EFFECT OF OZONE TREATMENT ON THE PHYSICOCHEMICAL PROPERTIES OF CORN, SAGO AND TAPIOCA STARCHES

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EFFECT OF OZONE TREATMENT ON THE PHYSICOCHEMICAL PROPERTIES OF CORN, SAGO AND TAPIOCA STARCHES

by

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LIST OF SYMBOLS AND ABBREVIATIONS

OGT	Ozone generation time
NaBr	Sodium bromide
DP	Degree of polymerization
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
WAXD	Wide−angle X−ray diffraction
DSC	Differential scanning calorimetry
TMA	Thermo-mechanical analysis
NMR	Nuclear magnetic resonance spectroscopy
RVA	Rapid Visco^{TM} Analyzer
GRAS	Generally recognized as safe
RVUs	Rapid Visco Units
DMSO	Dimethyl sulfoxide
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
DSP	Digital signal processing
RI	Refractive Index
T_o	Onset temperature
T_p	Peak temperature
T_c	Conclusion temperature
ΔH	Exothermic/endothermic energy
ΔH_g	Enthalpy of gelatinization
ΔH_r	Enthalpy of retrogradation
[η]	Intrinsic viscosity

η_{sp}	η of solution/η of solvent
C	concentration in g/mL
K	Huggin's constant
η_{sp}/C	y-axis of linear plot of the Huggin's equation
$k[\eta]^2$	Slope of linear plot of the Huggin's equation
Pa.s	Viscosity of fluid
O_3	Ozone
V	Volt
ppm	Part per million
CFU/g	Colony forming units per gram
O_2	Oxygen
$\overline{Mw}$	weight-average molar mass
μL	Micro liter
dn/dc	Differential index of refraction
σ	Shear stress, Pa
K	consistency coefficient, Pa s^n
γ	Shear rate, s^{-1}
n	Flow behavior index
r	Correlation coefficient
P	P-value
$\overline{Mn}$	number-average molar mass
$\overline{Mw}/\overline{Mn}$	Polydispersities

KESAN RAWATAN OZON KE ATAS CIRI-CIRI FIZIKALKIMIA KANJI JAGUNG, SAGU DAN UBI KAYU

ABSTRAK

Kesan pengoksidaan dengan menggunakan gas ozon ke atas ciri-ciri fizikalkimia kanji telah dikaji dengan menggunakan tiga jenis kanji yang berlainan tempat asal (jagung, sagu, dan ubi kayu). Kanji dalam keadaan serbuk kering telah didedahkan kepada gas ozon selama 10 minit pada masa penghasilan ozon yang berlainan (1, 3, 5, dan 10 minit). Bagi semua kanji, kandungan karbosil and karbonil meningkat secara nyata dengan peningkatan masa penghasilan ozon. Pengoksidaan secara signifikannya telah mengurangkan kuasa pembengkakan kanji sagu and kanji ubi kayu yang telah dioksida tetapi telah menunjukkan peningkatkan bagi kanji jagung yang telah dioksida. Keterlarutan kanji ubi kayu menurun dan kanji sagu meningkat selepas pengoksidaan. Walaubagaimanapun, perubahan keterlarutan kanji jagung yang telah dioksida adalah tidak signifikan. Kelikatan "intrinsic" bagi semua kanji yang telah dioksida menurun dengan signifikan, kecuali kanji ubi kayu yang dioksidakan pada 5 minit masa penghasilan ozon. Sifat-sifat pempesan bagi semua kanji yang dioksida mengikut tren yang berlainan apabila masa penghasilan ozon meningkat. Semua kanji yang telah dioksida menunjukkan kelakuan "non-Newtonian shear-thinning". Kelikatan kanji menurun secara drastik dengan peningkatan masa penghasilan ozon. "Young's modulus" bagi semua gel kanji jagung dan sagu yang disimpan selama 1 dan 7 hari pada suhu 4 ^{o}C meningkat dengan signifikan berbanding dengan kanji yang tidak dimodifikasi. Tiada perbezaan yang diperhatikan bagi suhu gelatinisasi dan entalpi gelatinisasi bagi semua kanji yang dioksida berbanding dengan kanji yang tidak dimodifikasi. Retrogradasi amilopektin

meningkat secara nyata bagi kanji jagung selepas pengoksidaan pada 1 min masa penghasilan ozon. Keputusan ini menunjukkan dalam keadaan rawatan ozon yang sama, tahap mengoksidaan kanji adalah berbeza antara jenis kanji yang berlainan.

EFFECT OF OZONE TREATMENT ON THE PHYSICOCHEMICAL PROPERTIES OF CORN, SAGO AND TAPIOCA STARCHES

ABSTRACT

The effects of oxidation by ozone gas on some physicochemical and functional properties of starch (corn, sago, and tapioca) were investigated. Starch in dry powder form was exposed to ozone for 10 min at different ozone generation times (OGTs: 1, 3, 5, and 10 min). Carboxyl and carbonyl contents increased markedly in all starches with increasing OGT. Oxidation significantly decreased the swelling power of oxidized sago and tapioca starches but increased that of oxidized corn starch. The solubility of tapioca starch decreased and sago starch increased after oxidation. However, there was an insignificant changed in the solubility of oxidized corn starch. Pasting properties of the oxidized starches followed different trends as OGT increased. All oxidized starches showed a non-Newtonian shear-thinning behavior. Starch viscosity decreased drastically with increasing OGT. Young's modulus for all oxidized corn and sago starch gels stored for 1 and 7 days at 4 ^{o}C increased significantly as compared to unmodified starch. No difference was noted in gelatinization temperature and gelatinization enthalpy of all oxidized starches as compared to unmodified starch. Retrogradation of amylopectin tended to increase markedly in corn starch after oxidation at 1 min OGT. These results show that under similar conditions of ozone treatment, the extent of starch oxidation varies among different types of starch.

