Physicochemical and emulsifying properties of pre-treated octenyl succinic anhydride (OSA) sago starch in simple emulsion system

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Abstract

A study was carried out to determine the physicochemical and emulsifying properties of pre-treated octenyl succinic anhydride (OSA) sago starch in simple emulsion. Sago starch was pre-treated with STARGEN enzyme (EN OSA: single pre-treatment), heat moisture treatment followed by STARGEN enzyme (HMT EN OSA: dual pre-treatment) before being esterified with OSA. The ability of the pre-treated OSA sago starch to stabilize emulsion was then investigated. Dual pre-treated starch, HMT EN OSA, had significantly highest degree of substitution (DS), (DS = 0.0179) compared to single pre-treated starch, EN OSA, (DS = 0.0159) and native OSA, N-OSA (DS = 0.0057). As compared to emulsions prepared by N-OSA and HMT EN OSA, EN OSA had significantly highest emulsifying activity throughout all starch concentrations and it produced a thick viscous emulsion layer directly after emulsification. This might be due to enzymatic pre-treatments may retained granule’s original shape and smooth appearance which allow having a better fit during the emulsification process. The highest emulsion stability was observed with the emulsion index values of EN OSA stabilized emulsions was the most stable for all starch concentrations throughout storage study. After the 8th week of storage study, the EN OSA remains the highest emulsion index from 0.37 to 0.56 for 200 mg/mL oil to 500 mg/mL oil starch concentration. Light micrograph of EN OSA showed that starch particles accumulated at the oil-water interface and cover the oil droplets with higher degree of coverage than the HMT EN OSA and control. No spaces were observed in the EN OSA stabilized emulsion which indicated that EN OSA modified sago starch can effectively stabilize oil in water emulsion.

1. Introduction

Oil droplets stabilized by dispersed particles in water were originated from Ramsden (1903) and Pickering (1907). The use of particles to stabilize emulsions, such as modified starch, has received substantial and increased research interest because of their distinctive characteristics and promising technological applications in a range of fields. Pickering emulsion is an emulsion that is stabilized by solid particles which adsorb onto the interphase between the oil and water interfaces (Matos et al., 2016).

In order to increase the hydrophobicity of starch, chemical modification of sago starch can be conducted by treatment with octenyl succinic anhydride (OSA). In the United States, FDA approved the addition of OSA for food applications since 1972 and must be no more than 3% based on the dry weight of the starch (Song et al., 2006). The substitution of hydrophobically OSA group into the starch glucose unit will change the starch properties from naturally hydrophilic to amphiphilic and thus obtain surface-active properties (Yusoff et al., 2017). Pre-treatment such as heat moisture treatment and enzymatically pre-treatment before OSA modification could facilitate the rapid entry of OSA into the granule interior, thus enhance the reaction between OSA and hydroxyl groups. The hydrophobic groups in the OSA starch granules introduced by OSA have an affinity for the oil phase, which can adsorb at the oil-water interface and form a physical barrier that prevents the separation of oil and water phases, and consequently affecting the functional properties of the modified starch (Jiranuntakul et al., 2014).

Octenyl succinic anhydride (OSA) modified starch
with hydrophilic and hydrophobic groups has been reported to be an excellent emulsifier in food, pharmaceutical, personal care, and biodegradable plastic industries. It has become increasingly popular due to its low price and excellent emulsification. By incorporating the lipophilic alkenyl groups from OSA into the hydrophilic starch molecules, which is the linear portion of amylose and branched portion of amylopectin, OSA modified starch thus obtains a specific surface activity (Simsek et al., 2015). Commonly, OSA modified starch is prepared with a low concentration of alkali as a catalyst in an aqueous medium. The reaction of OSA and starch carried out in the aqueous phase results in poor reaction efficiency and the uneven distribution of OSA groups (Ačkar et al., 2015).

Thus, the application of pre-treatment such as heat moisture treatment (HMT) and enzymatic pre-treatments before OSA esterification could facilitate the rapid entry of OSA into the granule interior and enhance the reaction between OSA and hydroxyl groups, resulting in an increase of hydrophobic groups and consequently affecting the functional properties of the modified starch (Jiranuntakul et al., 2014). Little work has been reported on the physicochemical and emulsifying properties of sago starch modification with OSA after single pre-treatment by enzyme and dual pre-treatment by heat moisture treatment and enzyme. Therefore, the objective of this work is to study the physicochemical and emulsifying properties of pre-treated OSA sago starch in simple emulsion system.

