



Research article

The role of breadfruit OSA starch and surfactant in stabilizing high-oil-load emulsions using high-pressure homogenization and low-frequency ultrasonication

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ARTICLE INFO

Keywords:

Food science
 Food analysis
 Food technology
 Breadfruit starch
 Emulsion stability
 Oil-in-water emulsion
 OSA starch
 High-oil-load emulsion

ABSTRACT

This study aimed to investigate the role of modified breadfruit starch in the presence of Tween 80 for stabilizing the oil-in-water emulsions. An ultra turrax homogenizer was used to produce coarse emulsions, followed by high-pressure homogenization (HPH) or low-frequency ultrasonication (LFU) for fine emulsions. The breadfruit starch was chemically modified using octenyl succinic anhydride (OSA) to produce modified breadfruit OSA starch (BOSA). The dispersed phase was a mixture of palm and lemon oil in a 9:1 ratio. Two BOSA (1% and 2%), three oil concentrations (10%, 25%, and 40%) and Tween 80 (1% of the total amount of oil) were examined based on the emulsion stability. The Fourier transform infrared spectroscopy (FTIR) indicated that starch modification was successful (Degree of Substitution-DS, 0.0241). The most stable coarse emulsions contained 40% oil and 2% BOSA starch. The same formula produced fine emulsions that remained stable for over 42 days, regardless of the homogenization method. BOSA starch and Tween 80 exhibit a mixed stabilization effect on the oil-in-water emulsions. HPH produced more uniformly sized emulsion droplets when compared with those produced using LFU.

Practical applications

Combination of breadfruit OSA starch (BOSA) and Tween 80 can be used to stabilize high-oil-load emulsions. Application of these mixtures can be used to substitute oil and fat for creating fat-reduced or low-fat food products such as margarine and butter. In addition, stabilization of emulsion-based food products such as in mayonnaise and salad dressing is also possible.

1. Introduction

The food emulsions are thermodynamically unstable and tend to break down over time; therefore, stabilizers should be incorporated into emulsions (McClements, 2015; Friberg et al., 2003). The stabilizers comprise emulsifiers and texture modifiers depending on their mechanisms for stabilizing the emulsions (Dickinson, 2003). Emulsifiers support the formation of emulsions and improve their stability by reducing the interfacial tension and building protective thin layers around droplets to prevent their aggregation (McClements, 2000).

Emulsifiers, such as surfactants, stabilize the emulsions by the adsorption of small surface-active molecules to the surface of the emulsion droplets. Surfactants reduce the surface and interfacial tension at the oil-in-water (O/W) interfaces and forming film layers, thereby preventing droplet aggregation. Tween 80 or polysorbate is a nonionic surfactant commonly used in food industries. Hsu and Nacu, 2003 studied behavior of soybean oil-in-water emulsion stabilized by various nonionic surfactants and found that at 10% (w/w) of Tween 80-oil proportion, the emulsion droplets were larger if the weight percentage of the oil were lower.

Certain proteins, polysaccharides, phospholipids, and solid particles are examples of emulsifiers (Stauffer, 1999). The texture modifiers stabilize the emulsions by increasing the viscosity of the aqueous phase to minimize the movement of oil (Bais et al., 2005). Many polysaccharides and proteins, such as starch and modified starch, cellulose and modified cellulose, pectin, alginate, carrageenan, gelatin, whey protein, caseinate, soy protein, and egg protein, are texture modifiers and can be used as thickening and gelling agents (Cui, 2005).

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Starches have been extensively used as stabilizers in food industries, mainly to create fat-reduced or low-fat food products. The stabilization of food emulsions using starch granules is renowned as a form of stabilization using solid particles, and such emulsions can be referred to as Pickering emulsions. This type of emulsion was initially investigated by Pickering (1907). During the previous decade, intensive research has been conducted to investigate emulsion stabilization using starch (Tesch et al., 2002; Timgren et al., 2013; Yusoff and Murray, 2011).