CHAPTER 1
INTRODUCTION

1.1 Background and Rationale

Starch is one of the most essential renewable resources for food and nonfood industries. However, uses of native starch in most applications are all the time limited by some unwanted characteristics. Therefore, starches must be modified chemically, physically, genetically, or enzymatically to enhance their positive attributes or to diminish their defects. Oxidation is one of the starch modification methods intended to overcome these problems. Many industries use oxidized starch to provide coating and surface sizing properties (e.g., the paper, laundry finishing, and building materials industries) (Chang *et al*., 2008). Oxidized starch has several unique functional properties, such as high stability, low viscosity, clarity, film forming, and binding properties (Kuakpetoon & Wang, 2006). In the food industry, oxidized starches are used in food products when low viscosity and/or neutral-tasting starch is required, such as in lemon curd, mayonnaise, and salad dressing (Adebowale & Lawal, 2003).

Oxidized starch is formed by reacting starch with a specific amount of oxidizing agent under controlled temperature and pH (Wurzburg, 1986). Starch oxidation took place in two main reactions. Initially, hydroxyl groups in starch molecules are oxidized to carbonyl (C=O) groups, which mainly take place at C-2, C-3, and C-6 (Wurzburg, 1986). Next, oxidation also causes depolymerization of starch molecules by cleaving α-(1→4)-glucosidic linkages (Wurzburg, 1986). Introducing of bulky carboxyl (COOH) groups minimizes retrogradation of oxidized starch (Wurzburg, 1986), while Chang and Cho (1997) reported that

depolymerization of starch causes loses of polymer properties and decreased paste viscosity. A number of oxidizing agents have been used to produce oxidized starch, such as sodium hypochlorite, sodium bromide (NaBr), gaseous chlorine, calcium hypochlorite, hydrogen peroxide, ammonium persulfate, potassium permanganate, oxygen, and bromide. However, alkaline hypochlorite is one of the most common methods used in industrial scale to produce oxidized starch.

Chemical oxidations have several drawbacks for starch modification due to safety and environmental issues. In the process, large quantity of waste water having a high concentration of salts causes waste water disposal problems (Kesselmans & Bleeker, 1997a). Furthermore, in alkaline slurry of hypochlorite oxidation process, the loss of small molecules produced by starch breakdown causes low yield of oxidized starch (Wing & Willett, 1997).

The use of ozone as green oxidant, requires limited purification procedures, would therefore be ideal over the use of hypochlorite. Moreover, in contrast to chemical oxidants, when ozone is introduced to a food product, it does not leave a residue. Ozone is a naturally occurring and industrially generated form of oxygen (Dillon *et al*., 1992). It is a more powerful oxidant than oxygen due to its extra oxygen atom, which it can share with other substances to oxidize them. Moreover, it is a clean oxidant that has an elevated thermodynamic oxidation potential, which means the reactions can be performed at low temperatures (Sahle-Demessie & Devulapelli, 2008).

A number of patents have been filed for a method of oxidizing dry starch (Kesselmans & Bleeker, 1997a) and polysaccharides (Kesselmans & Bleeker, 1997b) using ozone as an oxidizing agent. Recently, An and King (2009) studied the effect of ozonation and amino acids on pasting properties of rice starch , they reported that ozonated rice starch exhibited similar pasting properties to those oxidized starches treated with low concentrations of chemical oxidizing agents. In contrast, Lii *et al*. (2003) studied the effect of corona discharges on granular starches and reported that the discharged decomposed starches to low molecular weight fragments from terminals of long polysaccharide chains at the same time that oxidation of the polysaccharides took place.

To date, most oxidation in the literature was conducted using chemical oxidant but very little research has been done to study the effects of ozone treatment on starches. Therefore, the present work attempts to use gaseous ozone to treat dry native starches from different botanical origin with different X-ray diffraction pattern (Corn – Type A, Tapioca – Type B, Sago – Type C) and to characterize the effect of ozone treatment on the physicochemical and functional properties of starches. Rational of present work by treating starch in dry state is dry condition can help to save further drying cost, labour cost and reduce loss in final product. Our hypothesis thus, ozone treatment could replace chemical oxidant for the production of oxidized starches, the degree of oxidation of the starches would increase with increasing of ozone generation time.

1.2 Research Objectives

i. To prepare oxidized starch from different botanical origin (Corn – Type A, Tapioca – Type B, Sago – Type C) in dry form using gaseous ozone.

ii. To study the effect of ozone treatment on the physicochemical and functional properties of starches from different botanical origins.

CHAPTER 2
LITERATURE REVIEW

2.1 Starch

Starch is vital functional food biopolymer which contributes to the distinctive properties of food products. It is an important food ingredient and forms major constituent of the human diet. Starch exists in plants as a major carbohydrate storage product by depositing itself into discrete granules in the amyloplasts of plant storage organs. It is generally distributed in seed, roots, and tubers as well as in stems, leaves, fruits and even pollen (Kaur *et al*., 2004). As reported by Wang *et al*. (1998), most of the starch consumed worldwide derives from a relatively small number of crops, the most essential being corn, potato, wheat, and tapioca with smaller amounts from rice, sorghum, sweet potato, arrowroot, sago, and mung beans. According to Jane (2009), starch in the granular form, can be isolated easily by gravity sedimentation, centrifugation and filtration process. Starches from different origins differ in overall structure through shape, size distribution of the granules , amylose and lipid content, distribution of chain length in amylopectin and crystalline structure (crystallinity, crystal size, polymorphic type) (Ahmad *et al*., 1999).