2. Materials and methods

2.1 Materials

Sago starch (Metroxylon sagu) was obtained from SIM Company Sdn. Bhd. (Penang, Malaysia). Octenyl succinic anhydride (OSA) was purchased from Sigma Aldrich, USA. Corn oil was purchased from Tesco Stores (Malaysia) Sdn. Bhd. (Penang, Malaysia).

2.2 Enzyme

The commercial enzyme, STARGEN 002 enzyme was used to hydrolyze the starches. It is a product of Genencor International (Palo Alto, CA) containing Aspergillus kawachi α-amylase expressed in Trichoderma reesei and glucoamylase from Trichoderma reesei. The pH of STARGEN 002 enzyme ranged from 4.0 to 4.5. The specific gravity of STARGEN 002 enzyme is 1.13-1.16 g/mL. The recommended temperature for STARGEN 002 enzyme is 20-40°C. The minimum activity of STARGEN 002 enzyme is 570 GAU/g. GAU is defined as Glucoamylase Units.

2.3 Enzymatic pre-treatment of sago starch

The starch was mixed with sodium acetate buffer (25% w/v) with pH 4.4. The enzyme was added (1% w/v) into the starch suspension and the process was conducted in an orbital shaker (JEIO Tech, SI-600R, Seoul, Korea) at 45°C with the speed of 170 rpm. After 24 h, the process was stopped by adding a pre-determined amount of 2.0 M hydrochloric acid (HCl) until the pH was 1.5-1.6. The pH of starch suspensions was adjusted back to pH 5-6 by washing the starch with distilled water and the starch residues were dried in an air-drying oven at 40°C (Shariffa et al., 2017).

2.4 Dual pre-treatment of sago starch

Heat moisture treatment was carried out by autoclaving method. The moisture content of starch was adjusted to 20% (w/w wet basis) by mixing calculated amounts of water into the starch for 15 mins. Next, the mixed starch suspension was placed into a Duran glass bottle 250 mL for 100 g starch 1 hr before autoclaving at 120°C for 60 mins as an optimum condition for sago starch. Lastly, the bottles were cooled to room temperature and dried overnight at 40-45°C in a hot air oven and subsequently, the heat moisture treated starch was subjected to enzymatic pre-treatments (Adawiyah et al., 2017).

2.5 Preparation of OSA starches

Native and pre-treated sago starches (125 g) were dispersed in 400 mL distilled water with agitations. The pH of the slurry was adjusted to 7.20 by using 2% NaOH solution. To this mixture, OSA at 2.5% (based on dry starch basis) was added dropwise for 2 hrs. During the addition of OSA, the pH was maintained at the set point. The reaction was carried out for 9 h. After the reaction time was ended, the pH of the starch slurry was adjusted back to pH 5-6 by washing the starch with distilled water and acetone and centrifuged at 3000 rpm. The modified starch was then collected by filtration using Whatman filter paper No. 1 and dried in drying oven at 45°C for 24 hrs. After drying, the OSA starch was sieved with 0.35 mm siever and kept in an airtight container (Zainal Abiddin et al., 2015).

2.6 Determination of degree of substitution (DS) of OSA modified starch

The DS of OSA substitution was determined using a titration method with slight modification (Rayner et al., 2012). The DS was calculated by

\[
\text{OSA Substitution (\%)} = \frac{V \times M \times 210}{W} \times 100
\]

Where V is the volume in mL of NaOH required for the
sample and the blank titration (Vsample-Vcontrol), M is the molarity of NaOH (0.1 M), W is the dry weight of the starch in mg, 210 is the molecular weight of octenyl succinate group.

\[
\text{Degree of substitution (DS)} = \frac{162 \times \text{OSA substitution} \%}{21000 - \left[209 \times \text{OSA substitution} \%\right]}
\]

Where 162 is the molecular weight of the glucose unit, 21000 is 100 x molecular weight of the octenyl succinate group, 209 is the molecular weight of octenyl succinate group minus the molecular weight of hydrogen atom.

