV light for 1 h and increased significantly after 2 h exposure time. The sago: mung bean 25:75 starch film treated with UV for 2 h had the highest TS and YM value. While for WVP analysis, the UV treatment did not affect the properties of films. However, WVP for control films with different ratios showed

significant differences in which SM 75:25 gives the highest value of WVP. X-ray analysis showed a B-type pattern for control and treated films. On the other hand, the treated films showed more crystalline character than control films. This could be due to the disruption of amorphous part of the starch granules. Thermal study of starch films showed that the amount plasticizers affecting the properties of films where plasticizer less than 27% gave the antiplasticization effect. Moisture sorption isotherms for all films showed the sigmoidal shape. The control films having slightly higher curve than treated films indicating that they absorbed more water.

CHAPTER 1

INTRODUCTION

1.1   Background and rationale

Plastics made from petroleum have been widely used throughout the world. With increased applications, the disposal of waste plastics has become a serious problem (Zhai *et al*., 2003). These synthetic polymeric materials have wide applications in food packaging due to various advantages such as high strength, elongation, gas barrier properties, low cost, lightness and water resistance (Guilbert, 1986). According to Han (2001), among the 30% packaging waste, 13% is due to plastic materials which are convenient, safe, strong and economical; but do not biodegrade.

The issue of sustainability and concern for the preservation of our biospheres has been high on the worldwide agenda lately (Hedenqvist, 2001). It is suggested that an alternative to this problem is to use biobased packaging which contain raw materials originating from agricultural sources, instead of non-renewable sources (Guilbert, 2000; Hedenqvist, 2001). According to Bastioli (2001), biobased packaging materials are able to significantly reduce the environmental impact in terms of energy consumption and green-house effect. Today, some of the bioplastics available in the market are used in applications where biodegradability is required in sectors such as composting (bags and sacks), fast food tableware (cups, cutlery, plates, straws and etc), packaging (soluble foams for industrial packaging, film wrapping, laminated paper, food containers), agriculture (mulch film, nursery pots and plant labels) and hygiene (diaper back sheet and cotton swabs) (Bastioli, 2001). In addition, biopolymer based packaging is not able to protect food products from all

outside environmental effects because of relatively poor mechanical properties and high hydrophilicity. It is able to protect from some the environmental effects when used together with other packaging materials, e.g., by coating cardboard with biopolymers, gas barrier properties of cardboard-based package can be enhanced (Talja, 2007).

Biobased packaging materials include both edible films and edible coatings along with primary and secondary packaging materials (Petersen *et al*., 1999). The development of alternative edible and/or biodegradable packaging material to partially or totally replace synthetic polymers may offer opportunities to fulfill the consumers' demands and expectations of new food packaging system that are convenient and environmentally friendly (Han, 2005). The use of edible films and coatings has a multipurpose objective includes restriction of moisture, aroma and lipid migration between food components but also gradual release of enrobed flavor, aroma, antioxidant and antimicrobial agents where traditional packaging cannot function (Kim *et al*., 2001).

Many food products are sensitive to ambient environmental effects which may dramatically decrease quality and shelf life. It may possible to improve food quality and shelf life by coating low moisture food products with biomaterial based coatings. These coatings prevent or retard water transfer from surrounding atmosphere to the food products. This is important because water may initiate deteriorative changes in foods like crystallization of amorphous materials, collapse or stickiness of low moisture food components and increase microorganisms activity

(Roos, 1995) whereas, edible films can be used as barriers or retarders of water sorption of low moisture products, e.g., crackers (Bravin *et al*., 2006).

It is expected to be an advantage if a polymer produced by nature could be used as a matrix material. Starch is one such initiative, a biopolymer that can be converted into a useful thermoplastic (Yu *et al*., 1996; Nabar *et al*., 2006) and can be safely and effectively disposed of in the soil or by compositing operation (Scandola *et al*., 1998). Therefore, starch is a possible candidate for a natural matrix material and it is commercially available at an industrial scale (Jansson and Thuvander, 2004).

In general, starch is a mixture of 2 polymers, the linear D-glucan amylose and the highly branched amylopectin. These 2 polymers exhibit different behavior with respect to gelation and the development of crystallinity. In gel formation, amylose and amylopectin form inter- and intramolecular physical crosslinks to produce a macromolecular network (Miles *et al*., 1985a, 1985b). The physical crosslinkages in the macromolecular network of starch are formed mainly by microcrystalline domains of amylose, which contribute to the higher tensile strength (Rindlav-Westling *et al*., 1998). Starch from different sources has been studied as a potential film-forming agent including from yam (Mali *et al*., 2002), wheat (Delville *et al*., 2003), tapioca (Marques *et al*., 2006), maize (Vasques *et al*., 2007) and cassava (Henrique *et al*., 2007). Starch films are excellent oxygen barrier due to their tightly packed, ordered hydrogen-bonded network structure and low solubility (Mali *et al*., 2005b). Unfortunately, there are some limitations for starch-based films due to its poor mechanical properties and high moisture sensitivity (Veiga-Santos *et al*., 2005). Therefore, improving properties of starch films through various treatments is

desirable (enzyme, physical and chemical). The modification techniques are often complex and time consuming; however irradiation can provide a simple and environmental-friendly alternative to change the physical, chemical and/ or biological characteristics of a product. This treatment requires limited sample preparation, is fast, does not require catalysts and does not induce major temperature increase (Woods and Pikaev, 1994).