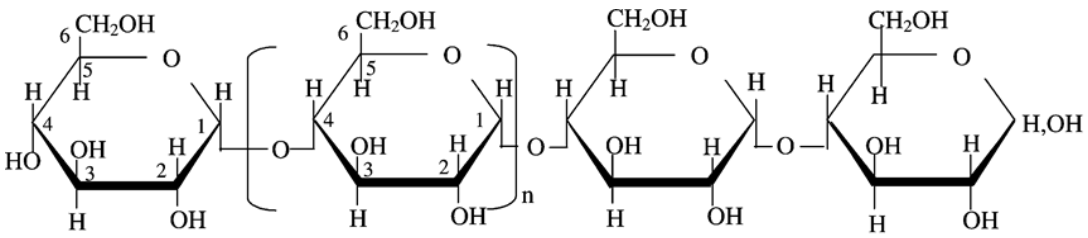
2.1.1 Starch Component

Starch comprises of two polysaccharides, namely, amylose and amylopectin. All starches are made up of these two polysaccharides. The proportion differs with the starch source but most starches comprise 20-30% amylose and 70-80% amylopectin (Ahmad *et al*., 1999; Chung & Liu, 2009). It has been reported that the _normal‘ starches contain 20–35% amylose, _waxy‘ starches less than 15%, while _high‘ (amylo-) amylose starches greater than about 40% (Tester *et al*., 2004). The

contents and the structures of these two polysaccharides play major roles in the functional characteristics of starch.

(a) Amylose

Amylose (Figure 2.1) is a linear molecule consisting of (1→4)-linked α-D glucopyranose unit. It has a molecular weight range of nearly 10^5−10^6 Da, comparable to a degree of polymerization (DP) of 1000−10,000 glucose unit (Copeland *et al*., 2009). The amylose content of starch increases with the maturation of the seeds and the size of starch granule (Morrison & Gadan, 1987). Generally, less than half the amylose will be branched and the amount of branch points will be less than 20 per molecule (Hizukuri *et al*., 1997). The number of branches of amylose molecules depends on the molecular size and botanical source of the starch (Hizukuri *et al*., 1981). Dissolved amylose has an affinity to form insoluble semi-crystalline aggregates due to low degree of branching, depending on the location of the branches in the structure (Copeland *et al*., 2009) and it was reported to has a high affinity to retrograde and produce hard gels and strong films (Jane, 2009; Pérez & Bertoft, 2010).



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. (Adapted from Tester & Karkalas (2002))

(b) Amylopectin

Amylopectin (Figure 2.2) is an extremely branched molecule containing of short chains of (1→4)-linked α-D-glucose with (1→6)-α-linked branches. It is a considerable larger polymer, with a higher molecular weight than amylose in approximately 10^8 and a DP that may go beyond one million (Copeland *et al*., 2009). Amylopectin comprises about 5% of branch points, which impart intense variances in physical and biological characteristics as compared to amylose (Pérez & Bertoft, 2010). Yoshimoto *et al*. (2000) reported that amylopectin molecules from high amylose starches have comparatively high amounts of very long chains. According to Moharram *et al*. (1998), amylopectin is the non-gelling portion of the starch which generally contributes a stringy consistency to food products due to its solubility. When dispersed in water, amylopectin is more stable and yields soft gels and weak films (Jane, 2009; Pérez & Bertoft, 2010).

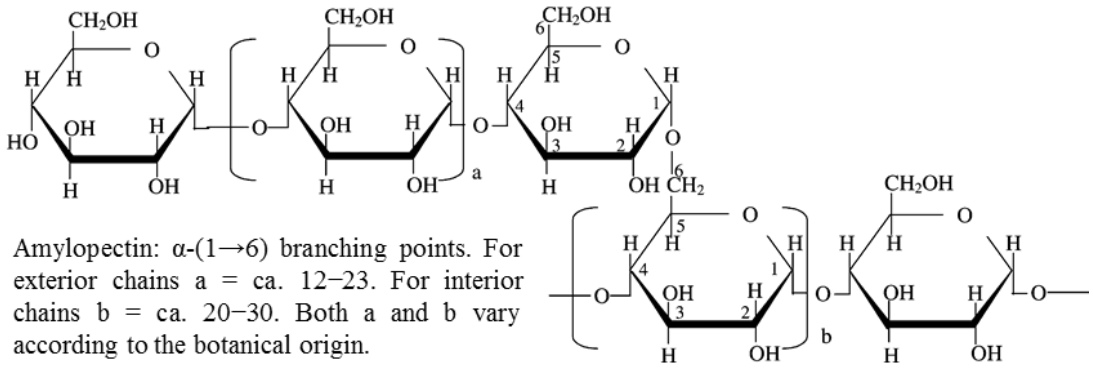


Figure 2.2 Structure of amylopectin. (Adapted from Tester & Karkalas (2002))

Some of the physicochemical properties of amylose and amylopectin are summarized in Table 2.1.

Table 2.1 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
Dilute aqueous solutions*	Unstable	Stable
Gels	Firm, irreversible	Soft, reversible
Films	Coherent	Not readily formed
Iodine colour	Blue	Reddish−purple
Digestibility by β−amylase	100%	Approx. 60%

*Amylose is, however, soluble in 0.5 mol/L KOH, DMSO and formamide.
(Adapted from Cornell (2004))

2.1.2 Starch Granule Structure

Starch granules from different botanical sources display different characteristic shapes, sizes and granular morphology (Jane *et al*., 1994) (Table 2.2). Starch granules have several shapes, including oval, spherical, polygonal, disk, kidney, and elongated shape and in size from < 1 μm in diameter (Jane, 2009).

Table 2.2 Starch granule characteristics

Starch	Type	Diameter microns (μm)	Morphology	Amylose content
Maize[a]	Cereal	5−30	Round polygonal	25
Waxy maize	Cereal	5−30	Round polygonal	<1
Tapioca	Root	4−35	Oval truncated ‗kettle drum'	17
Potato	Tuber	5−100	Oval spherical	20
Wheat	Cereal	1−45	Round lenticular	25
Rice	Cereal	3−8	Polygonal spherical compound granules	19
Sago	Pith	15−65	Oval truncated	26
High amylose maize	Cereal	5−30	Polygonal irregular elongated	50−90

[a] Maize is also often referred to as ‗corn', ‗dent corn' or ‗regular maize'.
(Adapted from Murphy (2000); Taggart & Mitchell (2009))

There have been several techniques used to identify starch granule structure. Transmission electron microscopy (TEM) has been used to study ultrastructural starch granules while scanning electron microscopy (SEM) is a widely used method to study starch granule morphology and also granule ultrastructure after fracture or partial hydrolysis (Gallant *et al*., 1997)). Wide−angle X−ray diffraction (WAXD) was used to study the packing within the crystals of the granule, allowing a complete analysis of the unlike polymorphs of amylose (Figure 2.3) (Imberty & Perez, 1988).