2.7 Preparation of emulsions

Starches (200-500 mg starch per mL oil) were suspended in distilled water, mixed with corn oil at the volume ratio of 2:1 in a beaker, and then the resultant mixture was homogenized using a high-speed homogenizer (Ultraturrax T25, IKA, Germany) at 20,000 rpm for 2 mins (Timgren et al., 2013; Saari et al., 2016).

2.8 Emulsifying activity

Emulsion (50 μL) was pipetted into 5 mL of 0.1% sodium dodecyl sulfate. The absorbance (emulsifying activity, EA) was measured at 500 nm using UV spectrophotometer (UV-1650PC, SHIMADZU, Kyoto, Japan) after the formation of emulsion (Lu et al., 2016).

2.9 Emulsion index and storage stability

Aliquots (20 mL) of above emulsions were accurately transferred into glass tubes, capped and photographed on 1st day, 2nd, 4th, 6th and 8th week (Timgren et al., 2013). The emulsions were monitored for 60 days and emulsion index was calculated according to the equation:

\[
\text{Emulsion index} = \frac{H_e}{H_s} \times 100
\]

Where \(H_e\) is the height of serum measured after a certain time of storage and \(H_s\) is the total height of the emulsion

2.10 Optical microscopic observation

Optical microscopic observation of the emulsions was performed using an Olympus BX41 light microscope (Plan, Olympus, Tokyo, Japan). An aliquot of the emulsions was placed in a clean dry microscopic slide and covered with a cover slip (Marefati et al., 2017).

2.11 Statistical analysis

All data were performed in triplicate and the results were expressed as the mean values ± standard deviations. The data were analysed using the analysis of variance (ANOVA) and Duncan’s multiple range test for significance at p < 0.05. One-way ANOVA was used for DS and two-way ANOVA was used for EA and emulsion stability. The statistical analysis was performed using IBM SPSS Statistics version 23.0 software.

3. Results and discussion

3.1 Degree of substitution of OSA modified starches

Single pre-treatment and dual pre-treatment of heat moisture treatment and enzyme hydrolysis had significantly affected the DS value of OSA modification of sago starch as shown in Table 1. The DS value increased significantly with the pre-treatment following the order: HMT EN OSA > EN OSA > control. Pre-treatments significantly enhanced the efficiency of starch reaction with OSA in accordance with FDA regulations for food starch (DS<0.02). These observations indicated that pre-treatment by enzyme and heat moisture treatment improved the efficiency of the subsequent OSA modification as HMT induced changes probably occur in the amorphous regions of the starch granules. HMT will cause more access to enzyme hydrolysis due to increased swelling power of starch granules and consequently increase DS values of starch (Zavareze and Dias, 2011; Chen et al., 2014).

| Sample   | DS        |
|----------|-----------|
| N-OSA    | 0.0057±0.00038^a |
| EN OSA   | 0.0159±0.00068^b |
| HMT EN OSA | 0.0179±0.00068^c |

Data are expressed as mean of 3 determinations ± standard deviations. Values with different letter in the same column are significantly different (p < 0.05). N: native; EN: enzyme; HMT: heat moisture treatment; OSA: octenyl succinic anhydride

The starches that were subjected to the dual pre-treatment exhibited significantly higher DS as to the starches with only single pre-treatment. This is because HMT resulted in the rearrangement of the starch structure and could increase the binding site of the enzyme, which promoted enzymatic hydrolysis and facilitated enzymatic accessibility to the amorphous regions and thus enhanced OSA droplets access to the granules’ interior (Xie et al., 2018).

Our results were in accordance with Xu et al. (2015), which showed that enzyme treatment had a thinning effect at the starch particle surface, accompanied by the appearance of the degree of polymerization 2-5 chains and the increasing percentage of α-1,6 linkages. During the esterification, the OSA reagents penetrate more readily into the less density areas and then grafted on glucan chain, resulting in more substituted OS and

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higher DS value. Lu et al. (2016) also reported that enzyme-treated starch nanoparticle modified with OSA was higher compared to the native starch nanoparticle treated with OSA.