Ultraviolet (UV) irradiation as a physical, cost effective, non-thermal and environmental friendly technology has received increasing attention during recent years, which has been successfully applied for preservation and decontamination of food products (Bintsis *et al*., 2000). It also has been used in medical and pharmaceutical research to crosslink collagen and gelatin films (Fujimori, 1965; Weadock *et al*., 1984; Tomihata *et al*, 1992). UV irradiation curing has become well-accepted technology because of its distinct advantages and found wide industrial applications such as fast-drying protective coatings, printing inks and adhesives (Masson *et al*., 2000). Besides, UV irradiation showed potential for modifying properties of different protein films such as solubility of sodium caseinate films, water vapor permeability (WVP) of egg albumen films and tensile strength (TS) of wheat gluten, corn zein and egg albumen (Rhim *et al*., 1999). The general aim of this work was to test the hypothesis that UV treatment could cross linked starch, enhance the mechanical and affect the physical properties of the films.

1.2   Research objectives

The general objective of this project are to develop edible films and study the combination of three types of starches; sago, mung bean and corn starches which represent various botanical sources; trunk, legume and cereal starches, respectively. Besides, the amylose content and pasting properties of sago, mung bean and corn starches also have been evaluated. Sago starch has been chosen as main material in this project as it has been considered as a potential crop in Malaysia and also to enhance and expand the application of sago starch. The films were treated with UV radiation as UV is a convenience tool for physical modification. Besides, UV radiation also can modify the properties of films. Specific objectives of this research are:

a) To investigate the effects of crosslinking via UV treatment on mechanical properties (tensile strength, elongation at break and young modulus) of starch blend films between combination of sago, mung bean and sago, corn starches.

b) To explore the effects of UV treatment on physical properties (water vapor permeability, water solubility, X-ray diffraction, glass transition and moisture sorption isotherms) of starch blend films.

1.3   Research outline

This study is focused on the effects of UV radiation on the mechanical and physical properties of edible films. The starch films were made by combination of those starches: sago, mung bean and corn starches according to the ratios (100:0, 75:25, 50:50 and 25:75) and were treated with UV light for 1, 2 and 3 h exposure time. Then, the films were evaluated the mechanical properties, water vapor permeability, water solubility, X-ray diffraction, sorption isotherms and glass transition analysis.

5

FTIR spectroscopy was also conducted to determine any interaction or changes on the UV treated samples. Overall methodology of this research has been shown in Appendix A.

CHAPTER 2

LITERATURE REVIEW

2.1   Starch

2.1.1   Introduction

Starch, an omnipresent bio-material is one of the most abundant and inexpensive polysaccharide sources which have the unique characteristics of 'biodegradability' (Cascone *et al*., 2001; Zhai *et al*., 2002). Starch is found in the leaves higher green plants and in the stems, seeds, roots and tuber of many higher plants, where it serves to store the chemical energy from the light energy of the sun in the process of photosynthesis. It also found in green unicellular algae (Robyt, 1998). Starch can be manufactured by the combination of grinding the starch-rich crop followed by wet separation technique. The starch granules will sediment in water due to their higher density (Chen, 2003).

The major botanical and commercial sources of starches are cereals, tubers, roots and legume starches. Corn (maize), wheat, rice, rye, barley and sorghum are the most common cereal starches. Starches from legumes such as broad bean, pea, cowpea (Tan *et al*., 2009), mung bean and lentil (Hoover and Zhou, 2003), tuber starches are potato, lily, canna and tulips whereas the root starches are sweet potato, tapioca, arrowroot, iris and taro (Dziedzic and Kearsley, 1984). Starch is the most abundant polysaccharide and is a major food reserve providing a bulk nutrient and energy source in human diet (Dewettinck et al., 2008). Besides, starch also 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 (Singh et al., 2003). In addition, starch also is one the most used in pharmaceutical formulations. It is used mainly in oral solid dosage forms as filler, binder or disintegrant (Kerf et al., 2001).

2.1.2 Basic starch chemistry

Starch is basically polymers of six carbon sugar of D-glucose which is often referred as 'building block' of starch. The structure of the monosaccharide D-glucose can be depicted in either an open chain or a ring form (Figure 2.1). The ring configuration is referred to as a pyranose, e.g., D-glucopyranose and can be in either α or β configuration. It is the most thermodynamically stable form (Thomas and Atwell, 1999).