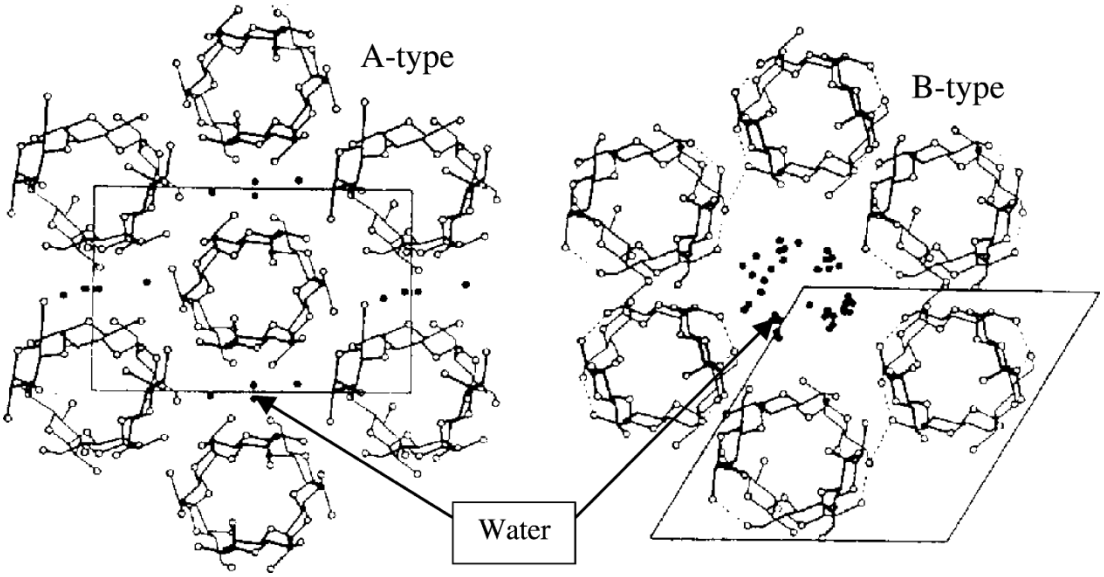


Figure 2.3 A- and B-type polymorphs of amylose. (Adapted from Wu & Sarko (1978))

There have been four different X−ray diffraction patterns reported. Cereal starches generate A−pattern, root, tubular, high amylose and retrograded starches, B−pattern, beans, peas, and some fruit and stem starches, C−pattern, and gelatinized lipid−containing starches generate V−pattern polymorph. (Eliasson & Gudmundsson, 2006; Sevenou *et al*., 2002). The A−, B−, and C−patterns are different polymorphic forms of starch that differ in the packing of the amylopectin double helices (Eliasson & Gudmundsson, 2006) and C−pattern represent a combination of A− and B−pattern polymorphs (Sevenou *et al*., 2002; Tester *et al.*, 2004).

Starch accumulates as a complex granular structure (Figure 2.4) by having a semi-crystalline structure, consist of crystalline and amorphous regions (Chung & Liu, 2009). Amylopectin branches are believed to be densely packed in the crystalline regions while amorphous regions of starch granule are considered to be present mainly by amylose (Gidley & Bulpin, 1989 cited in Chung & Liu, 2009).

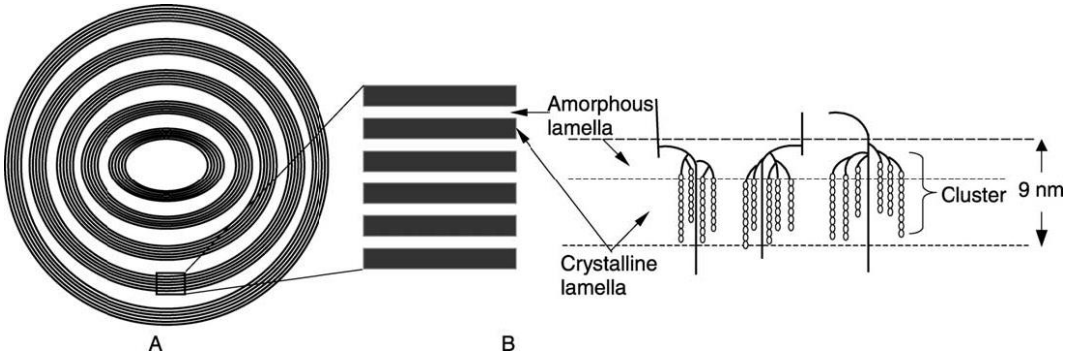


Figure 2.4 Diagrammatic representation of the lamellar structure of a starch granule according to (Adapted from Donald *et al*. (1997)). (A) Stacks of microcrystalline lamellae separated by amorphous growth rings. (B) Magnified view of the amorphous and crystalline regions.

Structure of amylopectin as shown in Figure 2.5 indicating the branching pattern of unit (1→4) −*α*−chains (A, B_1–B_3) joined together by (1→6) −*α*−linkages. The A-chains of amylopectin are (1→6)-*α*-linked by B-chains which in turn can be linked to other B-chains or the single reducing end carrier, C-chain (Hizukuri, 1986).