The modification of starch using octenyl succinic anhydride (OSA) became a central research interest during the previous decade. The OSA starches are recognized as important stabilizers because of their surface active properties (Bao et al., 2003). They exhibit unique characteristics because their hydrophilic sites gain hydrophobic elements in the form of octenyl groups, resulting in the whole molecules exhibiting amphiphilic characteristics. The stabilization mechanism based on OSA starches exploits the hydrophobic and steric contributions of OSA (Sweedman et al., 2013).

The OSA starches are scientifically proven to be excellent stabilizers for food, cosmetics, and pharmaceutical applications (Chanamai and McClements, 2001; Viswanathan, 1999), mainly because they are soluble in cold water. The OSA starches have also been used to stabilize the emulsion-based products exhibiting low oil contents, such as beverages (Chanamai and McClements, 2001), and those with high oil contents (more than 80%), such as mayonnaise and salad dressings (Hayati et al., 2009).

Among the starch sources, the breadfruit starch can be potentially used as a stabilizer in food emulsions. Anwar et al. (2016) explored the possibility of the usage of native starches, including jicama, rice, and breadfruit, as stabilizers in the O/W emulsions. Further, they observed that the breadfruit emulsions were more stable when compared with the rice and jicama emulsions. The breadfruit starch was further modified using OSA to improve its stabilization ability. In another study, Anwar et al. (2017) used a mixture of maltodextrin (7.5%) and breadfruit OSA starch (BOSA, 7.5%) to stabilize 10% fish or microalgal oils under both unheated and preheated conditions. Further, the results revealed that a preheated mixture of maltodextrin and BOSA provided the highest stabilization for both fish and microalgal oil emulsions. This study also proved that BOSA was potentially suitable for usage as a stabilizer for emulsions containing polyunsaturated fatty acids.

Based on the previous observations, this research was designed to examine the ability of the BOSA starch to stabilize the O/W emulsions in the presence of Tween 80 as a surfactant using a high-pressure homogenizer and a low-frequency ultrasound processor. The emulsions were prepared using different oil concentrations (25% and 40%) for coarse emulsion, (10%, 25%, and 40%) for fine emulsion, and the stabilization ability of the low BOSA starch concentrations (1% and 2%) was tested.

Previous studies related to the interaction of polymer – surfactant in emulsion revealed that micellar aggregation of surfactant and cloud point can be altered by the presence of polymer in surfactant aqueous solution (Alami et al., 1996). The interaction mechanisms of polymers particularly protein and polysaccharide with surfactant might occur through electrostatic and hydrophobic interactions (Singh and Nilsson, 1999). Combination of hydrophobic and hydrophilic interactions was reported occurred between xanthan gum and of Tween 80 as well as the formation of hydrogen bond between them. The surface tension measurement indicated that the interaction of polymer-Tween 80 mainly in the bulk. The interaction mechanisms were also affected by the added polymer concentration (Krstonošić et al., 2019).

2. Materials and methods

Breadfruit starch was extracted from the local breadfruits cultivated in the Aceh Province, Indonesia. 2-octen-1-ylsuccinic anhydride was purchased from Sigma-Aldrich (Singapore). Tween 80 (polysorbate) was purchased from Merck, Darmstadt, Germany. The dispersed phase contained palm oil (purchased from a local supermarket in Banda Aceh, Indonesia) and Class A lemon oil (purchased from CV Karunia, Atsiri

Harapan, Surabaya, Indonesia). Other chemicals were of analytical grade. Further, double-distilled water was used to prepare all the emulsions in this study.

2.1. Breadfruit starch extraction and modification

The breadfruits were peeled, cut into small pieces, washed, and blended to form a porridge. Water was then added to the starch porridge in a 2:1 ratio, and the mixture was left for 24 h. There was no bleaching treatment during the extraction process. Further, the starch slurries were separated from the water by natural gravity. The sediment was decanted and dried in an oven for 7 h at 50 °C. Subsequently, the dried starch was collected and sieved at 60 mesh.