Starch mainly consists of D-glucopyranose polymers linked together through α-1, 4 and α-1, 6 glycosidic bonds (Figure 2.2) (Thomas and Atwell, 1999). This glycosidic bond is stable at high pH but hydrolyzes at low pH (Marc *et al*., 2002). These bonds are formed when carbon number 1 (C1) on a D-glucopyranose molecule links to either carbon number 4 (C4) or carbon number 6 (C6) from an adjacent D-glucopyranose molecule (Thomas and Atwell, 1999). Starch polymers always have one reducing end because the aldehyde group on one of starch polymer is always free, while the other end is the non-reducing end. There could be a large number of non-reducing ends in a starch molecule depending on the number of polymeric branches present. These non-reducing ends and the glycosidic linkages are usually in α-configuration (Thomas and Atwell, 1999).
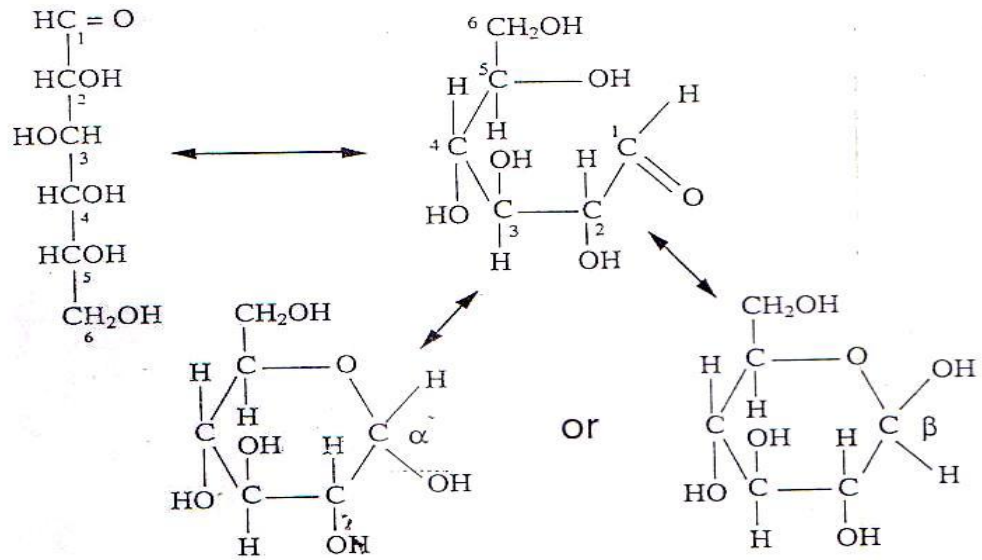
Figure 2.1: Open-chain and pyranose ring structures of the hexose sugar, D-glucose (Thomas and Atwell, 1999).
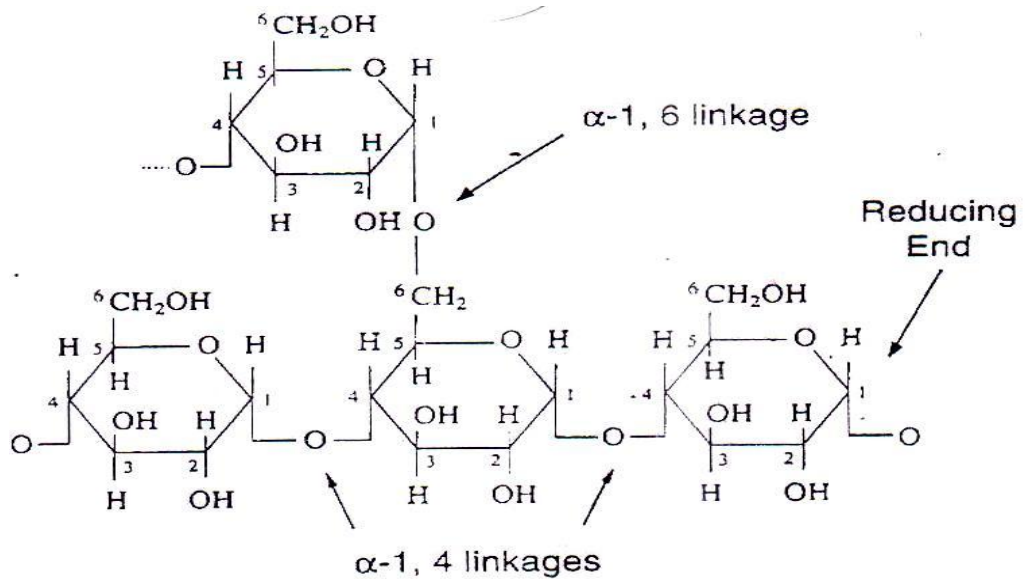
Figure 2.2: α-1, 4 and α-1, 6 glycosidic bonds of starch (Thomas and Atwell, 1999).

2.1.3 Chemical composition

Starch granules composed of a mixture of two polymers: amylose and amylopectin (Figure 2.3). The ratio of the two polysaccharides varies according to the botanical origin of the starch (Tester *et al*., 2004). Amylose is a linear polymer composed of

