Effect of Octenylsuccinylation on Morphological, Particle Size and Surface Activity of Octenyl Succinic Anhydride (OSA) Modified Sago Starch

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Abstract—Sago starch (Metroxylon sagu) and Gelose 80 was chemically esterified with Octenyl Succinic Anhydride (OSA) to produce hydrophobically starch which can act as emulsifier in oil-in-water emulsion (O/W). The modification of sago starch and Gelose 80 was conducted in aqueous following parameter: phase the optimum OSA concentration 5.0%, pH 7.20 and reaction time of 9.65 h. The study was conducted to investigate the morphological properties, particle size and surface activity of octenyl succinic anhydride (OSA) modified sago starch. The OSA sago starch had a degree of substitution (DS) of 0.0120. The morphology of starch granule examined by scanning electron microscopy (SEM) showed some rough surface and a few changes in shape of OSA starches. The particle size of both OSA starches significantly (p < 0.05) increased (OSA sago starch = 29.05 μ m, OSA gelose 80 = 20.37 μ m) due to structural disorder that occurs after modification. OSA sago starch showed an increase in surface tension from 58.40 to 60.80 mN/m with increased in OSA sago starch concentration from 0.01 to 1.0% w/v. The surface tension for OSA gelose 80 was 54.87 mN/m. The modification has improved native sago starch and Gelose 80 becoming surface active molecules which will be useful to be used in emulsion.

Index Terms—Sago starch, Gelose 80, Octenyl Succinic Anhydride (OSA), degree of substitution, particle size, surface activity

I. INTRODUCTION

Starch is a combination of amylose and amylopectin molecules with different ratio depending on the botanical origin of the starch. Starch from sago was derived from the trunk of the palm (*Metroxylon spp.*), in which it has become important raw materials and food stuffs in South Asia [1]. Traditionally, sago starch was used in cooking for the making of pudding, jellies and soups. Even though sago starch has quite similar properties as other common starch, it also has some unique properties like easy to gelatinise, high viscosity, easily moulded and low gel synerisis. Malaysia is one of the sago starch producing country in the world, however the application of sago starch in commercial is still limited. Similar to other starches, native sago starch also need to be modified to improve the properties of sago starch in order to meet industrial applications. Modification of starch with octenyl succinic anhydride (OSA) has gain many interest as it acquire improved properties and fulfill the requirements of the food products. The esterification of starch with dicarboxylic acids was first patented by Caldwell and Wurzburg in 1953 and the product was known as starch sodium octenyl succinate (SSOS) [2]. Native starch has three free hydroxyl groups at carbon 2, 3 and 6 of the anhydroglucose unit which make the substitution of starch with OSA is possible. The substitution of hydrophobically OSA group into the glucose unit of starch will change the properties of starch from naturally hydrophilic to amphiphilic and thus obtain surface active properties. Octenyl succinic anhydride starch has found its application in various products like beverages, oil in salad dressings, gel production, coatings and as encapsulating agent for flavour and fragrances [3]. Modified starch including OSA starches has been approved to be safe for human consumption by Food and Drug Administration (FDA).

The esterification of various type of starch with OSA has been extensively studied over the past few years, in which the effect of reaction conditions on the synthesis of OSA starch have been a great interest to be studied. There are few factors that will affect the synthesis of OSA starch, such as pH, temperature, concentration of OSA, time of reaction, starch concentration and also interaction of these factors [4]-[6]. The basic principle on preparation of esterified starch is suspends the starch granules in water and OSA reagent was added under alkaline conditions [7]. The product of the esterification process was known as sodium starch octenylsuccinate and it has been coded as E1450 [8].

To our knowledge, there still lack of studies on preparation and physicochemical properties of OSA sago starch have been done. The objective of the present study

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is to investigate the morphological properties, particle size and surface activity of octenyl succinic anhydride (OSA) modified sago starch. The result may give a complete understanding into the effect of esterification on the properties of sago starch. The results reported was compared with the OSA commercial starch and OSA gelose 80 which has been modified in a similar way as OSA sago starch.

II. MATERIALS AND METHODS

A. Materials

Food grade sago starch was purchased from Nee See Ngeng & Sons Sago Industries Sdn Bhd (Sarawak, Malaysia). Gelose 80 is unmodified high amylose maize starch that contains approximately 80% of amylose and was donated by Ingredion (Bridgewater, New Jersey). Octenyl succinate anhydride commercial starch was a gift of N-Starch Sdn Bhd (Shah Alam, Malaysia) and was used without any further treatment. Octenyl succinic anhydride (OSA) was obtained from Dixie Chemical Company, Pasadena, Texas, USA. Sodium hydroxide, hydrochloric acid, acetone and dimethyl sulfoxide were of analytical grade and purchased from Sigma-Aldrich.