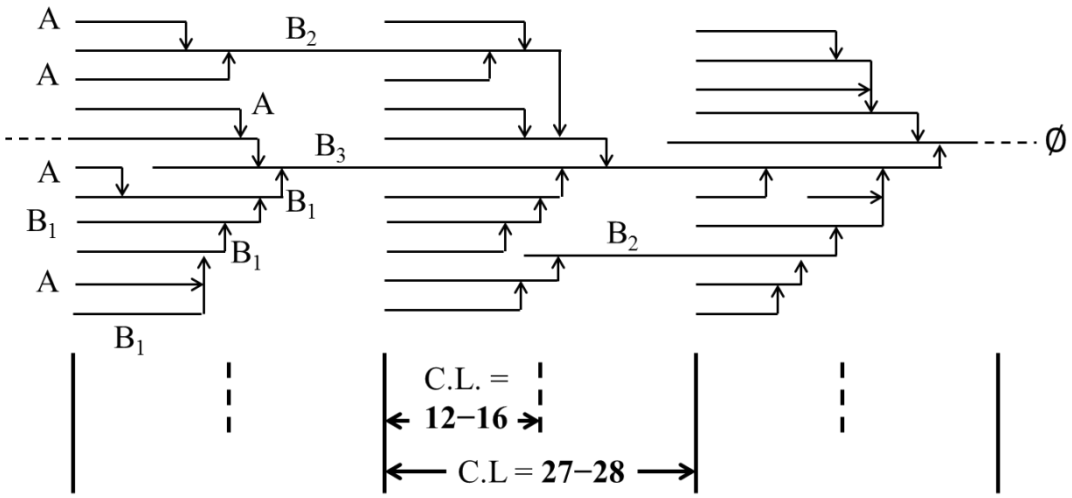


Figure 2.5 Schematic representation of a section of amylopectin indicating the branching pattern of unit (1→4) −*α*−chains (A, B_1–B_3) joined together by (1→6) −*α*−linkages (branch points). The chain carrying the reducing end (Ø) is the C chain. (Adapted from Hizukuri (1986))

Relationship between average chain length of the starches and the crystalline starch‘s X−ray diffraction pattern for a variety of starches has been studied by Hizukuri (1985) and Jane (2009). Generally, amylopectin molecules of A−pattern

starches have both long and short chains that are smaller (short average chain length) than those of the B−pattern starch while C−pattern starch consist of mixture of A− and B−type branch patterns and have both very long and very short chains with the molar ratios of short and long branch−chains of 8−12 for A−type starches, 3−7 for B−type starches, and 7−9 for C−type starches (Hizukuri, 1985; Jane, 2009). Jane *et al*. (1997) reported that A−pattern starches having considerably more individually branched molecules than those B−pattern starches and had branch points distributed in both amorphous and crystalline regions. In contrast, B−pattern starch had most branch points gathered in the amorphous region, making them more prone to the modification reagent.

2.1.3 Type Of Starch

(a) Corn

Corn (*Zea mays L*) is the commonly grown grain crop and stable food in the Americas with ~316 million metric tons grown yearly in the United States alone (Food and Agriculture Organization of the United Nations, 2010). Corn starch is a valuable ingredient to the food industry, being extensively used as a gelling agent, water retention agent, thickener, and bulking agent (Singh *et al*., 2003). Corn starch can be found from the endosperm of the corn kernel by a process called wet milling. Dry and unmodified corn starch is a powder with white to pale yellow in color. Corn can be separated into normal, waxy and high amylose based on the different amylose and amylopectin ratio (Sandhu & Singh, 2007). Native corn starch granules were less smooth and smaller in size (5–18 μm), with rounded and angular shaped (Singh & Singh, 2003). Murphy (2000) reported corn starch contained ~25% amylose.

According to Morrison (1995), corn composed of 0.8% lipid and 0.4% protein. According to Jane *et al.* (1999), corn starch has an A-type X-ray diffraction pattern.

(b) Sago

Sago starch is possibly the only one of a commercial starch obtained from the stem of palm (sago palm). Sago palm (*Metroxylon spp*.) contains a huge quantity of starch in its trunk (Nor Nadiha *et al*., 2010). It is predictable that about 60 million tons of sago starches, extracted from sago palms, are produced per annum in Southeast Asia (Wang *et al*., 1996). Sago starch has been used in the cooking of several types of dishes such as soups, jellies, puddings, biscuit, noodle, sago pearls, and many more (Karim *et al*., 2008). Sago starches are well-established in Malaysia especially in the State of Sarawak (Leong *et al.*, 2007). Sago starch consists of oval granules with diameters in the range of 20–40 μm. The apparent amylose content (starch with lipid) was in the range of 24% to 30%, while the total amylose contents (lipid free starch) in sago starches was slightly higher in the range of 24% to 31%, the moisture contents of the sago starch ranged between 10.6% and 20.0%, the quantity of crude fat ranged from 0.10% to 0.13% and the crude protein content was between 0.13% and 0.25% (Ahmad *et al.,* 1999). The starch was found to have a C-type X-ray diffraction pattern consisting of A-type to B-type crystalline forms ratio of 65:35 (Ahmad & Williams, 1998).

(c) Tapioca

Tapioca (*Manihot esculenta Crantz*) starch is derived from the roots of the cassava plant, which is found in equatorial regions between the Tropic of Cancer and the Tropic of Capricorn (Breuninger *et al*., 2009). In many tropical countries, tapioca

is an economically important crop as a source of starch. According to Food and Agriculture Organization of the United Nations (2010), the present global tapioca production is around 200 million tons, of which Africa accounts for half (51%), while Asia and Latin America produce 34% and 15% respectively. Tapioca is high yielding and is well adapted to poor soils, drought, and high temperature (Liu *et al*., 2011). Tapioca starch is widely used in the food, feed and industrial sector owing to its low gelatinization temperatures, low retrogradation rate after cooking, high slurry viscosities, bland taste, neutral flavor, easy degradability, low cost and easy extractability (Han & Sosulski, 1998; Sajeev *et al*., 2003). The extraction of starch from fresh tapioca tubers includes several unit processes such as washing, peeling, washing of peeled tubers, rasping, screening, settling, purification, pulverization and drying (Sajeev *et al*., 2003). The maximum moisture content of native tapioca starch is 13% (Breuninger *et al*., 2009). Tapioca starch has low level of protein, fat, and lower amylose content than other starches. According to Swinkels (1985), tapioca starch consists of granules with diameters in the range of 4–35 μm, 17-20% amylose, 0.1% protein and 0.1% lipid. Tapioca starch was found to have an A-type X-ray diffraction pattern (Jane *et al*., 1999).