glucopyranose units linked through α-D-(1, 4) glycosidic linkages, while the amylopectin is a branched polymer with one of the highest molecular weights known among naturally occurring polymers (Karim *et al*., 2000).
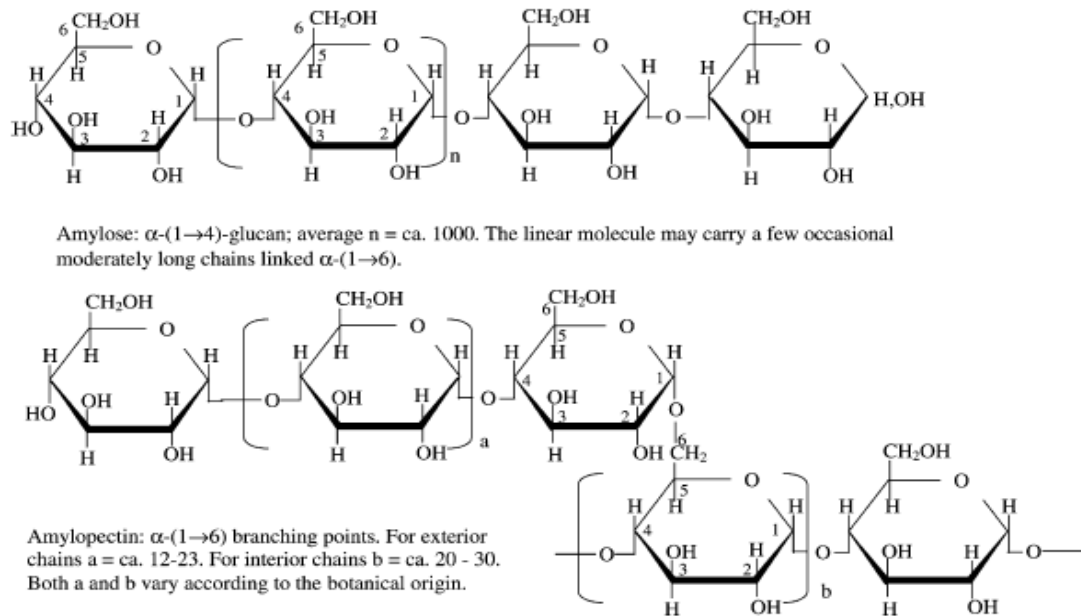
Figure 2.3: Structure of amylose and amylopectin (Tester and Karkalas, 2002).

2.1.4   Major components

2.1.4.1 Amylose

Amylose is smaller and linear fraction of the two components with the degree of polymerization (DP) around 500-5000. It possesses a very small degree of branches, 9-20 per molecule and the chain length is between 4 to > 100 glucose units (Oates, 1997). Basically, amylose is longer and relatively more linear compared to amylopectin which contains around 99% α-(1, 4) and 1% α-(1, 6) linkages and the molecular weight of amylose is between 32 400 − 113 400 (Tester *et al*., 2004). Although typically illustrated as a straight chain structure for the sake of simplicity, amylose is actually often helical (Fennema, 1985). According to Whistler and BeMiller (1997), this helical shape is due to the axial-equatorial position coupling

the α-(1, 4) D-glucopyranosyl units. The inferior of the helix is lipophilic as it contains mostly hydrogen atoms whereas the exterior coil has hydroxyl groups positioned on it (Whistler and BeMiller, 1997).

The ability to form helical inclusion complexes gives rise to the typical deep blue color in the presence of polyiodide ions (Biliaderis, 1998). Simplified models for the structure of amylose are shown in Figure 2.4. The content of amylose in starch varies from 0 to 100% depending on the botanic origins (Gerard *et al*., 2001). Table 2.1 showed the amylose and amylopectin content of starches. However, certain mutant plants, commonly called waxy because of the waxy appearance of the seed endosperm have a much lower content or even lack the amylose content completely (Jane *et al*., 1999). Other type posses increase amylose content called high-amylose starch (Morrison *et al*., 1984). BeMiller and Whistler (1996) reported that wheat starch contains around 25% amylose and corn has about 25-27% amylose with the remainder being amylopectin. High amylose corn starch can run as high as 80% of amylose and waxy starches have virtually none. According to Hung *et al*. (2006) and Regina *et al*. (2006), starches with varying amylose content are often interest for food processing because of the potential to modify the texture and quality of the end-use products, moreover, starches with increased amylose content are of nutritional interest because they contribute to slow digesting and resistant starch which are associated with beneficial physiological effects.
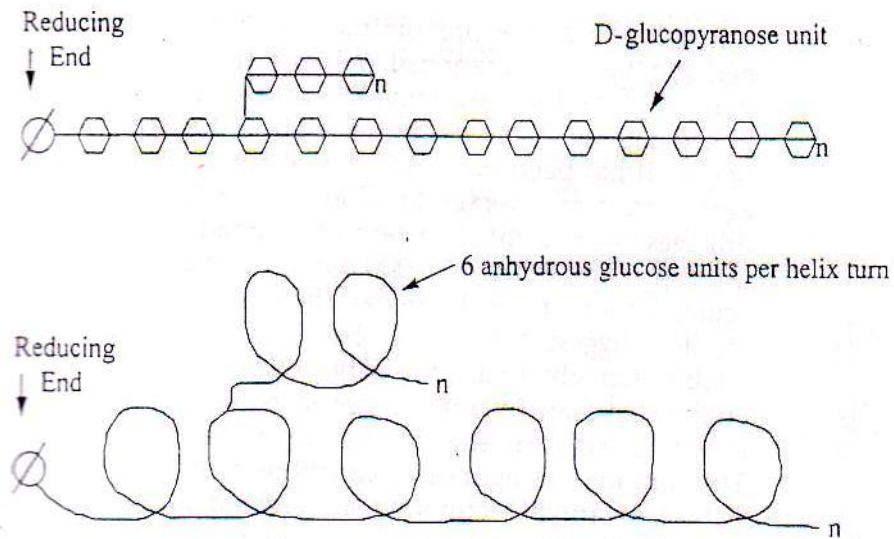
Figure 2.4: Amylose model. Amylose can be depicted as either a straight chain or a helix (Thomas and Atwell, 1999).