The modification of the breadfruit starch was initiated by dissolving 125 g of starch in distilled water, and the pH was adjusted using 2% NaOH to 8.0 ± 0.2. 3% OSA was carefully added dropwise into the starch suspension. During the modification process, the pH should be maintained between 8.0 - 8.5 by adding NaOH and continuously stirring for 2 h. Finally, the pH was reduced to 6.5 using HCl. The solution was washed thrice using 250 ml of water, centrifuged at 5,000 rpm for 2–3 min, and dried in an oven at 40 °C for 24 h (Bhosale and Singhal, 2006).

2.2. Determination of DS of BOSA

Determining the DS in BOSA involves the estimation of the average number of hydroxyl groups substituted per glucose unit, which was performed using the method of (Whistler and Paschall, 1967). Briefly, OSA starch solution was prepared by dilution 5 g of OSA starch into 50 ml distilled water. Twenty-five ml of 0.5 N NaOH solution was added into the modified starch solution and shaken for 24 h. Phenolphthalein was used as an indicator before titration of excess alkali was performed with 0.5 N HCl. Similar procedure was done for native unmodified starch to prepared a blank. The calculation of % OSA substitution was following the equation:

$$\%OSA\ substitution = \frac{(V\ blank - V\ sample) \times 0.1 \times N \times 100}{W} \quad (1)$$

where W = weight of sample

N = Normality of HCl solution

V_{blank} = volume of HCl required for blank titration

V_{sample} = volume of HCl required for sample titration

The degree of substitution (DS) was determined from % OSA substitution as follows:

$$DS = \frac{162 \times \%OSA\ substitution}{21,000 - (209 \times \%substitution)} \quad (2)$$

where 162 = molecular weight of glucose unit

21,000 = 100 x molecular weight of octenyl succinyl group

209 = molecular weight of octenyl succinyl group

The breadfruit starch was analyzed before and after modification with OSA to characterize the degree of substitution (DS), and substitution analysis was performed using Fourier transform infrared spectroscopy (FTIR).

2.3. FTIR analysis

Hydroxyl group substitution by the carbonyl groups of OSA was confirmed using FTIR (Wang et al., 2013). FTIR (Agilent Resolution Pro Cary 630) was used to obtain the IR spectra of native starch and BOSA. Briefly, the sample (1.5 mg) was ground with potassium bromide (KBr) and pressed to form a pellet disk. The disk was placed in the sample compartment and scanned over the wavelength range of 500–4600 cm⁻¹.

2.4. Preparation of coarse oil-in-water emulsions

In case of coarse emulsions, the aqueous OSA starch solutions (1% and 2%) were prepared by dispersing the OSA starch in water at room temperature, which was followed by heating to 60 °C for 10 min while stirring to ensure the complete hydration of starch before homogenization. The dispersed phase contained a mixture of palm and lemon oils at a ratio of 9:1 (palm oil:lemon oil). Tween 80 was added (1% of the total amount of oil) to the oils, and the mixture was homogenized using an ultra turrax T25 homogenizer at 10,000 rpm for 1 min. Finally, 25% or 40% oil was slowly added to the starch solution, and homogenization was continued at 14,000 rpm for 3 min.

2.5. Preparation of fine emulsions

Fine emulsions were produced using high-pressure homogenization (HPH) and low-frequency ultrasonication (LFU). Two BOSA (1% and 2%) and three oil concentrations (10%, 25%, and 40%) were examined based on the emulsion stability. In both cases, emulsification was initiated by the formation of a coarse emulsion (as mentioned above). For HPH, the coarse emulsion was continued homogenized by passing the emulsion thrice (three cycles) through a high-pressure homogenizer (GEA Niro-Soavi Panda PLUS, Italy) at 300 bar. In case of LFU (20 kHz), a laboratory-scale batch ultrasonic apparatus (Qsonica Q700 Sonicator, USA) was used to further homogenized the coarse emulsion. The ultrasonic probe was centrally immersed in an 85-ml coarse emulsion. The immersion depth was 1 cm with a sonication time of 180 s and an ultrasonic amplitude of 35%.