2.1.4 Physicochemical and Functional Properties of Starch

Starch characteristics depend mainly on physical and chemical properties such as granule size distribution, mean granule size, amylose/amylopectin ratio and mineral content (Madsen & Christensen, 1996). Physicochemical properties such as swelling power, amylose content, water binding capacity, and percent light transmittance were associated with the average granule size of the starches from different botanical origin (Singh & Singh, 2001; Zhou *et al*., 1998).

(a) Swelling Power and Solubility

Hydrogen bonds stabilizing the structure of the double helices in crystallites are broken when starch is heated in additional water. Broken hydrogen bonds are replaced by hydrogen bonds with water (with the unprotected hydroxyl groups of amylose and amylopectin). This phenomenon causes an increase in granule swelling and solubility (Hoover, 2001; Tester & Karkalas, 1996). Swelling power and solubility provide indication of the extent of interaction between starch chains within the amorphous and crystalline domains. Degree of swelling and solubility of starch granules is affected by the amylose/amylopectin ratio, molecular weight distribution, conformation, and degree and length of branching (Hoover, 2001) and amylose-lipid complexes (Swinkels, 1985). According to Tester and Morrison (1990), swelling power is contributed by the content of amylopectin, while solubility is contributed by the content of amylose. Singh *et al*. (2003) found that the swelling power and solubility of starches from different botanical origins differ significantly.

(b) Pasting Properties

The pasting behavior of starch pastes is influenced by the physical and chemical properties of the starch such as the amount and type of starch, the presence of lipids, protein, and low molecular weight solutes, which was associated to genotype and was affected by environmental factors during crop growth (Dang & Copeland, 2004). The Rapid Visco^{TM} Analyzer (RVA) is a popular instrument for studying the pasting properties of cooked starch and flour in terms of changes in viscosity during a programmed heating-cooking-cooling cycle (Blazek & Copeland, 2008; Mohan & Malleshi, 2006). Pasting properties in the RVA are measured with the application of heat and shear to interrupt the granules (Blazek & Copeland, 2008).

It involves starch-swelling, gelatinizaton, paste formation, total disruption of the gelatinized granules, and finally retrogradation of amylose during cooling. Parameters that can be measured from the pasting curve are (i) peak viscosity (the maximum apparent viscosity achieved during pasting); (ii) peak viscosity temperature; (iii) the paste stability or resistance to breakdown (it illustrates the stability of paste during cooking); (iv) setback (indicated by the apparent viscosity of the paste after cooling), and (v) stability of the cooked paste (indicated by the apparent viscosity after stirring at a certain temperature for a certain periods of time) (Karim *et al*., 2000; Sandhu & Singh, 2007). Understanding of pasting properties is often an essential indicator for the measurement of quality and acceptability of foods and their constituents in industrial application, it can help a processor in optimizing ingredient concentrations and temperature-pressure-shear limits to accomplish desired product (Dang & Copeland, 2004).

(c) Gelatinization

Starch gelatinization can be defined as the changes of the ordered-structure to inordered-structure in starch granules due to the loss of crystallinity and swelling of the granules which takes place during heating in excess water (Donovan, 1977; Hermansson & Svegmark, 1996). This process involves a heat absorption (to weaken the hydrogen bond held between molecules in starch granules), hydration of starch (hydrogen bond formed between water and starch granules molecules), loss of crystallinity (due to dissociation of amylopectin double helices within the crystallites regions), loss of the anisotropic order (birefringence) due to swelling of starch granules, and disruption of granular structure if shear is involved (Donovan, 1979; Hermansson & Svegmark, 1996). According to Randzio & Orlowska (2005), starch

gelatinization is a process involving the hydration of an amorphous region and subsequent melting of crystalline arrays. Starch granules gelatinize in water when the temperature is raised to 60–70°C (Singh *et al*., 2003) and different temperature ranges were reported for different starches**.** According to Donovan (1979), the granule envelopes are formed during gelatinization and they could degrade into amylopectin-rich ghost remnants after leaching all the starch content (linear amylose) from swollen granules (Atkin *et al*., 1998) while amylose leaching out from swollen granules during and after gelatinization makes up the continuous gel phase outside the granules (Hermansson & Svegmark, 1996).

There are several techniques have been used to study starch gelatinization process include differential scanning calorimetry (DSC), thermo-mechanical analysis (TMA), X-ray scattering, light scattering, optical microscopy (light and electron microscopy), and nuclear magnetic resonance spectroscopy (NMR) (Jenkins & Donald, 1998; Perry & Donald, 2002; Tananuwong & Reid, 2004).

DSC has been commonly used to study the thermal behavior of starch including gelatinization (Derycke *et al*., 2005; Gonera & Cornillon, 2002; Liu *et al*., 2009; Tran *et al*., 2007) and retrogradation (Liu *et al*., 2010; Tran *et al*., 2007). Data that can be obtained from DSC thermogram are (i) T_o which represents the onset temperature at which starch start to swell; (ii) T_p which denotes peak temperature of the starch granules; (iii) T_c represents conclusion temperature which shows the completion of gelatinization process, and (iv) ΔH represents the exothermic/endothermic energy during the measurement.

(d) Retrogradation

Starch retrogradation has been defined as the changes that take place in gelatinized starch, from an amorphous state to a more organized or crystalline state (Eliasson & Gudmundsson, 2006), causing effects such as gelation, precipitation, and changes in opacity and consistency (Hermansson & Svegmark, 1996). There are several factors that affect the rate of retrogradation including the starch concentration, structure of the amylose and amylopectin molecules, molecular ratio of amylose to amylopectin, presence and concentration of other ingredients, and botanical origin of the starch (Jacobson *et al*., 1997). According to Jane and Robyt (1984), during retrogradation, amylopectin crystallization happens by reassociation of the outermost short branches (DP = 15) while amylose forms double-helical associations of 40–70 glucose units (Ring *et al*., 1987). Jacobson *et al*. (1997) have reported that retrogradation of starch is a rearrangement process involving both amylose and amylopectin, with amylose retrograde faster than amylopectin.