Table 2.1: Amylose and amylopectin contents of some common starches

Starch source	Amylose content (%)	Amylopectin content (%)
Maize	26	74
Wheat	25	75
Rice	17	83
Sorghum	26	74
Potato	24	76
Sago	27	73
Waxy maize	1	99
Waxy sorghum	1	99
Tapioca	17	83

(Source: Howling, 1974)

2.1.4.2 Amylopectin

In contrast to amylose, amylopectin molecule is much larger and branched. It is the predominant molecule in most normal starches (Thomas and Atwell, 1999). The molecular weight of amylopectin is around $1 \times 10^7 - 1 \times 10^9$ (Biliaderis, 1998) and highly branched with the composition of 95% α-(1, 4) linkages and 5% α-(1, 6) linkages. Like amylose, the molecular shape, size, structure and polydispersity of amylopectin molecules vary according to its botanical origin. Amylopectin unit

chains are relatively short compared to amylose molecules with a broad distribution profile. They are typically 18-25 units long on average (Morrison and Karkalas, 1990; Hizukuri, 1993; Mua and Jackson, 1997). The different properties of amylose and amylopectin components are summarized in Table 2.2. The size of the molecule and its branched structure is able to slow down the retrogradation process and can either delay or prevent gel formation. Pastes from starches that contain essentially all amylopectin (waxy starches) are considered to be non-gelling but typically have a cohesive and gummy texture (Thomas and Atwell, 1999). Furthermore, amylopectin does not give a starch-iodine (amylose-iodine) blue color but a purple and sometimes reddish-brown color depending upon its source.

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

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

(Source: Eliasson, 2004).

Models for structure of amylopectin are illustrated in Figure 2.5. Amylopectin has three types of α-1, 4 chains (Peat *et al*., 1952). According to Oates (1997), A-chains are unbranched and attached to the molecule by a single linkage; meanwhile B-chains are branched and connected to two or more chains. A single C chain may also present at an amylopectin molecule, which contains a sole reducing group (Oates, 1997). The A- and B- chains are situated at the external and form double helices (crystallites) within the native granules. These properties may be correlated with the fact that starches with 'A type' crystallinity (most cereals) have shorter chain lengths (CL) of 12-16 compared to 'B type' starches with CL 20-24 starches (Hizukuri *et*

al., 1996). These chains are assembled in a 'cluster structure' based on the model proposed by Nikuni (1969).

2.1.5   Minor components

Though the major components (amylose and amylopectin) have received much attention, the existence of minor components such as lipids, proteins and minerals are also affecting the behavior of starch in various applications (Table 2.3).

Moisture

Water or moisture content of starch varies significantly in its native state. Usually, starch as prepared commercially contains on the average approximately 12% moisture (Eliasson, 2004).

Lipid

The minor components of starch granules that have received much attention are the lipid materials and particularly the lipids within the granule matrix. According to Tester and Karkalas (2002), lipids in starch may be present in the form of integral lipids which comprise of lysophospholipids (LPL) and free fatty acid (FFA). LPL contains phosphate groups and this structure allows it to form a complex with amylose wherein the FFA is aligned in the core of the amylose helix. This complex is very stable and dissociates only at very high temperatures (Thomas and Atwell, 1999). The complex reduces the accessibility of the active site of the enzyme making the amylose chain less likely to be hydrolyzed. However, according to Tester *et al*., (2006), lipids not actually confer complete resistance to hydrolysis, but just prolong the whole digestion process. A clear distinction between potato, maize and wheat starches can be made on the basis of internal lipids.
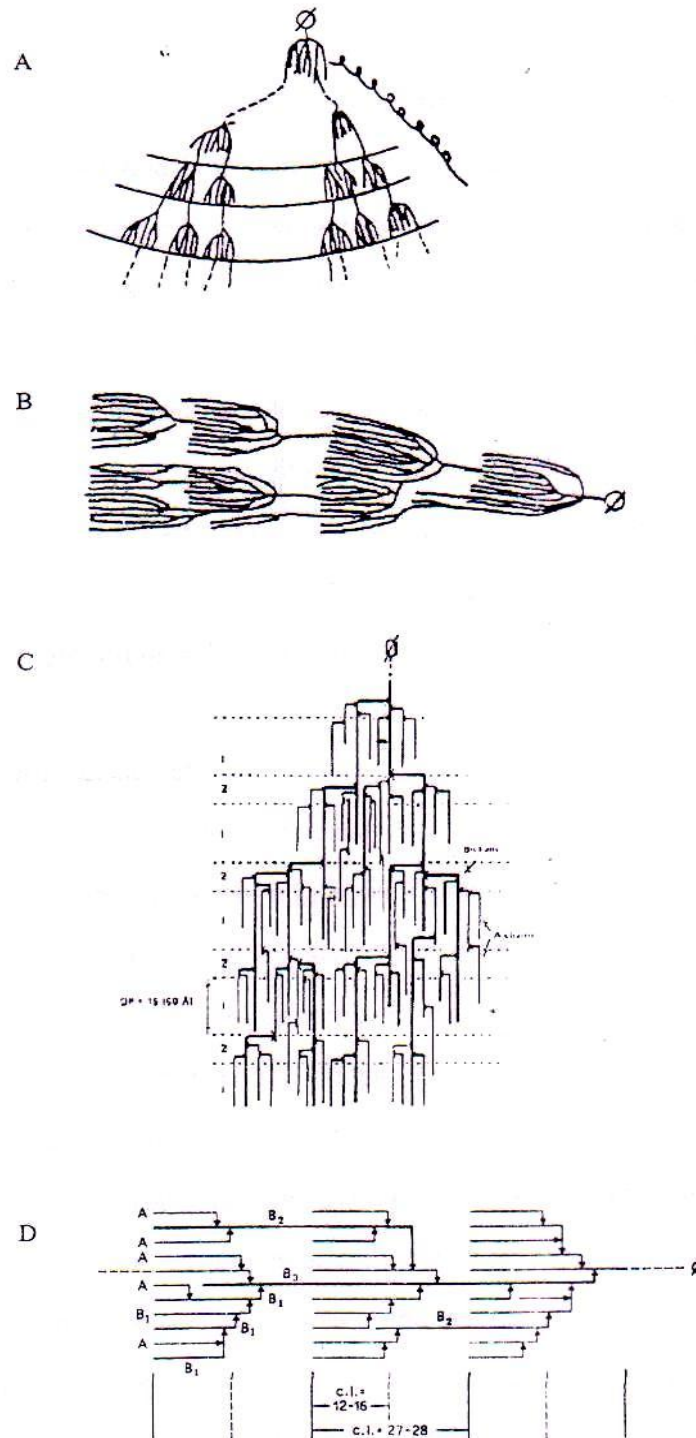
Figure 2.5: Models for amylopectin-cluster as proposed by (A) Nikuni (1969), (B) French (1972), (C) Robin *et al.* (1974) and (D) Manners and Mathesons (1981). DP: degree of polymerization.

