PHYSICOCHEMICAL AND EMULSIFYING PROPERTIES OF PRE-TREATED OCTENYL SUCCINIC ANHYDRIDE (OSA) SAGO STARCH IN EMULSION

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by

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LIST OF SYMBOLS

α	Alpha
β	Beta
<	More than
>	Less than
°	Degree
%	Percentage
Ɵ	Angular displacement
G’	Storage modulus
G”	Loss modulus
ha	Hectare

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
a*	Redness
BD	Breakdown viscosity
b*	Yellowness
C	Celcius
DS	Degree of substitution
EN	Enzyme
FDA	Food and Drug Administration
HMT	Heat moisture treatment
L*	Lightness
mg/mL	Milligram per milliliter
N	Native
NSS	Native sago starch
OSA	Octenyl succinic anhydride
O/W	Oil in water
RVU	Rapid Visco Units
SB	Setback viscosity
SEM	Scanning electron microscope
W/O	Water in oil

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SIFAT-SIFAT FIZIKOKIMIA DAN KEBOLEHAN MENGEMULSI KANJI SAGU OKTENIL SUKSINAT ANHIDRAT (OSA) PRA-RAWAT EMULSI

ABSTRAK

Kanji sagu banyak terdapat di Malaysia dan ia berpotensi untuk dijadikan sebagai bahan pengemulsi. Walau bagaimanapun, kanji asli secara amnya bersifat hidrofilik dan terdapat keperluan untuk meningkatkan tahap kehidrofobikannya dengan membuat pengubahsuaian bagi meningkatkan keupayaannya untuk menstabilkan emulsi. Oleh itu, penyelidikan ini direka untuk meningkatkan tahap kehidrofobikannya serta keberkesanan kanji sagu sebagai bahan pengemulsi. Dalam kajian ini, kanji sagu telah dirawat terlebih dahulu dengan rawatan kelembapan haba (HMT) (pra-rawatan tunggal), enzim STARGEN (pra-rawatan tunggal), dan rawatan kelembapan haba diikuti dengan enzim STARGEN (dwi pra-rawatan) sebelum diesterifikasi dengan oktenil suksinat anhidrat (OSA). Keputusan menunjukkan bahawa kanji sagu OSA dengan rawatan enzim (EN OSA) memberikan nilai darjah pengantian (DS) tertinggi (0.0152), diikuti oleh OSA kanji sagu dengan rawatan kelembapan haba-enzim (HMT EN OSA) (0.0119) dan OSA kanji sagu dengan rawatan kelembapan haba (HMT OSA) (0.0105). Mikroskop imbasan elektron (SEM) menunjukkan bahawa semua OSA kanji sagu menghasilkan permukaan kasar dan kehilangan beberapa sisi definisi. Di antara semua sampel, saiz partikel EN OSA adalah paling kecil, di mana saiz partikel EN OSA teleh menurun dari 31 μm kepada 28 μm. Keupayaan untuk menstabilkan emulsi oleh semua kanji sagu OSA pra-rawat kemudian dikaji. Berbanding dengan kanji lain, EN OSA mempunyai aktiviti pengemulsi yang paling tinggi dan menghasilkan lapisan emulsi yang tebal selepas

emulsifikasi. Selepas penyimpanan dalam bilik suhu selama 56 hari (8 minggu), EN OSA menunjukkan kestabilan tertinggi dengan mempamerkan indeks emulsi tertinggi, iaitu antara 0.37 (kepekatan kanji 200mg / mL) sehingga 0.56 (kepekatan kanji 500 mg / mL). Mikroskopik cahaya EN OSA menunjukkan bahawa granul kanji didepositkan di antara permukaan air dengan minyak, meliputi titisan minyak, yang boleh menghalang titisan minyak daripada bergerak secara bebas yang akan membawa kepada ketidakstabilan emulsi. Sifar-sifat fizikal mayonis yang disediakan dengan penggantian separa oleh 6% EN OSA kanji sagu menunjukkan kestabilan emulsi tertinggi dan ia mempunyai partikel saiz lemak yang terkecil (11 μm). Mikrostruktur cahaya menunjukkan bahawa emulsi yang stabil dengan saiz titisan yang kecil adalah diperhatikan dalam sampel 3% dan 6% N-OSA dan EN OSA. Tingkahlaku viscoelastic menunjukkan bahawa semua sampel penggantian kuning telur mempamerkan tingkah laku seperti pepejal. Kajian ini menunjukkan bahawa pra-rawatan telah meningkatkan kecenderungan kanji sagu untuk OSA modifikasi. Kanji sagu OSA pra-rawat dalam bentuk granul telah menunjukkan sifar-sifat fizikokimia dan fungsi yang lebih baik dan EN OSA menunjukkan aktiviti emulsi tertinggi dan dapat menghasilkan emulsi yang stabil. Kuning telur dalam mayonis boleh digantikan sehingga 6% EN OSA kanji dan berupaya menunjukkan kestabilan emulsi yang sangat baik.

PHYSICOCHEMICAL AND EMULSIFYING PROPERTIES OF PRE-TREATED OCTENYL SUCCINIC ANHYDRIDE (OSA) SAGO STARCH IN EMULSION

ABSTRACT

Sago starch is abundant in Malaysia and it has the potential to be used as an emulsifier. However, native starch is generally hydrophilic and there is a need to increase its hydrophobicity by modification in order to increase its ability to stabilize emulsion. Therefore, this research was designed to increase the hydrophobicity as well as effectiveness of sago starch as an emulsifier. In this study, sago starch was pre-treated with heat moisture treatment (HMT) (single pre-treatment), STARGEN enzyme (single pre-treatment), and heat moisture treatment followed by STARGEN enzyme (dual pre-treatment) prior to octenyl succinic anhydride (OSA) modification. Enzyme-treated OSA (EN OSA) showed the highest degree of substitution (DS) (0.0152) followed by heat moisture-enzyme-treated OSA (HMT EN OSA) (0.0119) and heat moisture-treated OSA (HMT OSA) (0.0105). SEM micrograph revealed rough surface with their edges lose some definition for all OSA modified starches. Among all samples, EN OSA showed the smallest particle size, where the particle size of EN OSA had reduced from 31 μm to 28 μm. The ability of all pre-treated OSA sago starches to stabilize emulsion was then investigated. Compared to other starches, EN OSA had significantly highest emulsifying activity and produced a thick viscous emulsion layer after emulsification. After 56^{th} days (8^{th} week) of storage at room temperature, EN OSA showed the highest stability by exhibiting the highest emulsion index which is between 0.37 (starch concentration 200 mg / mL) to 0.56 (starch concentration 500 mg/mL). Light microscopy of EN OSA revealed that starch granules

deposited at the oil-water interface, covered the oil droplets, which could inhibit the oil droplets from moving freely that will lead to emulsion instability. The physical properties of mayonnaise prepared by partial substitution of 6% EN OSA sago starch showed the highest emulsion stability and it had the smallest fat particle size (11 μm). Light microstructure showed that stable emulsion with small droplet size was observed in samples with 3% and 6% of N-OSA and EN OSA. Viscoelastic behaviour showed that all egg yolk substituted samples exhibit solid-like behaviour. This study showed that pre-treatments had increased the susceptibility of sago starch to OSA modification. The pre-treated OSA sago starch in granule form had showed an improved physicochemical and functional properties and EN OSA showed the highest emulsifying activity and produced a stable emulsion. Egg yolk in mayonnaise can be substituted by up to 6% EN OSA starch and was able to showed an excellent emulsion stability.