B. Preparation of OSA Starches

Sago and gelose 80 starches were subjected to esterification using OSA by treating with the optimum condition obtained through response surface methodology (RSM). The optimum condition for the preparation of OSA starches was at 5.00% OSA concentration, pH 7.20 and reaction time of 9.65 h. Preparation of OSA starches and determination of degree of substitution (DS) were based on study from [4] and has been reported in our earlier work [9]. OSA gelose 80 and OSA commercial starch were analyzed as a comparison with OSA sago starch.

C. Morphological Properties

The morphological characteristics of native and OSA starches were observed through scanning electron microscope (Leo Supra 50VP Field Emission SEM, Carl Zeiss SMT, Germany). The starch samples were make sure to be completely dry before imaging to avoid any interference of moisture. The dried samples were mounted on double-sided adhesive tape on an aluminium stub and the starch was sputtered with a layer of gold in vacuum evaporator. A magnification of 2000x was used for sago and commercial starch while 5000x for gelose 80 starch [10].

D. Particle Size Distribution

The particle size distribution was determined with static multi-angle light scattering method using Mastersizer Hydro 2000 (Malvern Instrument, Malvern, UK) equipped with Scirocco Dry Powder Feeder. The starch samples should be free from moisture as it can make the starch powder clotted and give incorrect measurement. For each measurement, about 2 g of starch samples were added into the feeder and was measured three times. The particle size of starch was characterized by Sauter mean diameter (d_{32}) [11].

E. Surface Tension

The Wilhelmy plate technique was used to determine the surface tension of OS A starches and the measurement was carried out with K20 Kr üss tensiometer (Hamburg, Germany). The platinum plate was rinsed with distilled water and acetone, and burnt directly under blue flame prior to measurement. Three different concentrations (0.01, 0.1 and 1.0 wt.%.) of OSA starch samples were prepared and measured [12].

III. RESULTS AND DISCUSSION

A. Optimisation of OSA Sago Starch and Degree of Substitution (Ds)

The sago starch was subjects to three variables namely as octenyl succinic anhydride (OSA) concentration, pH and reaction time according to reaction conditions that was suggested by Response Surface Methodology (RSM). The response for these esterification reactions was Degree of Substitution (DS) analysis. To examine the effects of three variables on the DS of OSA sago starch, a Central Composite Design (CCD) was performed and 20 experiments was performed with different combination of variables.

According to the result from RSM and as reported in our previous work [9] the optimum condition for the preparation of OSA sago starch was at 5.00% OSA concentration, pH 7.20 and reaction time of 9.65 h with the resulting DS of 0.0120. For the comparison purpose, gelose 80 starch was subjected to similar optimum condition and the resulting DS was 0.0145. Besides, commercial OSA starch was also used as comparison in order to confirm whether OSA modification on sago starch have the similar properties as the commercial ones.

The first independent variable that was studied was OSA concentration which show increase in DS with increasing in the addition of OSA. This observation can be explain as increasing OSA concentration will make more OS group being available for the substitution, making more hydroxyl group (OH) can be replaced by OS group [13]. During the mixing process, it can be seen that the OSA droplet was floated on the surface of the solution as on oily droplets and due to this, OSA was known as insoluble in water making the reaction to be heterogeneous [14]. Moreover, starch hydroxyls are immobile and the substitution was dependent on the availability of OSA around the starch molecule [4]. For the above reason, it make the use of OSA in higher concentration is more favourable in order to make the starch more accessible to the OSA, increase molecule collision and favour the formation of derivative.

During the modification, the pH of the solution was also controlled and the optimum pH suggested was pH 7.20. Our result showed an increase in DS with increasing pH but subsequently decreased after pH 7.50. This is supported by the study from [15] which explain that lower pH will promote esterification whereas too high pH will favour side reactions and promote de-esterification. In addition to this, it is required to use low pH as hydrolysis of the OSA starch was likely to occur when the pH was too high [5].

Another important factor for esterification was reaction time. In the present study, succinvlation of sago starch was optimum at short reaction time which is 9.65 h. As the reaction time increased, it can be seen that the DS was likely to decrease. This agreed with the finding from [10] and [16] which found that short reaction time resulted in higher DS than long reaction time. The most likely explanation is that the concentration of OSA was limited, and during the reaction, the concentration of OSA will keep decreased due to esterification process and hydrolysis. Extending the reaction will only cause the side reactions to become dominant. The second explanation is that every starch has its own morphology especially the size and granular structure, and also difference in botanical origin which can also contribute to the effect of time on DS. This was confirmed by the previous work from [4], who study on two difference starches and it was found out that each starch will have its own reaction time in order to achieve maximum DS.