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
Sago[c]	27	0.1	0.1	0.02

(Source: a: BeMiller and Whistler, 1996; b: Hoover *et al*., 1997; c: Singhal *et al*., 2008).

From of all starch granules that have been examined, only those from cereal grains including maize and wheat contain significant amounts of internal lipid, starch from tuber (e.g., cassava) and legume (e.g., peas and beans) does not contain this type of internal lipid. Although maize and wheat starch contain internal lipid, the nature of the lipids in the two species is quite different (Galliard, 1987).

Protein

Protein content varied based on the source of starch. All protein analysis is reported as percent nitrogen. It can be categorized into starch surface associated proteins and integral proteins (Tester and Karkalas, 2002). Surface associated proteins can be removed with salt solutions whereas integral proteins need a more intense treatment, for example with sodium duodecyl sulfate, a type of detergent (Thomas and Atwell, 1999). 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. The presence of surface protein (friabilin) has received much attention because of its proposed association with grain hardness (Baldwin, 2001; Morris, 2002). In conversion of starch to

hydrolysis products such as glucose, maltose and fructose syrups, the presence of proteins may lead to flavors and colors due to formation of Maillard products between hydrolysed protein and reducing sugars. The surface of proteins can lead to foaming in starch processing (Galliard, 1987).

Phosphorus

Phosphorus is found in the three major forms; phosphate monoesters, phospholipids and inorganic phosphates (Blennow *et al.*, 1998, 2000a, b, 2002). Based on the study done by Singh *et al.* (2003) and Noda *et al.* (2004), phospholipids and phosphate monoesters have enormous effects on swelling and pasting properties. Potato starch which consists of a high amount of phosphorus carries a net negative charge due to the presence of this mineral. These negative charges on the individual polymer chains repel each other and these may enhance the hydration and swelling hence producing a higher viscosity (Blanshard, 1987). Most cereal starches contain very small amount of phosphorus and if present, it is usually analyzed as phospholipids (Morrison, 1989). Phosphorus plays an extremely important role in starch functional properties such as paste clarity, viscosity, viscosity consistency and paste stability (Eliasson, 2004). While, phospholipids present in starch have a tendency to form a complex with amylose and long branched chains of amylopectin, which resulted in limited swelling (Singh *et al.*, 2003).

Trace elements

In addition to other compounds present in starch, can and does contain very small amounts of minerals and inorganic salt. During the isolation of starch products for commercial application, these compounds are assayed for and reported collectively as 'ash'. Ash content varies on the native starches and this variance is primary dependent upon source or origin of the starch. The ash content for most commercial

17

starches is reported to be <0.5% based on dry starch basis (Eliasson, 2004).Starches also contain relatively small quantities (< 0.4%) of minerals (calcium, magnesium, phosphorus, potassium and sodium) which are, with the exception of phosphorus of little functional significance (Tester *et al*., 2004).

2.1.6 Structure and organization of starch granules

Native starches are in the form of semi-crystalline where the arrangement of the two main components; amylose and amylopectin make up the internal organization. The amylose and amylopectin do not exist freely in nature but as components of discrete, semicrystalline aggregates called starch granules (Thomas and Atwell, 1999; Donald, 2004; Tester and Karkalas, 2005). In tuber and root starches, the crystalline regions are solely composed of amylopectin, while the amylose is present in the amorphous regions. While, in cereal starches, amylopectin is also the most important component of the crystalline regions (Marc *et al*., 2002).