CHAPTER 1

INTRODUCTION

1.1 Background

Emulsions are used in assorted sectors, such as in food processing companies, pharmaceuticals industry, petroleum processing, and petroleum refining. An emulsion is formed by the combination of two incompatible liquid phases where one phase is dispersed into another (Placentine, 2014). Generally, two main kinds of emulsions are commonly available, namely oil in water emulsion (O/W) and water in oil emulsion (W/O), and the process of emulsion formation is known as emulsification (Akbari & Nour, 2018). Foods such as milk, mayonnaise, sauces and ice cream contain emulsion droplets. The emulsion is not stable thermodynamically and will slowly break down over time. Emulsion instability is caused by aggregation of the dispersed phase accompanied by separation of the product (Khan *et al.*, 2011). Phase inversion, creaming and flocculation are examples of emulsion instability. Emulsifier is an important substance necessary to make an emulsion stable over a period of time (Madaan *et al.*, 2014).

In the emulsion field, the words surfactant and emulsifier are usually used interchangeably. Surfactants, also called surface-active agents, are an amphiphilic compound consists of both hydrophilic head groups and hydrophobic tails, adsorb at the interface between two phases, therefore lowering the interfacial tension between two liquids or the surface tension between a liquid and a solid. On the other hand, an emulsifier is a surfactant that coat droplets within an emulsion and prevent coalescence occur in order to stabilize emulsions (Villiers, 2008).

Conventional emulsions are stabilized by the addition of emulsifying agents such as amphiphilic polymers or surfactant molecules. However, the artificial surfactants, such as sorbitan esters, ethoxylated sorbitan esters, sucrose esters, and non-artificial surfactants, for instance egg yolk lecithin and numerous milk proteins, are expensive and yet some of them may cause an allergy-like reaction and carcinogenic (Tadros, 2013). Scientific studies have shown that the ingestion of surfactants in foods such as mono- and diglycerides citric acid esters of fatty acids may increase the absorption of many environmental toxins, including endocrine disruptors (Csáki, 2011; Lerner & Matthias, 2015). Besides that, some surfactants are potent carcinogens and may promote numerous diseases such as autoimmune diseases by affecting intestinal barrier function and altering intestinal microbiota (Csáki & Sebestyén, 2019).

Recently, starch have been studied to be used as an emulsifier (Chivero *et al.*, 2016; Kasprzak *et al.*, 2018; Zhao *et al.*, 2018). Timgren *et al.* (2013) reported that the emulsions which were stabilized using quinoa starch remained stable for more than two years. In addition, according to the findings by Zhao *et al.* (2017), oil-in-water emulsions could be stabilized using kudzu starch without the addition of commercial emulsifier. The benefits of using starch which is derived from natural resources include cheap, naturally non-toxic, and tasteless that enable its formulation application in food (Saari *et al.*, 2019). In contrast to conventional emulsifiers, being abundant, starch is a natural commodity that can be derived from different botanical sources (Tang *et al.*, 2015; Yang *et al.*, 2017). In cereal grains, starch is the major supply of stored energy. It is generated in plants as a reserve carbohydrate and comprise a significant supply of energy for humans worldwide. Diverse cereals, roots, rhizomes, along with tubers are the most essential supply of starch for humans.

Sago palm has a high production capacity of starch, between 20-25 tons/ha/year. Sarawak state in Malaysia is the main sago starch producer and every year, Malaysia export more than 40,000 tonnes of sago starch from sago palm to Peninsular Malaysia and to various countries (Amin *et al.*, 2019). Sago starch is substantially underused if compared with potato and maize starches. However, to the best of our knowledge, no research on the utilization of sago starch as an emulsifier has been carried out. Sago starch has the ability to be used as an emulsifier, but it needs to be modified due to its broad particle size range and hydrophilic character. Physical and chemical modifications may help to increase its hydrophobicity and thus increasing its ability to stabilize emulsions.

Sago starch can increase its hydrophobicity with chemical modification by employ octenyl succinic anhydride (OSA). The OSA addition must be limited to 3 % based on the starch dry weight if it is to be employed in food application as stated by Food and Drug Administration (FDA). With the introduction of dual-functional hydrophobic and hydrophilic groups, the substitution of the hydrophobic OSA group into the glucose unit of starch would shift the properties of the starch from naturally hydrophilic to amphiphilic, thereby obtaining surface active-properties (Jiang *et al.*, 2016). Pre-treatment of starch, for example heat moisture treatment (HMT), enzymatic pre-treatment, as well as dual pre-treatment before OSA modification will promote the speedy introduction of OSA inside the granule, thereby increasing the response enclosed by the OSA and hydroxyl groups (Jiranuntakul *et al.*, 2014; Lu *et al.*, 2016; Sun *et al.*, 2015). In the OS-starch granules introduced by OSA, the hydrophobic groups have the preference for the oil phase that could accumulate at the interface of the oil-water by forming a physical barrier that prevents the aggregation of the oil phase and subsequently affects the functional properties of the modified starch.

Physical alteration by heat moisture treatment (HMT) is a method which changes the starch physicochemical properties without rupturing the granular structure of starch. It is a process in which important criterion that must to be monitored are the ratio of starch to moisture, heating time and temperature. It is conducted at higher temperatures (90 - 120˚C) under a restricted moisture content (10 - 30 %) which is above the point of gelatinization. Due to structural changes in granules crystalline and amorphous areas, HMT is often worked as a pre-treatment in which HMT makes the granule sensitive to chemical and enzymatic changes (Alcázar-Alay & Meireles, 2015). It has been shown that heat moisture treatment reduces the relative crystallinity and/or improves the starch crystalline pattern (Zavareze *et al.,* 2010). This phenomenon was also due to the dissolution and amylopectin chains rearrangement during HMT. Therefore, the employment of HMT prior to OSA esterification may facilitate the rapid entry of OSA into the interior of the granule, strengthen the reaction between the OSA and hydroxyl groups, lead to an increase in degree of substitution (DS) and thus affect the functional properties of the modified starch (Jiranuntakul *et al.,* 2014).