B. Morphological Properties and Particle Size of Starches

The morphological of both native and OSA starches were analysed using scanning electron microscope (SEM) as shown by Fig. 1. The sago starch was presented in Fig. 1(a) as oval and some having round shaped granules with smooth surface. This is in agreement with previous reports on sago starch granules [17] and [18]. There are no observable pores and their edges were well defined but some granules having truncated end. Esterification on sago starch cause only little changes, most of the granules retained its original shape and smooth appearance as shown by Fig. 1(b). However, it can be seen that some granules having rough surface, most likely can be said as erosion with lost definition of their edges.

The result shown in Fig. 1(c) and (d) was a microstructure of gelose 80 starch. The native gelose 80 [Fig. 1(c)] was depicted to have various shape and size, with no visible fracture, holes and the surface was smooth like sago starch. The gelose 80 was originally from maize starch, the only difference from normal maize starch is it contains higher amylose content up to 80 %.

Our result from SEM for gelose 80 was similar with the previous report by [19], also reported that high amylose maize starch show three different shapes which are individual, elongated and aggregate granules. Like sago starch, OSA treatment was found to alter the morphology of gelose 80 especially in the surface.

In Fig. 1(d), the starch was seen to have a rough surface but clearly, there are no pores or cracks were observed on the surface of starch. The change in morphology of OSA gelose 80 is more pronounced than OSA sago starch, and this coincide with the DS result. The DS for OSA gelose 80 is higher than OSA sago starch, indicate that the substitution of OSA was occurred at higher rate in gelose 80. The granular structure of OSA

commercial starch [Fig. 1(e)] was observed to be smooth with hollow in the middle of the granules. The granule size and shape was irregular, and the granules were observed to be aggregate.

In three types of starch that has been analysed, it can be seen that there are variation in size and shape and this might be due to the biological origin [8]. All three starches seemed to be less affected when treated with OSA, probably due to the absence of pores and cavities that might help the penetration of OSA into the starch molecules. The damage of granule only can be observed to occur on the surface of the granule and not all granules was affected. This is due to OSA presence as low water solubility and it was suggested that the reaction was occur at the surface of the starch granules.

The diffusion of OSA into the starch molecules was also depend on the droplet volume of OSA, which explained the uneven distribution of OSA into the starch molecules [20]. According to [21], the rough surface and fusion of granules that occur on OSA starches was presumably happen as a result of surface gelatinization due to maintaining alkali condition during the addition of OSA.

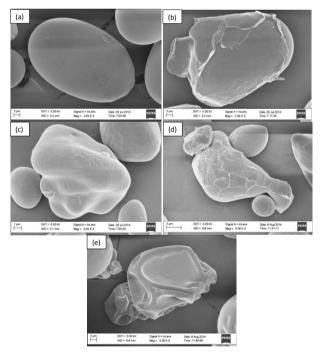


Figure 1. Scanning electron micrographs of native and OSA starch: (a) native sago starch and (b) OSA sago starch at 2000x, (c) native gelose 80 starch and (d) OSA gelose 80 at 5000x, and (e) OSA commercial starch at magnification of 2000x.

The lesser damage on the sago and gelose 80 granules suggesting that concentration of OSA used in this study was not high enough to cause the rupture on the granules and both starches was found to be resistant to the succinvlation reaction at this level.

From the morphology image of native and OSA starches, the change in particle size of starch after esterification was not really significant. However, the measurement via multi angle light scattering Mastersizer Hydro 2000 shows otherwise. Fig. 2 shows clearly that

the esterification modification significantly increased (p>0.05) the particle size of native starch.

Amongst the starches, the largest increment in particle size was found for gelose 80 starch which is from 9.793 μ m to 20.375 μ m. Even though the increase in size for sago starch after modification was not very evident (29.052 μ m to 29.889 μ m), it is still give a significant difference. The size distribution for all starches shows a unimodal distribution except for OSA gelose 80 and OSA commercial starch that shows bimodal distribution. OSA commercial starch has the largest particle size (44.244 μ m), however, the increment in size cannot be compare to the other starches in this study because the particle size of the commercial starch before modification was unknown.