2.1.6.1 Crystalline phase of starch granules

The native starch granule structure has crystallinity levels from 15 to 45% depending on the starch origin (Zobel, 1988b). Starch granules in their native form have a complex structure, with parts that are very densely packed and others that are loosely packed. It was shown that the crystalline parts in a starch granule are formed by short outer chains (DP 15-18) of amylopectin molecules (French, 1984). According to Chung and Liu (2009), the crystalline regions are believed to be densely packed structures, which are thought to involve the regions of amylopectin branches. The densely packed areas are measurable by X-ray techniques. By this method, different types of crystal structures can be analyzed and the amount of

crystalline and amorphous material measured (Colonna *et al*., 1982; Zobel, 1988a). The cluster arrangement of these branches make up the ordered, tightly packed, parallel glucan chains and they are organized in a manner that alternates with less-ordered regions that are predominantly composed of branch points. Therefore, an amylopectin cluster can be regarded as comprising a amorphous region which contains most of the tightly spaced branches (amorphous lamella) and a thin crystalline region which consists of the parallel glucans (crystalline lamella) (Oates, 1997).

Under microscope, starch granules illuminated with polarized light, show a characteristic Maltese cross pattern. The phenomenon is also known as birefringence (Murphy, 2000). Amylose does not appear to have any significant effect on crystallinity in normal and waxy starches (which may be virtually free of amylose) both of which display strong birefringence (Hoover, 2001; Tester and Karkalas, 2002). In high amylose starches, however the amylose may contribute significantly to the crystallinity (Banks and Greenwood, 1975; Tester *et al*., 2000) although the exact nature of the crystalline polymorphs may be different (Matveev *et al*., 2001).

Native starches can be divided into three types: A, B and C according to their wide-angle X-ray diffraction patterns (Zobel, 1988a, b; Biliaderis, 1991a, b) as shown in Figure 2.6 which are formed in cereals, potato and legumes, respectively. An additional form, known as V-type crystallinity corresponds to structures of helical inclusion complexes of amylose occurs in swollen granules (Hoseney, 1994). These polymorphs are different in the way they are packed and organized. A-type polymorphic starch has a monoclinic unit cell, which is closely packed whereas B-

type polymorphs has hexagonal unit cell, which is relatively loosely packed with an open channel of water in the unit cell (Jane, 2006). Even though A-type starches are more densely packed, they are more susceptible towards hydrolysis compared to the loosely packed B- and C-type starches (Gallant *et al*., 1992).
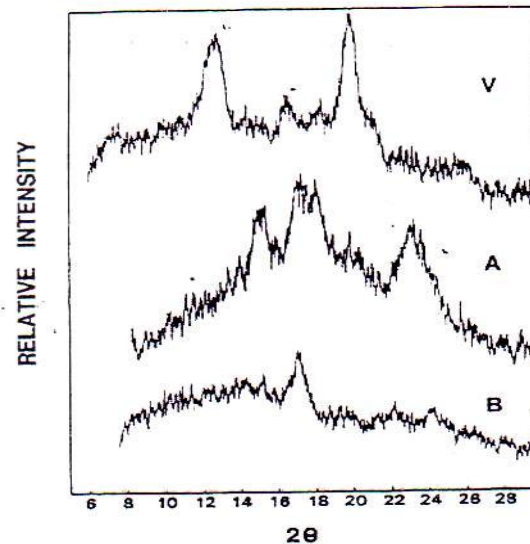
Figure 2.6: Three general X-ray patterns of various starches (Hoseney, 1994).

In addition, Jane (2006) had also reported that the A-type starch consists of shorter branch chains whereas B-type starch consists of longer B chains. The short crystalline structure in A-type polymorphs are less stable and more susceptible for rearrangement and therefore generate more loosely packed areas of voids. On the other hand, B-type polymorphs and some C-type polymorphs have long chains, which extended through two or more clusters and stabilize the internal structures of granules. Recent studies on the structure of starch by small angle neutron scattering (SANS) revealed that the starch granules can be described as containing three regions (Figure 2.7) namely semicrystalline stacks containing alternating crystalline and amorphous lamellae, which are embedded in a matrix of amorphous materials (Jenkins, 1996). Small angle neutron scattering (SANS) is used to understand

architectural aspects of the starch granules and complements the X-ray techniques (Tester *et al*., 2004).

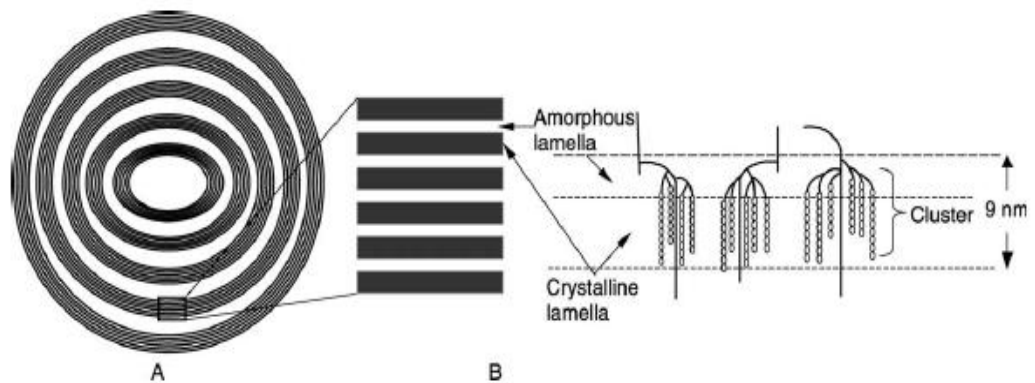Figure 2.7: Diagrammatic representation of the lamellar structure of a starch granule according to Donald *et al*., 1997. A) Stacks of semicrystalline lamellae are separated by amorphous growth rings. B) Magnified view of one such stack, showing that it is made up of alternating crystalline and amorphous lamellae. C) Double helical structures formed by adjacent chains of amylopectin give rise to crystalline lamellae. Branching points constitute the amorphous regions.