Besides, enzymatic pre-treatments before OSA esterification might also boost the response between OSA and hydroxyl groups (Lu *et al.,* 2016; Huang *et al.* 2010). This is because, after enzymatic pre-treatments, the formation of pore in the granules of starch can increase the starch particles surface area, thus promote the penetration of the water-insoluble OSA reagent into the inner sections of the starch. The most common enzymes used for this function are α-amylase, β-amylase, isoamylase, glucoamylase, and pullulanase. Enzymes attack α -(1,4) or α -(1,6) bonds during hydrolysis process, depolymerizing starch into maltose, glucose, or oligosaccharides (Gonzalez Conde, 2017).

In food industries, modified starch can act as an excellent emulsifier or thickener. Due to the low cost and abundance in Malaysia, sago starch has great potential to be further altered. Previous research by Abiddin *et al.* (2016) was focused on the preparation and physicochemical properties of OSA modified sago starch. Another research by Naseri *et al.* (2019) was on the physicochemical and functional properties of the film prepare with OSA-modified sago starch. Azfaralariff *et al.* (2020) reported that Pickering emulsion with good stability could be obtained by using sago starch which modified with acid hydrolysis method to obtained round and oval shaped sago starch nanocrystals. From the literature, no research was performed to study the modification of sago starch to be used as food emulsifier. This study was planned to increase the hydrophobicity and efficacy of sago starch as an emulsifier. This research offers valuable information on the effects of pre-treatments on OSA sago starch modification and the enhancement of the physicochemical and functional properties of pre-treated OSA sago starch as a food product emulsifier.

1.2 Objectives

1. To study the effects of pre-treatments namely heat moisture treatment (single pre-treatment), enzymatic pre-treatment (single pre-treatment), and combination of heat moisture treatment and enzymatic pre-treatment (dual pre-treatment) on the susceptibility of sago starch to OSA modification.

2. To study the physicochemical and functional properties of pre-treated OSA sago starch in granule form and in emulsion system.

3. To study the effects of pre-treated OSA sago starch substitution on the physical properties of mayonnaise.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch

Over long periods, starch is generated for energy storage by green plants. It is synthesized into special organelles, plastids, in a granular shape as two main forms. During photosynthesis, a short term storage of starch is produced in chloroplast, while long-term storage is produced in amyloplasts. Starch granules are primarily found in seeds, tubers, roots, leaves, stems, fruits, and as well as pollen (Pfister & Zeeman, 2016). Depending on their botanical origin, starch granules are available in all shapes and sizes, from oval, circle, ogival, or elongated to flat, lenticular or polyhedral, ellipsoids, irregular tubules, polygons, platelets, spheres and have diameters varying from 0.1 to 200 microns (Pérez & Bertoft, 2010).

Starch is a soft, white amorphous powder that cannot dissolve in ether, water, alcohol, and is a non-reducing carbohydrate containing a combination of two glucans, amylose as well as amylopectin. Depending on starch sources, most starches contain 10 to 20 percent water-soluble amylose and 80 to 90 percent of water-insoluble amylopectin (Pokhrel, 2015).

The degree of polymerization, which is the length of the amylose molecules in starch, will vary accordingly. The way longer amylose molecules associate appeared to make the texture of a product more viscous. The amylose molecular weight also influences gel elasticity. Longer molecules tend to combine more strongly and create stronger, more brittle gels, but there is a limit to this effect. Starch composition varied with starch source. For instance, starches contain phosphorus in some form or another. The performance of starch has been impacted by the presence of phosphorus. Lipids

together with proteins are also present in very small quantities in granules of starch. Protein in the granules is mostly linked to starch biosynthesis. Certain proteins are just loosely bounded to the granules, while other proteins are tightly associated within the granules. In most of the tuber as well as root starches, lipids are uncommon. However, lipids are more widespread in cereals in the lysophospholipids forms or free fatty acids that make amylose-complexed inclusions (Bertoft, 2017). The composition of native starch granules from various botanical sources are presented in Table 2.1.

Table 2.1. Composition of native starch granules from various sources.

Species	Amylose (%w/w)	Lipid (%w/w)	Protein (%w/w)	Phosphorus (%w/w)
Barley[a]	19.0-22.1	0.7-1.2	0.2-0.4	0.02-0.06
Cassava[a]	23.6-23.8	0.2	0.3	0.01
Corn[a]	28.5	0.6-0.8	0.4	0.31-0.35
Potato[a]	29.1-29.5	0.1	0.1	0.60
Rice[a]	21.0-25.0	0.6-1.4	0.1	0.10
Sago[b]	32.2-33.98	0.38-0.46	0.73-0.77	0.01
Sorghum[a]	23.7-27.6	0.8	2.3	0.21
Wheat[a]	24.6-26.6	0.08-0.12	0.2-0.3	0.40

[a]: Alcázar-Alay & Meireles, 2015, [b]: Polnaya *et al.*, 2012.

2.1.1 Starch chemistry

Two main types of polymers can be found in starch molecules are amylose and amylopectin. These polymers can be identified by separation process followed by granule solubilization process (Pérez *et al.*, 2009). Starch chemical structures, for instance the amylose molecular sizes, the amylopectin branch-chain lengths, and the ratio of amylose and amylopectin may affect the starch functional properties. When amylopectin interacts efficiently with amylose, greater gel strength together as well as viscosity are produced. Moreover, the starch paste's viscosity and gel strength will also increase directly in proportion to the starch's amylose content. Amylose alone (amylose

that does not form inclusion complex with other molecules) and high amylose maize starch produce strong films (Jane, 1995).

2.1.1(a) Amylose

Figure 2.1 shows the α-1,4 linkages of amylose. Amylose is known as a substantially linear polysaccharide whereby anhydroglucose units are composed predominantly of α-D-1,4 linked glucan.