3.2 Emulsion properties: emulsifying activity (EA)

The EA of the modified starches is shown in Table 2. The emulsifying activity of OSA sago starch were in the range 0.04 – 0.54 throughout four starch concentration and varied among samples. From the results, all concentration of pre-treated OSA starch had higher EA compared to control sago starch. These results revealed that pre-treatment enhanced the modification with OSA and increased the hydrophobicity of starch. Single and dual pre-treatments by enzyme and heat moisture treatment will increase the susceptibility of granular starch to modification with OSA as those pre-treatments create pinholes on the surface of starch granules, thus, more OH groups are substituted by OSA and resulting in an increased of the starch hydrophobicity (Wang et al., 2017; McNamee et al., 2018). In contrast, the reaction between OSA and native sago starch is retarded because of poor penetration of the big oily droplets of OSA into starch granules in an aqueous suspension, thus, less octenyl succinic groups were grafted into the starch chains (Chen et al., 2014).

As concentration increase, EA also increases as there is a higher amount of starch allowed the stabilization of larger surface area, which is in line with previous investigations by Rayner et al. (2012) and Lu et al. (2018).

According to the study of Lu et al. (2016), OSA-starch with an amphiphilic character was an efficient emulsifier, where it could generate a high surface load of 10 mg/m² and forms a very thick layer of macromolecules at the O/W interface. Starch modified with OSA resulted in molecules with both hydrophilic and hydrophobic properties as the hydrophilicity of the starch is retained during modification process and can attach strongly to the O/W interface (McNamee et al., 2018). Thus, the presence of polar carboxyl groups and non-polar acetyl groups in the modified sago starch resulted in an enhancement of EA of OSA modified starch. Kasprzak et al. (2018) showed that the emulsion stabilized with the OSA starch showed no signs of flocculation and the droplets were visibly smaller and less polydisperse compared to non-OSA starch.

3.3 Effect of starch concentration on emulsion stability

Emulsion stability can be observed by calculating the emulsion index (EI) and by visual observation. Referring to Table 3, a significant difference for EI values was observed with the highest values recorded for EN OSA, followed by HMT EN OSA and the least for N-OASA. Throughout storage study, the highest decline EI values were found for HMT EN OSA, followed by N-OASA and EN OSA. As the starch increase in concentration, the cream volume as the viscosity of the aqueous phase also increased. For instance, for starch concentration at 200 mg / mL oil, EI values for HMT EN OSA decrease by 92.5%, followed by N-OASA decreased around 57.1% and EN OSA which decrease by 41.3%.

Table 2. Emulsifying activity (EA) of 2.5 % OSA modified sago starch.

| Sample       | Starch concentration (mg mL oil) | 200  | 300  | 400  | 500  |
|--------------|---------------------------------|------|------|------|------|
| N-OASA       | 0.04±0.00^a                      | 0.07±0.01^ab | 0.12±0.00^ab | 0.18±0.00^ab |
| EN-OASA      | 0.32±0.07^ab                     | 0.42±0.05^bc | 0.51±0.01^c  | 0.54±0.02^cd |
| HMT EN OSA   | 0.16±0.02^ab                     | 0.22±0.01^b  | 0.27±0.01^bc | 0.31±0.00^bd |

Data are expressed as mean of 3 determinations ± standard deviations. Values with different letter in the same column are significantly different (p < 0.05). N: native; EN: enzyme; HMT: heat moisture treatment; OSA: octenyl succinic anhydride.

Figure 1 and Figure 2 shows the photo of the obtained emulsions after one day of productions and after 8th week of storage study, respectively. After 24 hours of production, an oil layer was observed on top of the emulsion layer stabilized by both N-OASA (Figure 1a) and HMT EN OSA (Figure 1c). However, the oil layer was not observed for the emulsions stabilized by EN OSA (Figure 1b). Most of the samples contain an upper layer composed of creamed emulsion droplets and a bottom layer composed of sedimented free starch and some emulsion droplets. A thin emulsion layer was produced by N-OASA (Figure 1a) and HMT EN OSA (Figure 1c), and both were observed to be stable after 24 hours. However, the layer became thin and the amount of free starch high after the 8th week of storage study (Figure 2a and 2c). In contrast, emulsions stabilized by EN OSA produced thick, creamy layers directly after emulsification (Figure 1b). As storage time increased, the cream volumes decreased for all pre-treated OSA modified sago starch as shown in Figure 2. EN OSA starch were somewhat better stabilizers than the native starch and dual pre-treated HMT EN OSA modified starch.