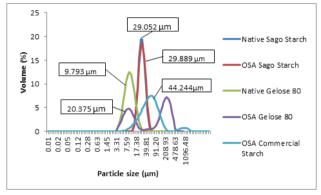


Figure 2. Particles size of native and OSA starches.

Slightly basic condition used during esterification and introduction of OSA group will caused the disruption of granule structure, which in turn caused the swelling of starch and increased the size of particle [8], [22]. These result also can be relate with DS, the smaller starch particle (gelose 80) have a higher DS than larger particle (sago starch). The possible explanation is that small particle size will have high surface area, which means that there are larger contact areas available for the reaction of octenyl succinic anhydride with starch granule. As particle size increases, the surface areas exposed for esterification decreases, hence reduce the DS and reaction efficiency. Additionally, small starch particle have a high tendency to agglomeration, confirm that the bimodal distribution observed for OSA gelose 80 starch [23].

C. Surface Properties of Octenylsuccinylated Starch

To better understand the surface active properties of OSA starch, determination of surface tension was done. Esterification of native starch with OSA has altered the properties of starch from naturally hydrophilic to amphiphilic molecule. The surface tensions of OSA starch with different concentration are shown in Table I.

In general, all esterified starch shown surface active properties and have the ability to lower the surface tension. For OSA gelose 80 and OSA commercial starch, it shows a similar trend which increase in concentration of starch from 0.01 to 1.0% will decrease the surface tension. However, OSA sago starch shows an opposite trend, increase in starch concentration will increase the surface tension from 58.40 to 60.80 mN/m.

During the analysis, the increased in addition of OSA sago starch will produce an uneven dispersion. It can be seen visually that higher concentration of OSA sago starch tends to sediment at the bottom of the vessel wall and some of them aggregate at the surface of Wilhelmy plate even though the sample has been stirred. By comparing the surface tension of all the derivatives, it can be concluded that OSA gelose 80 revealed to be as the best surfactant. It was believe to be related to the DS of the sample as OSA gelose 80 starch (DS: 0.0145) exhibited more hydrophobic properties than OSA sago starch (DS: 0.0120). It is also can be related to the particle size of starch itself as gelose 80 starch is smaller than sago starch, means that there are more surface available at gelose 80 starch to be coated with OSA than sago starch [24].

 TABLE I. THE SURFACE TENSION OF AQUEOUS DISPERSION WITH

 DIFFERENT CONCENTRATION OF STARCH

Starch Type	Concentration	Surface Tension
	(wt.%)	(mN/m)
OSA Sago Starch	0.01	58.40 ± 1.13^{b}
	0.1	59.60 ± 0.95^{ab}
	1.0	60.80 ± 0.52^{a}
OSA Gelose 80	0.01	54.87 ± 0.72^{a}
	0.1	52.87 ± 0.11^{b}
	1.0	$43.80 \pm 0.52^{\circ}$
OSA Commercial Starch	0.01	61.77 ± 0.61^{a}
	0.1	56.50 ± 0.70^{b}
	1.0	$46.83 \pm 1.24^{\circ}$

Values are mean ±standard deviation

Mean within each row with different superscripts letter are significantly different at p < 0.05 indicated by small letter is the effect of different concentration of starch on the surface tension.

The surface tension of OSA commercial starch lies between OSA sago starch and OSA gelose 80, which indicated that it have amphiphilic character less than OSA gelose 80 but higher than OSA sago starch. The normal and widely used surfactant such as sodium dodecyl sulfate (SDS) has a surface tension of 39.5 mN/m [25]. Comparing with our study, even though the value of surface tension for OSA sago starch and OSA gelose 80 was not close with SDS, it still show some surface active properties.

IV. CONCLUSIONS

The extent of esterification was determine through degree of substitution and esterification has shown to had significant effect on morphology, particle size and surface tension of sago starch. OSA sago starch has shown to have improved properties than native sago starch. The particle size of OSA sago starch was increased from 29.052 µm to 29.889 µm and only little change can be seen to occur on the surface of native starch after modification. It shows that concentration of OSA used in this study only have the ability to alter the surface of the granules. OSA substitution imparts hydrophobic properties, which have lower the surface tension of all OSA starches. The size of the granules had a large impact as the smallest granules (gelose 80) was shown to have better degree of substitution and physicochemical properties than that large granule (sago

starch). The modification has improved native sago starch becoming surface active molecules by the addition of hydrophobic chain to the naturally hydrophilic molecule. By this means OSA sago starch will be useful to be used in emulsion. Future work is needed to study the effect of OSA sago starch on the emulsification properties.

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