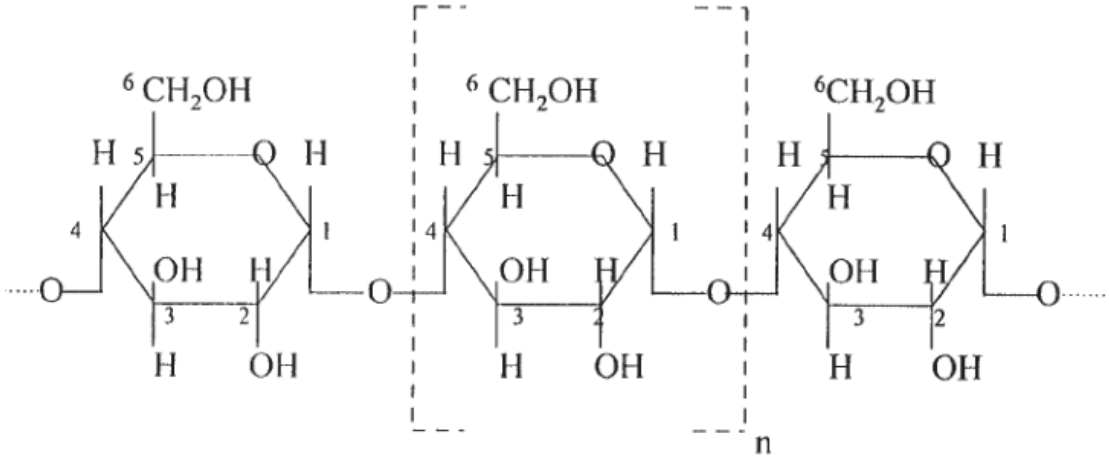

Figure 2.1: α-1,4 linkages of amylose.

Nevertheless, recent evidence has indicated that amylose polymer might have certain branches, but only fewer of α-1,6 linked branches (0.3 to 0.5%) may be present. Both branched and linear amylose may contain long chains with several hundreds or even thousands glycosyl unit. For instance, by depending on the types of starch, amylose molecules might contain around 200 to 6000 anhydroglucose units. In common native starch, amylose makes up around 15 to 30%. For example, potato and maize starch contains 21% and 28% of amylose respective (Tiefenbacher, 2019). The amylose content of sago starch varied between 24% and 31% due to harvesting sago at different growth stages (Karim *et al.*, 2008).

Botanical origin and starch maturity under study affects the degree of polymerization of amylose, which is the polymer molecular size that refers to the number of α -1,4-linked D-glucopyranose units in the starch chain, which is about 1,500-6,000 (Thomas & William, 1999). Starches typically have a very large molecular weight distribution of their amylose fraction, with average weight of amylose values much higher than the average number of amylose values. The molecular weight range of amylose is quite broad, which is between 8×10^4 to 10×10^5. Long side branches and high molecular weight of amylose makes the amylose performs and can be treated as an unbranched linear polymer. Natural helical structure of amylose molecules able to interact with iodine, that gives rise to blue colour stain, or with monoglyceride molecules such as organic alcohols and fatty acids. The formed complexes are usually referred to as helical inclusion complexes whereas if fatty acids are enclosed, the formed complex is known as an amylose-lipid complex. One of the easiest way to extract amylose is using hot water (Joye, 2018).

Single amylose molecules form helices capable to form inclusion complexes by reacting readily with a broad variety of different compounds, for example fatty acids, iodine, or various alcohols (Bertoft, 2017). The structure of the starch-iodine complex formed was single left-handed helix accommodates with polyiodide chain where there is a hydrophilic outer surface and a hydrophobic helical channel that accommodates the guest molecules as determined by X-ray and titration investigations, which contributes to the blue colour and the change of colour with the change in chain length of the amylose (Tan & Kong, 2019). However, the change of colour is largely depending on the chain length and the characterization of starch-iodine complex, and this can be tested out by Raman spectroscopy and UV Vis spectroscopy by determine the presence of starch (Du *et al.*, 2013).

Another widely known feature of amylose is that they are excellent film formers that result in a firm gel during cooked. This attribute is obvious in the behaviour of some starches that containing amylose. Sanyang *et al.* (2015) showed that the addition of plasticizing agent to sago starch film will produce a strong barrier property compared to other biodegradable starch based films, which also suggests that sago starch films could be an acceptable material for future food packaging applications. After cooking, the re-association of solubilized starch polymers would mainly lead to gel formation and can happen very quickly with the linear polymer amylose (Tiefenbacher, 2019).

2.1.1(b) Amylopectin

The chemical structure of a part of amylopectin is shown in Figure 2.2. Amylopectin composed of linear glucose unit chains bound by α -1,4 glycosidic bonds and is strongly branched with relatively short chains at the α-1,6 positions by small glucose chains at 10 nm intervals along the molecule axis. Amylopectin makes up between 70% to 85% for most starches (Alcάzar-Alay & Meireles, 2015).

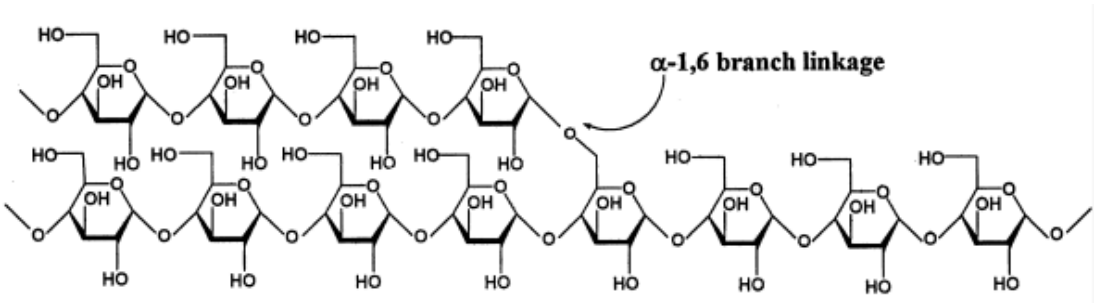

Figure 2.2: The structures of segment of amylopectin (Robyt, 2008).

The amylopectin branches can be divided into three categories according to their pattern of substitution: A-chains exist as unsubstituted, while B-chains can be substituted by other chains and could be even further divided into B2-chains, B3-chains or ultimately longer chains, whereas C-chain holds the single reduction end category of macromolecule, nevertheless it is otherwise identical to the B-chains (Bertoft, 2017).

Hizukuri (1986) used enzymes to debranch amylopectins, size-exclusion HPLC was then used to determine the branch size distribution, two separated main peaks will be possibly observed by using this method. Amylopectin chains are categorized into two main categories, namely short chain and long chain. The separation among the groups is normally at polymerization degree of 36. Short chains are the most noticeable and the molar ratio between starches varies for long chain and short chains, with cereals typically having the greatest ratios. Short chains that are associated in crystal formation in the granule of starch produce double helices and are secured to the long chains, which act as the interconnecting chains and are primarily confined to the amorphous lamellae (Bertoft, 2017).