2.6. Measurement of the coarse emulsion stability

2.6.1. Viscosity

The emulsion viscosities were measured using a Brookfield DV-II + Pro Viscometer. Briefly, the emulsion was poured into a 600 ml beaker glass and measurement conducted at ± 28 °C with speed of 100 rpm. All measurements were performed in triplicate and the viscosity was expressed in centipoise (cP).

2.6.2. Creaming index (CI)

The emulsion stability was determined by testing the acceleration using centrifugation (at 4500 rpm for 5 min) before calculating the creaming index (CI, McClements, 2007). The emulsion CI was calculated according to the following equation:

$$CI = H_S/H_E \times 100\% \quad (3)$$

Here,

H_S = Height of the serum layer (ml)

H_E = Height of the initial emulsion (ml)

2.6.3. Emulsifying activity (EA)

Additionally, the stability was examined by measuring the separation of the cream and serum layers during storage for ten days and by calculating the emulsifying activity (EA) according to the method published by Wu (2001). The emulsions were kept in measuring cylinders (10 ml) and stored at ambient temperature (± 25 °C). Observation was done daily by reading the separation volume of cream and serum layers. EA was calculated as:

$$EA(\%) = \frac{\text{Height of emulsified layer}}{\text{Total height of mixture in measuring cylinder}} \times 100\% \quad (4)$$

2.6.4. Droplet imaging via photomicroscope

In order to observe the droplets size and distribution, a drop of emulsion was placed onto the microscope slide and covered carefully. Images were then captured using Olympus photomicroscope (type

BX41TF - Tokyo, Japan) which was equipped with Olympus DP12 camera. The magnification of 40x was selected to produce photomicrographs.

2.7. Measurement of the fine emulsion stability

2.7.1. Average droplet diameter

The emulsion was prepared by dilution with distilled water in a 500-ml beaker and gentle agitation using a glass rod. The emulsion droplet diameter was subsequently determined by dynamic light scattering using a Zetasizer Nano Particle Analyzer (Malvern Instruments, UK). A helium–neon laser (4 mW) was operated at 633 nm with the scattering angle being fixed at 173° and the temperature being maintained at 25 °C. All the measurements were performed in triplicate (Li et al., 2010).

2.7.2. Monitoring the visible boundaries during storage

The emulsions were placed in 10-ml measuring cylinders, wrapped, and stored at room temperature. The emulsion separation was observed daily for 42 days to observe any visible boundary formation. Any separation of oil, emulsion, and serum layers in the tube observed during the storage was recorded.

2.7.3. Emulsion droplet imaging via transmission electron microscopy

The emulsion samples were diluted with distilled water 100 times and were subsequently dropped on a Formvar/carbon 400-mesh Cu grid and settled for 1 min before absorbing the excess water using a filter paper and allowing them to dry. Two percent aqueous uranyl acetate was used for negative staining. The grid was further placed in a sample holder, inserted in the transmission electron microscopy (TEM) apparatus (FEI Tecnai 2 Spirit 1200) and observed at a high tension of 120 kV. Images were recorded using a MegaView G2 CCD camera (Olympus) (Xiang et al., 2016).

2.8. Statistical analysis

All the experiments were performed in duplicate or greater. The significant differences with respect to the coarse emulsion viscosity, CI, EA, and average droplet size of the fine emulsions were analyzed using two-way analysis of variance (ANOVA, $P < 0.05$). Statistical analysis was conducted using SPSS version 20 (SPSS Inc., Illinois, USA). Significantly different groups were compared using the least significant difference (LSD) tests at a 95% confidence level.