Dual pre-treated HMT EN OSA was not able to enhance the stability of emulsion as compared to single pre-treated EN OSA. This might be due to a part of...
substituted octenyl succinic groups in HMT EN OSA starch could not interact with the dispersed phase, as some starch granules were not completely broken (Jiranuntakul et al., 2014). On the other hand, single pre-treatment EN OSA starch may have retained its smooth appearance and original shape after enzymatic pre-treatment process, which were believed to have a better fit and maximize the packing at oil-water interphase (Saari et al., 2016). N-OSA was able to stabilize oil-in-water emulsions as the starch had modified to impart amphiphilic characteristics. However, the emulsifying abilities were not as good as EN OSA stabilized emulsions as without pre-treatments, less octenyl succinic groups were grafted into the hydrophobic starch chain.

3.4 Microstructure of the emulsions

Table 3. Effect of OSA starch concentration on emulsion stability.

| Sample (mg starch/mL oil) | Day 1       | Day 14      | Day 28      | Day 42      | Day 56      |
|---------------------------|-------------|-------------|-------------|-------------|-------------|
| N-OSA 200                 | 0.07±0.01   | 0.06±0.00   | 0.05±0.01   | 0.04±0.00   | 0.03±0.01   |
| N-OSA 300                 | 0.08±0.00   | 0.07±0.01   | 0.06±0.00   | 0.05±0.01   | 0.04±0.00   |
| N-OSA 400                 | 0.09±0.01   | 0.08±0.00   | 0.07±0.01   | 0.06±0.00   | 0.05±0.01   |
| N-OSA 500                 | 0.10±0.00   | 0.09±0.01   | 0.08±0.00   | 0.07±0.01   | 0.06±0.00   |
| EN OSA 200                | 0.63±0.01   | 0.56±0.00   | 0.54±0.01   | 0.48±0.00   | 0.37±0.01   |
| EN OSA 300                | 0.63±0.00   | 0.59±0.01   | 0.55±0.00   | 0.53±0.01   | 0.41±0.00   |
| EN OSA 400                | 0.62±0.01   | 0.61±0.00   | 0.59±0.01   | 0.58±0.00   | 0.45±0.00   |
| EN OSA 500                | 0.77±0.00   | 0.69±0.01   | 0.69±0.00   | 0.59±0.01   | 0.56±0.01   |
| HMT EN OSA 200            | 0.67±0.01   | 0.28±0.01   | 0.24±0.00   | 0.24±0.01   | 0.20±0.01   |
| HMT EN OSA 300            | 0.76±0.01   | 0.66±0.00   | 0.48±0.00   | 0.19±0.00   | 0.09±0.00   |
| HMT EN OSA 400            | 0.80±0.00   | 0.71±0.01   | 0.58±0.01   | 0.24±0.01   | 0.11±0.01   |
| HMT EN OSA 500            | 0.80±0.00   | 0.74±0.00   | 0.66±0.01   | 0.59±0.00   | 0.14±0.01   |

Data are expressed as mean of 3 determinations ± standard deviations. Values with different letter in the same column are significantly different (p < 0.05). N: native; EN: enzyme; HMT: heat moisture treatment; OSA: octenyl succinic anhydride.
OSA, although the oil droplets were observed to be the largest, however, the starch granules are clearly seen on the drop surface with no gaps between the oil droplets. A higher degree of coverage for these drops has been formed by EN OSA.

A three-dimensional network had been formed, which could prevent the oil droplet from freely moving. Our results were consistent with Song et al. (2015), where the starch granules were observed to stabilize the oil-water interface in a closely packed layer. Among the samples, EN OSA shows the best emulsifying abilities as the drops were covered by the OSA modified sago starch granule and no gaps have been observed between the droplets.

4. Conclusion
Hydrophobic sago starch granules, especially when modified with OSA, could be used to stabilise emulsions. OSA modified sago starch with enzymatic pre-treatment had the highest emulsifying activity, higher and stable creaming index, and able to completely cover the oil droplets. Results revealed that it is possible to create an emulsion with OSA modified sago starch granule and no gaps have been observed between the droplets.

Conflict of Interest
The authors declare no conflict of interest.

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Figure 3. Micrographs of the O/W emulsions stabilized by (a) N-OSA modified (b) single pre-treated EN OSA modified (c) dual pre-treated HMT EN OSA modified sago starch after one day of storage at room temperature (starch concentrations 400 mg/mL oil).
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