The amylopectin molecules can be two dimensional and three dimensional. Three-dimension structure are forming with the formation of intermolecular hydrogen and hydrophobic bonds by stacking of the molecules in the water insoluble granule and also partially when in an aqueous solution (Robyt, 2008). Figure 2.3 shows a schematic drawing of amylopectin, its clustered branches and branched chains. The crystalline regions in the starch granules are majority composed of amylopectin molecules, whereas the amorphous areas are composed of the clustered branch of amylopectin.

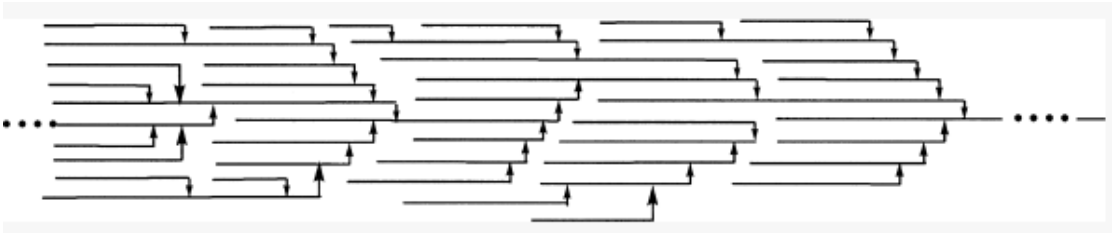

Figure 2.3. Schematic diagram on a segment of amylopectin, looking from the top down, showing the clustering of the α-1,6 branch linkages in a repeating sequence (Robyt, 2008).

Table 2.2 shows the characteristics of amylose and amylopectin. Amylopectin are highly branched molecules with a vast number of short branches and relatively large

molecular weight, ~10^{7-8} Da (Li *et al.*, 2017). Given the size and the structure of the molecules, retrogradation can be slowed and gel formation can either be delayed or aborted. Starches pastes that contain essentially all amylopectin are non-gelling, at the same time have a cohesive and gummy structure. A much larger amylopectin molecule has a degree of polymerization of about 300,000-3,000,000. The molecular weight of amylose and amylopectin fractions are varying for every specific plant source, at the same time affected by the method of polymer isolation and method of molecular weight determination.

Table 2.2: Characteristics of amylose and amylopectin.

Property	Amylose	Amylopectin
Shape[a]	Linear	Highly branched
Linkage[a]	α-1,4	α-1,4, α-1,6
Degree of polymerization[a]	1,500-6,000	300,000-3,000,000
Molecular weight[b]	~10^{5-6} Da	~10^{7-8} Da
Iodine colour[a]	Dark blue	Red
Gel formation[a]	Firm	Non-gelling to soft
Films[a]	Strong	Weak

[a]: Thomas & William (1999), [b]: Li *et al.* (2017).

2.1.2 Sources of starch

The typical commercial starches are usually derived from cereals (maize, wheat, rice and sorghum), tubers (potatoes and sweet potatoes), roots (cassava) and legumes (mung bean and green pea). Sago starch is another example of starch obtained from another source, the stem of sago palm similar with sugar palm starch that derived from sugar palm tree (*Arenga pinnata*) (Sahari *et al.*, 2014).

Starches from different origins usually have different functional properties, such as difference in terms of gelatinization onset temperature, final paste viscosity, and formation of two phase pastes or paste stickiness and thus vary in their end usage

(Pfister & Zemean, 2016). Thus, different sources of starch have their own beneficial properties and will meet various industrial demands. For example, sago starch is good in terms of elasticity, softness, flexible to use and less adhesive. Tapioca starch has a very cohesive texture despite of its better cold stability. Wheat starch has been used in breadfruit cookies formulation and it was also found to have gelling properties to food products. Potato starch was found to possess a good pasting and gelling agent and was able to produced potato starch film. This application of starch is not only applicable to the food industry, but also applicable to the pharmaceutical industry (Yazid *et al.*, 2018).

2.1.2(a) Sago starch

Sago starch (*Metroxylon* spp.) are widely distributed throughout South East Asia. Sago palm is a significant resource particularly for people in rural areas as sago palm has different uses, specifically in the starch production, either as sago flour or sago pearl. Around 100 to 500 kg of flour can be produced by a mature sago palm. In South East Asia, it is predicted that around 60 million tonnes of sago starch are produced annually. Compared to other crops, sago can compete economically on output and cost, for instance the sago starch output is 2000 to 3000 kg/Ha Yr compared to 2000 kg/Ha Yr of cassava and 1000 kg/Ha Yr of maize (Ahmad *et al.*, 1999). The sago palm is hapaxanthic, that is, it flowers once and dies shortly thereafter (Singhal *et al.*, 2008). In Malaysia, sago industry is well established in Sarawak state. The production capacity of sago palm varies from 2.00 – 5.00 tons of dry starch / ha in the wild to 10.0 – 25.0 tons / ha in cultivated plants (Jong *et al.*, 2018; Amin *et al.*, 2019).

Traditional process still plays a vital role in the production of starch, even though modern factories utilize a completely mechanical process (Ahmad *et al.*, 1999). In the modern factory, sago processing begins by feeding a part of sago log into a slicer

in order to separate pith and bark. To produce finer pieces, the debarked section is fed into a mechanical rasper, which is then fed via conveyor belt into the hammer mill. The resulting starch slurry is passed through a series of centrifugal sieves, which containing coarse fibre. In order to obtain very pure starch, further purification is achieved by the separation process with sieve bands in a nozzle separator and a series of cyclone separators. Starch dewatering is conducted using a rotary vacuum drum dryer followed by a hot air drying process (Kamal *et al.*, 2016). Figure 2.4 shows the production of sago starch through traditional process.

(a) (b)

(c) (d)

(e) (f)

Figure 2.4: Traditional sago starch production (a) Ready harvested sago trunk was felled down. (b) The trunk is then cut into logs. (c) Debarking and splitting the log (d) Pieces of sago log called batten was ready to be rasped. (e) Sago log pieces is being rasping. (f) Rasped sago log / sago pith called repas was then processed further to get the starch. (Darma *et al.*, 2017)

The growth stages of sago palm are characterized into five stages. Plawei is the first growth stage where the palm was at maximum vegetative growth. The second stage is the Plawei Manif, where the evolution of inflorescence occurs. The third stage is the Bubul, where the development of inflorescence takes place. Angau Muda and Angau Tua is where flowering happens, and fruiting ensues respectively. Plawei, Plawei Manif and Bubul stages show no significant differences in terms of starch yield while the Angau Muda stage show the greatest starch yield per trunk, which is 39 to 41% on dry weight basis (Lim *et al.*, 2019). Vegetative stage is the stage happens just before the flowering process, where the plant converts its stored nutrients into starch, which fills the trunk (Singhal *et al.*, 2008). Figure 2.5 shows the five developmental stages of sago palm.