Rearrangement process occurred during retrogradation causes syneresis or phase separation between polymer and solvent. This process causes a major influence on the texture of starch-based foods including increase in firmness or rigidity and staling of bread other baked products (Eliasson & Gudmundsson, 2006). However, certain starch-based products required retrogradation process to modify the structural, mechanical or organoleptic properties such as in the production of breakfast cereals and parboiled rice due to hardening and reduced stickiness resulted from retrogradation process (Karim *et al*., 2000).

Several techniques have been used to study retrogradation of starch including DSC, X-ray diffraction, rheological measurements, NMR, turbidimetric methods, compression method, and pasting properties. In pasting properties determination, setback values obtained from Rapid Visco Analyzer (RVA) are indicative of the retrogradation tendency of starch and are related to the retrogradation tendency of amylose (Karim *et al*., 2000). Thermal properties and uniaxial compression tests were used to study the retrogradation of aging starch gel. In thermal study by using DSC, ΔH gives a quantitative measure of the energy transformation that happens through the melting of recrystallized amylopectin together with data of the transition temperatures of this endothermic occurrence (Karim *et al*., 2000). Meanwhile, uniaxial compression test was carried out to study gel deformation of aging starch gel and relatively firm gels are required.

(e) Intrinsic viscosity

Intrinsic viscosity [η] is basically a measure of the internal resistance or friction or hydrodynamic volume of high polymeric molecules in solution. It is a properties of macromolecules which is directly associated to their ability to interrupt flow and indirectly to the shape and size of the molecules (Islam *et al*., 2001; Tanglertpaibul & Rao, 1987). There are several factors that affected [η] of a solution including concentration of solution, temperature of medium used, molecular weight, structure or chain rigidity (Islam *et al*., 2001; Kar & Arslan, 1999; Nayak & Singh, 2001).

[η] is determined by graphic extrapolation from a linear plot of the Huggin's equation, which is applicable for very dilute solutions (when concentration tends to zero),

$$\eta_{sp}/C = [\eta] + k[\eta]^2C$$

where η_{sp} = (η of solution/η of solvent), C = concentration in g/mL, and k is the Huggin's constant (Curvale *et al*., 2008). η_{sp}/C is the y-axis and C is the x-axis. The y-intercept = [η] and the slope = $k[\eta]^2$.

(f) Flow behavior

Flow behavior of a material is one of the rheological properties that resulted from mechanical properties in the presence of a stress. It is normally expressed in terms of stress, strain and time effects. The viscosity (*Pa.s*) of fluid is defined as the resistance of fluid to flow. There are two types of viscous fluids that have a tendency to deform continuously under the effect of an applied stress, namely Newtonian and non-Newtonian fluids. The viscosity in Newtonian fluids is constant and independent of shear rate. While for non-Newtonian fluids, its viscosity may increase (shear thickening or dilatant fluids) or decrease (shear thinning or Pseudoplastic fluids) with increasing shear rate. For plastic fluids (Bingham and non-Bingham fluids), yield stress which is defined as the lowest shear stress is required to begin the flow. Thixotropic fluids are fluids that exhibit shear thinning with time at a fixed shear rate (Sahin & Sumnu, 2006). Figure 2.6 shows the flow curves for Newtonian and non-Newtonian fluids.

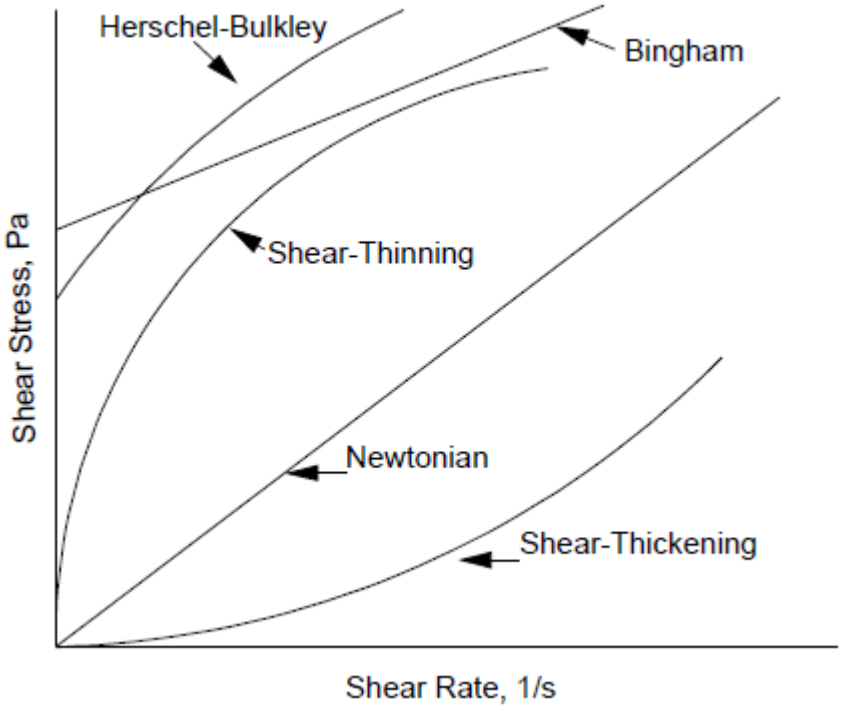


Figure 2.6 Typical flow curves for viscous and plastic fluids. (Adapted from Steffe (1996))

2.2 Modification of Starch

2.2.1 Introduction

Native starches have been used ever since long-ago as a raw material to formulate different products. For most application, the physicochemical properties of native starches are incompatible. Native starch has a low thermal decomposition, low shear stress resistance, high syneresis, and retrogradation. Starches modifications have been used to remove or reduce these unwanted characteristics. Starch may be modified chemically, enzymatically or physically to impart unique characteristics (Tester *et al*., 2004).

With aiming to improve properties of native starch, several studies have been carried out for chemical modification of native starch. According to Hebeish *et al*.