2.1.6.2 Amorphous phase of starch granules

According to Blanshard (1987), 70% of starch granule is in amorphous regions and consist mainly of amylose components. This fact is supported by Gidley and Bulpin (1989), who reported that amylose, is present predominantly in the amorphous regions. However, there is no sharp differentiation between the crystalline and amorphous phase of starch granules and it is believed that some or all the starch molecular chains run continuously from one phase to another. This must true for the amylopectin component of starch, in as much the individual molecules are far larger than the crystallites (French, 1984). Thus, much amylopectin exist in the amorphous state also.

In addition, Thomas and Atwell (1999) also mentioned that amorphous phase or gel phase may be the area of starch that easily penetrates by water and low-molecular

weight water-soluble solutes. With water uptake, the gel phase undergoes limited reversible swelling with consequent swelling of the entire granule. Loss of water from the gel phase during severe drying cause formation of interchain and intrachain hydrogen bonds which may generate strain and distort the granule structure sufficiently, and lead to visible cracks develop.

2.1.6.3 Size and shape of starch granules

According to Tharanathan and Saroja (2001), starch present in semicrystalline granules that varies in composition, size, shape and functionality when obtained from different botanical sources. Table 2.4 showed the size and shape of some starch granules. The sizes of starch granules are vary from submicron to more than 100 microns in diameter.

The smallest granules are rice and amaranth starches (1 μm in diameter) and the largest granules are potato and tapioca starch granules with a 100 μm diameter (Bello-Pérez *et al*., 1998). The shapes of starch granules include spherical, disk, oval, polygonal, dome-shape, elongated rod shape and compound starch (Jane, 2006). Starch can be subdivided into few classes; tuber starches are generally large, ellipsoidal or spherical with apparent shells around an eccentric hilum. Some of the examples of this particular class are yam tubers (Gallant *et al*., 1982). Cereal starch granules are small and polyhedric. These starches occur as single granules as in normal or waxy corn starches, or as compound granules as in oats or rice whereas, in wheat, barley, rye and triticale, 10% of the granules are lentil-shaped. However, amylomaize starch consists of irregular and filamentous granules (Gallant *et al*., 1982). Most cereal starches exhibit 'A' type X-ray diffraction pattern (Tester and

Karkalas, 2002) whereas legume starches are kidney-like, ovoid shaped with well defined shells centered along an elongated hilum. There are some exceptions for wrinkled pea, where sphero-pyramidal units are associated (Gallant *et al*., 1992). Some granules, such as those in oats and rice have a higher level of structure in which many small, individual granules are cohesively bound together in an organized manner. They are called compound starch granules (Thomas and Atwell, 1999).

Table 2.4: Approximate size and shape of common food starch granules.

Starch	Type	Shape	Distribution	Size (µm)
Barley	Cereal	Lenticular, spherical	Bimodal	15-25, 2-5
Rye	Cereal	Lenticular, spherical	Bimodal	10-40, 5-10
Wheat	Cereal	Lenticular, spherical	Bimodal	15-35, 2-10
Maize (waxy and 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 (single) 80 (compound)
Rice	Cereal	Polyhedral	Unimodal	3-8 (single) 150 compound)
Pea	Legume	Rentiform (single)	Unimodal	5-10
Potato	Tuber	Lenticular	Unimodal	5-100
Tapioca	Root	Spherical/ lenticular	Unimodal	50-45
Sago	Trunk	Oval	Unimodal	20-40

(Source: Tester and Karkalas, 2002).

2.1.7 Types of starch

Starch granules are mainly found in seeds, roots, tubers, stems, leaves and fruits. The differences are so distinct that only a few parameters are needed to determine the origin of the native starches.

2.1.7.1 Sago starch

Sago is an aquatic palm of tropical Asia (Steinbüchel and Rhee, 2005). It is derived from sago palm (*Metroxylan spp*.) which is better known as 'rumbia' and distributed throughout South East Asia. The sago palm is a species of the genus *Metroxylon* belonging to the Palmae family (Singhal *et al*., 2008). Some of the important species widely used in the sago starch production includes *M. longispinum*, *M. sylvestre*, *M. microcanthum*, *M. sagu* and *M. rumphii*. The word 'sago' is originally Javanese and means starch containing palm pith (Flach, 1997). In Malaysia, sago starch ranks fifth among the highest terms of agricultural revenue after pepper, palm oil, cocoa and rubber. The sago starch prepared from the pith of several genera of palms in the form of small whitish, pinkish or brownish grains are exported to Europe and America, where they are used mainly for soup thickening and making of puddings (Abd Aziz, 2002). The sago palms are hapaxantic that is it flowers once and die shortly thereafter. During the vegetative stage, just before flowering, the plant converts its stored nutrients into starch which fills the trunk (Abd-Aziz, 2002).

Sago palm is an important resource especially to the people in rural areas because it has various uses, especially in the production of sago flour or sago pearl (Ahmad *et al*., 1999).There are some important properties of sago starch such as its ease to gelatinize, high viscosity if properly exacted and the ease with which it can be