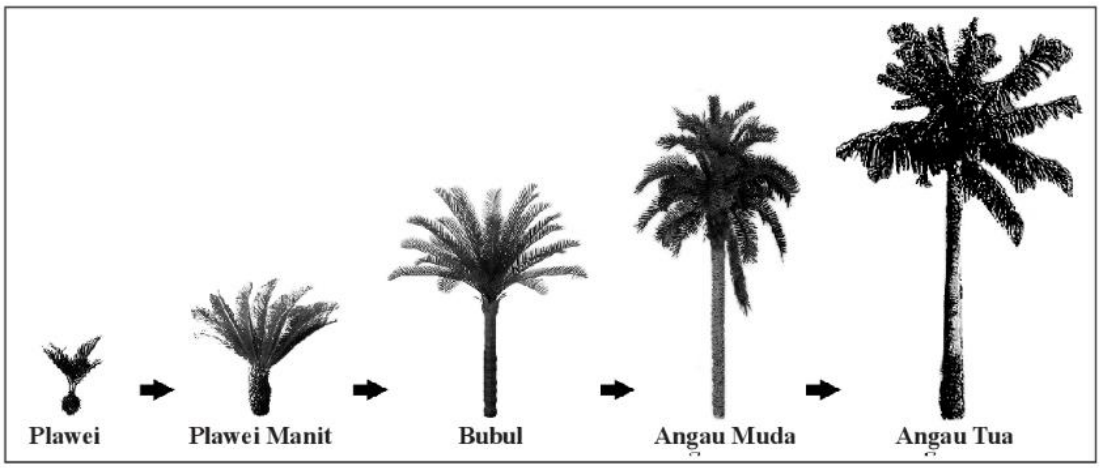

Figure 2.5: The five developmental stages of sago palm, from Plawei, Plawei Manit, Bubul, Angau Muda to Angau Tua (Lim et al., 2019).

Sago starch has been used in a broad variety of traditional food products as well as in other industrial applications in food products. Sago starch has the ability to be employed as a thickener in the soups and baby food production, and can be used as an additive in various food products (Chulavatnatol, 2002). In non-food industries, sago starch can be used in the making of biodegradable plastic, in the production of ethanol, monosodium glutamate, cyclodextrin and in the lactate industry (Karim *et al.*, 2008).

According to a study by Ahmad *et al.* (1999), sago starch as determined with scanning electron microscope has the mean diameter of about 30 μm with oval shapes. It exhibits a Maltese cross, signifying the existence of some common internal ordering. The moisture content affects the degree of a crystallinity within the granule, granule size and the amylose to amylopectin ratio. As determined by using differential scanning calorimeter, the gelatinization temperature and enthalpy of sago starch are 69.1-70.1˚C and 15.1-16.1 J g^{-1} (Okazaki, 2018). X-ray diffraction studies showed that a C-type diffraction pattern, which is a mixture of 65 percent A-type and 35 percent B-types crystalline forms, was exhibited by all the sago starch. Proximate composition analysis found that the sago starch has the moisture content ranged from 10.6 - 20.0%, amylose content between 24 - 31%, ash between 0.06 - 0.43%, crude fat between 0.10 - 0.13%, fiber between 0.26 -0.32% and crude protein varied between 0.19 - 0.25% (Ahmad *et al.*, 1999).

2.1.3 Granular structure

The crystallinity of granules from native starch differs from 15 to 45 percent, so majority of the native starch granules when discovered using a polarized light microscope reveal a Maltese cross. The Maltese cross is described as on a dim background, the birefringent native starch granules revealed as a cross radial light after exposure to polarized light (Cornejo-Ramirez *et al.*, 2018). Starch crystallinity degree shown that most polymers in the granules of starch are in amorphous situation. Native starch granules, however, produce x-ray diffraction patterns that can be used to classify the various allomorphs although they are typical of low quality (Pérez *et al.*, 2009).

2.1.3(a) Amorphous structure of starch granules

Semi-crystalline material in starch consisted of two main zones: crystalline and amorphous regions (Song *et al.*, 2017). The amorphous regions are consisted by non-ordered amylopectin branches and amylose (Kelly *et al.*, 2009). The least dense properties of amorphous region in starch granule make it more sensitive toward enzymatic hydrolysis and has good water absorption at the temperatures which is below the gelatinization temperature.

2.1.3(b) Characteristics of starch crystallites

In starch granules, the double helices of the glucan chains can differentiate in two various forms, A- and B-type polymorph. The A-type and B-type polymorph consists of a monoclinic and hexagonal unit cell, respectively (Figure 2.6) (Ai, 2013).

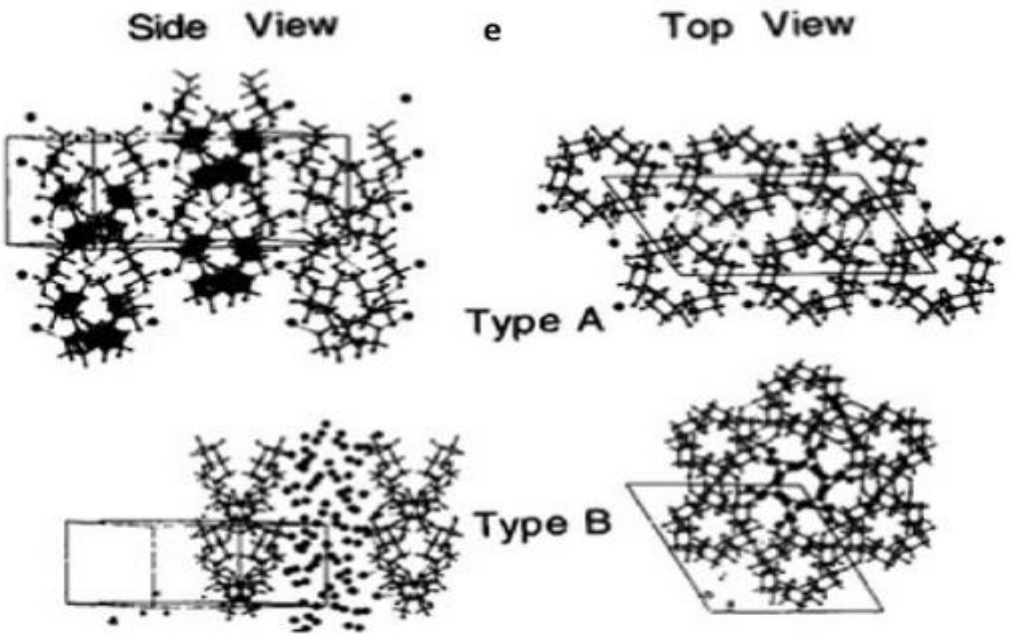

Figure 2.6: Crystal structures of the starch polymers, a and b crystalline polymorphism