(1989), chemical modifications have been carried out to enhance the water holding capacity of starch dispersion at low temperature, to change the gelatinization and cooking properties of granular starch, to reduce the retrogradation and gelling tendencies of amylose containing starches, to impart hydrophobic properties, to increase hydrophilic character, and/or to introduce ionic substituents for use as thickening, binding adhesive, gelling, and film-forming charactiristics. Modification methods involved are hydroxypropylation, cross-linking, acetylation, and oxidation. Figure 2.7 shows chemical and biochemical modifications of starch.

Hydroxypropylation has been reported to enhance freeze-thaw stability, decrease gelatinization and pasting temperatures, and increase paste clarity (Pal *et al*., 2002). Hydroxypropyl starch derivatives are formed by introduction of hydroxypropyl groups onto the polymeric chain of starch by treating starch with the etherifying reagent 1, 2-epoxypropane (propylene oxide) (Lawal *et al*., 2008).

Cross-linking provides more stable pastes at high temperature and at low pH (Liu *et al*., 1999). Cross-linking is made by reacting granular starch with bifunctional or multifunctional reagents which have ablility to form ether or ester linkages with hydroxyl groups in starch. These bifunctional or multifunctional reagents include monosodium phosphate, sodium tripolyphosphate, sodium trimetaphosphate, phosphoryl chloride, epichlorohydrin, a mixture of succinic anhydride and vinyl acetate, and a mixture of adipic and acetic anhydrides (Gui-Jie *et al.*, 2006). Yeh & Yeh (1993) reported that dual-modification, hydroxypropylations and cross-linking can overcome higher retrogradation and syneresis in native starch. Wattanachant *et al*. (2003) found that starch that was hydropropylated with 10-12% propylene oxide

and crosslinked by a mixture of 2% sodium trimetaphosphate and 5% sodium tripolyphosphate produced modified starch with the most desirable characteristics by exhibiting high acid resistance, no viscosity breakdown, improved gel texture, and high freeze-thaw stability.

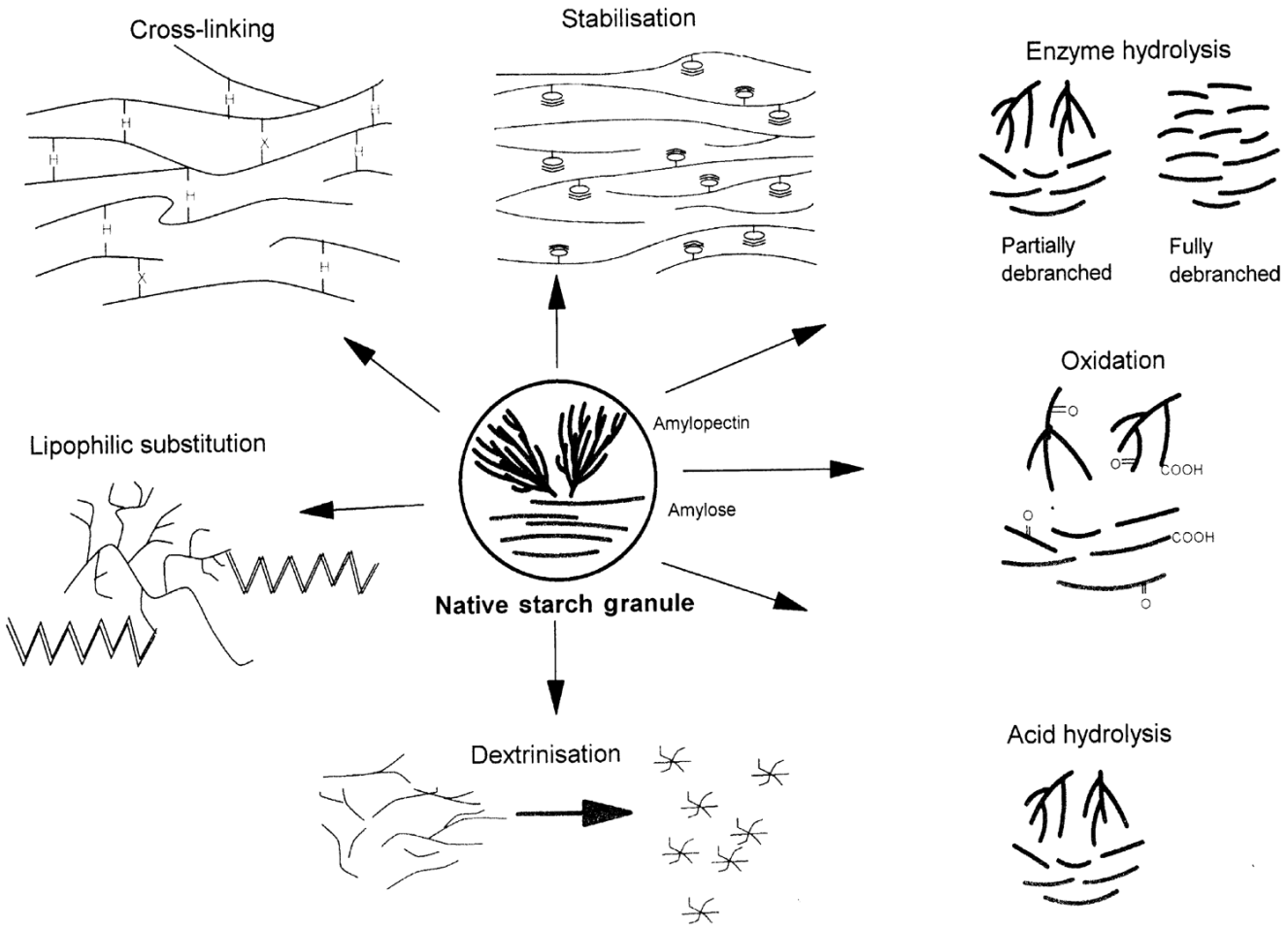


Figure 2.7 Chemical and biochemical modifications of starch. (Adapted from Taggart (2004))