The A- and B-type crystalline structures has a parallel double helix in a hexagonal configuration, in which twelve glucose molecules are the primary crystalline elements. Cereal starch have an A-type crystallite structure and it is a dense packed contains four water molecules. Tuber and high amylose content starches have a B-type crystallite structure where it contains 36 water molecules. Roots, legumes, and some fruits and starches have a C-type structure in which the C-type structure is a mixture of the A- and B-type structures (Cornejo-Ramírez *et al.*, 2018). The C-type starches can be further subdivided into C-type, C_A-type, and C_B-type, where C_A-type predominantly A-type polymorph in granules, closer to A-type XRD pattern and C_B-type predominantly B-type polymorph in granules and closer to B-type XRD pattern (Guo *et al.*, 2017). Singular helices and amylose complexed by other compounds have the V-type structure where it is constituted of starches with amylose-lipid complex (Cornejo-Ramírez *et al.*, 2018).

Recently, Cai *et al.* (2014) studied the X-ray diffraction pattern of sago starch and classify sago starch as C_A-type structures because it showing peaks at around 5.6, 17, 18 and 23˚, which corresponds to 1.58, 0.521, 0.492 and 0.386 nm. Thus, sago starch has more A-type structure characteristics than B-type crystallite structure. A weak diffraction peak at $2\theta=5.67^\circ$ and broad peaks at $2\theta=15.30^\circ$, 17.12˚, 18.08˚ and 23.46˚ was researched by Polnaya *et al.* (2013) for native sago starch in Indonesia, which once again indicated that sago starch exhibits C-type structure. Besides that, sago starch examined from 14 local varieties of sago palm in Vanuatu was of the C or C_A-type also studied by Srichuwong *et al.* (2005). Generally, sago starch is classified as C type crystallite structure which containing a mixture of A and B types of starch (Okazaki, 2018).

Starch crystallinity is affected by amylose content whereby insufficient of amylose will increases the crystallinity degree. By intertwining amylopectin outer chains, he formation of double helices has a crystalline structure and give rise to semi crystalline properties. Besides that, amylopectin with longer chains appears to form a more stable crystalline structure. This is because short chain amylopectin molecules are unable to produce stable double helix structures, and are thus easier to denature at lower temperatures by heat. On the other hand, for medium and long chained amylopectin, a relatively stronger crystalline network can be formed. High molecular weight of amylopectin in waxy starch increases the degree of crystallinity, such as in the case of waxy wheat in comparison to normal wheat. Diversity of starch granules has its own unique crystallinity. A-type starch granules contain a higher percentage of amylose than those of B-type granules, which produces lower percent of crystallinity in the A-type starch granules (Ao & Jane, 2007). Furthermore, crystallinity degree also affected by industrial manufacturing processes as structure of starch may also be denatured physically. The milling process, for example, causes the starch granules to experience physical damage. In the denature stage, the crystalline amylopectin breakdown to amorphous amylopectin and produce some low molecular weight molecules. As an example, the A-type crystalline structure in wheat starch was the major with a substantial degree of B-type crystallites in the large granules. Softer wheat observed to contain large quantities of amylose-lipid complex due to the extension of the amylose complexed with lipids or crystalline/amorphous complex (Cornejo-Ramirez *et al.*, 2018).

2.2 Starch modifications

Native starches are limited for industrial application due to their inherent imperfect properties, such as water insolubility and their high tendency to retrograde and undergo syneresis, therefore forming highly unstable gel and paste. Besides that, instability under various pH, temperatures, and shears conditions had also limit their applications in various industries. Additionally, most starch granules are chemical inactive due to lack of specific functional group, insoluble at ambient temperature, highly resistant to enzymatic hydrolysis (Eltaboni & Alabidi, 2017). In order to meet the demanding technological needs, the properties of starch are modified by scientist to revamp the structural characteristics and functional properties for specific targeted applications. The objective of starch modification is to decrease retrogradation, gelling tendencies of pastes and gel syneresis while improve clarity of paste and sheen, paste and gel texture, film formation and adhesion as well as to stabilize starch granules during processing. By increase the stability of starch granules, starch becoming more suitable for various food and non-food industrial applications thus enhance its versatility and consumer demand satisfaction (Chen *et al.*, 2018; Neelam *et al.*, 2012).

Starches and modified starches can be used to improve the foods physical properties. Modified starches are used in many processed foods due to their improved functional properties over those of the native starches. Starch are modified through physical process (heat moisture treatment), chemical process (succinylation) and enzymatic ways (STARGEN enzyme) (Karmakar *et al.*, 2014). Physical modification involves the use of heat, moisture and temperature which are relatively safe for edible food products. On the other hand, chemical modification involves the introduction of functional groups into the starch molecule, resulting in markedly altered physico-chemical properties (Korma *et al.*, 2016).

In food applications, starch can act as an emulsifier providing that modification of starch must be first carried out. When make use of starch as an emulsifier, starch is usually gelatinized. In addition, native starch is not hydrophobic, is typically failed to adsorb at the oil-water (O/W) interface and is therefore unable to stabilize an emulsion. Meanwhile, hydrophobicity can be increased by starch modification to achieve sufficient adsorption at the oil-water interface (Timgren *et al.*, 2013). Ye *et al.* (2017) had discovered that modification of sugary maize soluble starch accumulated at the oil/ water interface in the form of a barrier layer, thus a gel-like appearance and three-dimensional network was formed which have impressive long term stability.

2.2.1 Octenyl succinic anhydride modified of starch

Native starch can undergo chemical modification through treatment with various forms of alkenyl succinic anhydride, for instance, octenyl succinic anhydride (OSA) that is permitted for employments in foods with a maximum added quantity of 3% based on dry starch weight. The group of hydrophobic octenyl, sodium carboxylate or carboxyl enhanced the possibility of starches for stabilize emulsions (Timgren *et al.*, 2013). Generally native starch tends to form large aggregates at air/water interfaces and a two-dimensional film of particles could not possible to form with starch particles at an air/water interface. However, with the OSA modification, the aggregation of the starch particles can be reduced by introduce the amphiphilic functional group on the starch particle (McNamee *et al.*, 2018). Caldwell and Wurzburg was the first documented starch modification with OSA in 1953, which has been applied to multiple areas for more than a half-century (No *et al.*, 2019). Moreover, Matos *et al.* (2016) reported that OSA modified quinoa starch served as a good emulsifier and possessed an excellent stability in Pickering emulsion.

Starch esterified with OSA exhibited desirable emulsifying and stabilization properties as the starch hydroxyl groups are replaced by OSA in an aqueous dispersion. OSA starch is strongly amphiphilic and surface active. During homogenization, OSA starch tends to migrate to the O/W interface where the hydrophobic groups binds with the oil phase and the hydrophilic groups with the water phase. The emulsifying capabilities of OSA starch depends not only on the newly attached of hydrophobic groups, but also the original starch structure (Nilsson & Bergenståhl, 2006). Therefore, different molecular structure of OSA starches display various emulsifying properties and have been used in numerous countries, such as in United States, as certified food additives (Zhao *et al.*, 2018).

OSA starches are commonly produced by esterification of starch with OSA in aqueous media under alkaline conditions (Figure 2.7).

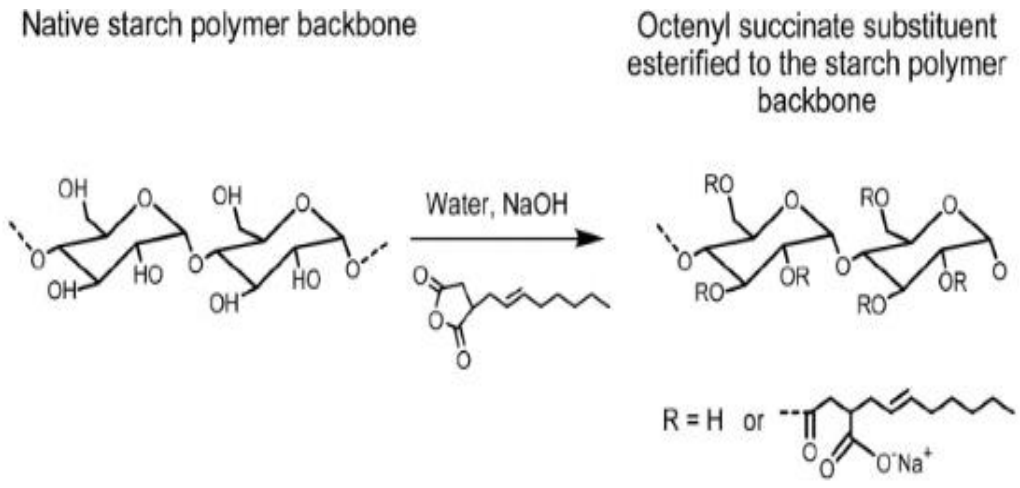

Figure 2.7: Modification of starch by OSA (McNamee *et al.*, 2018).

Generally, the esterification reaction with starch occurs on the particles surfaces due to the low solubility of OSA in water, and the problems such as separation of acid anhydride and weak reaction efficiency easily occur due to uneven distribution of OS groups (Altuna *et al.*, 2018). As a consequence of the heterogenous character of the

system and the semicrystalline nature of the starch granules, characterization of OS-starches shows that the reaction is mainly a surface phenomenon, with esterification reaching mostly the amorphous region of the granules. Thus, pre-treatment such as HMT will help structural modification at amorphous and crystalline regions on the starch granules, which make the granule susceptible to enzymatic pre-treatment and chemical modification via OSA (Alcάzar-Alay & Meireles, 2015). On the other hand, the use of anhydride at high temperature often produce other by-products in the process of chemical reaction. Pre-treatments need to be done before OSA modification of starch to minimize the problems occurred (Tian *et al.*, 2018). Chen *et al.* (2014) showed that OSA groups could penetrate deep into the granules interior and be distributed throughout the starch granules by an ultrasound or hydrothermal treatment. Besides that, high-speed shear conditions can be applied to enhance the starch modification reaction efficiency by OSA and improve the modified starches clarity and freeze thaw stability (Ačkar *et al.*, 2015).

As stated by Abiddin *et al.* (2015), the physicochemical properties of native sago starch have been altered after being esterified with 5% OSA, at pH 7.20 for 9.65 hours. Sago starch was esterified with OSA to regulate its shortcomings by adding amphiphilic properties. There is a reduction in amylose content for esterified sago starch, indicating that modification happened mainly in the amorphous areas and disrupt the amylose chains linearity. There is an increase in particle size which correspond to swelling activity compared to their native starches. Scanning electron micrograph showed that OSA starch developed slightly rough surface and lost definition at their edges. The modified starch obtains surface active properties which will be useful in stabilizing emulsion (Abiddin *et al.*, 2015).

However, the reaction of OSA and starch is restricted due to poor diffusion of the large oily droplets of OSA into the starch granules in an aqueous suspension, and the site of reaction is limited to the granule surface (Wang *et al.*, 2015). As a results, the OS groups are not distributed uniformly throughout the starch granule. Pre-treatment such as heat moisture treatment (HMT), enzymatic pre-treatment or dual pre-treatment of heat moisture treatment and enzyme before OSA modification of starch could help to solve the problems mentioned (Huang *et al.*, 2010).

2.2.2 Heat moisture treatment (HMT)

HMT is a thermal treatment in the presence of less than 35 percent w/w of water, heating between 15 min to 16 h, at temperature between 100 to 120˚C (Neelam *et al.*, 2012; Alcάzar-Alay & Meireles, 2015). It is an ecologically approachable and lower cost technique use to helps OSA enhancement of starch (Barua & Srivastav, 2017). The effects of HMT on the starch granules physicochemical as well as morphological properties was affected by the starch granule origin together with the structure and organisation of amylopectin and amylose. This changes involve major improvements in swelling ability, gelatinization, retrogradation, crystalline structure along with pasting properties. It is typically applied as pre-treatment due to structural changes of the granules into amorphous and crystalline regions, which make the granule sensitive to acid and enzyme hydrolysis as well as chemical modification (Alcάzar-Alay & Meireles, 2015).

The rigid glassy state of the amorphous regions is altered to a moveable rubbery state when heating. HMT caused changes that are likely to occur in the amorphous regions of the starch granules, which are more available to hydrolysis (Zavareze & Dias,