3. Results and discussion

3.1. Starch characteristics

The modification of breadfruit starch resulted a degree of substitution (DS) of 0.0241. The FTIR spectra of the breadfruit starch before and after modification are presented in Figures 1 and 2. Generally, both the spectra exhibit similar profiles, where broad peaks around 3400 cm^{-1} indicate the presence of hydroxyl groups (O–H) as reported in many other literatures (Bai et al., 2009; Cui, 2005; Shingel, 2002). The peaks at approximately 2900 cm^{-1} represent the C–H stretching vibration, whereas those around 1600 cm^{-1} represent the adsorbed water bending vibration (Cui, 2005; Miao et al., 2014; Simsek et al., 2015). Five peaks in the fingerprint region of starch, renowned as the characteristic peaks of starch, appear at 800–1200 cm^{-1} , indicating the stretching of the C–O stretching (Miao et al., 2014).

Figure 2 confirms that the esterification was successful. This was proved by the existence of two additional absorption bands at 1720 and 1560 cm^{-1} . As reported by many other studies, the peak at 1720 cm^{-1} represents the C=O stretching vibration of an ester carbonyl group, whereas that at 1560 cm^{-1} corresponds to the stretching vibration of the carboxylate RCOO^- (Wang et al., 2013; Miao et al., 2014). These results denote that the hydroxyl groups in the starch are substituted with the ester carbonyl and carboxyl groups of OSA (Simsek et al., 2015).

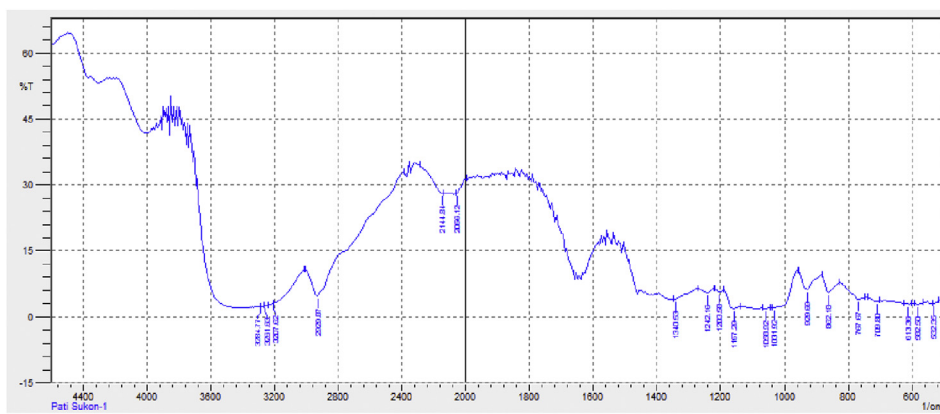


Figure 1. The Fourier transform infrared spectroscopy spectra of the native breadfruit starch.

3.2. Stability of the coarse emulsion

3.2.1. Viscosity

Viscosity is defined as the flow resistance of the fluid and the gradual deformation of the fluid by shear stress; in case of liquids, it is related to the concept of thickness (Viswanath et al., 2007). Figure 3 presents the effects of the oil and BOSA starch concentrations on the coarse emulsion viscosity. Statistical analysis has denoted that both the variables significantly affect the emulsion viscosity. An increase in the oil content increased the viscosity. A viscous emulsion prevents the movement of droplets and minimizes the droplet coalescence. This is consistent with the observation of Dokić et al. (2012), who denoted that a large amount of dispersed phase in an emulsion that was stabilized by the OSA starch reduced the separation of cream in an emulsion.

In this research, viscosity is also related to the amount of added surfactant (Tween 80), which was 1% of the total oil. In an O/W emulsion, stabilization by a surfactant can be attributed to its lipophilic tails, which strongly interact with oil, and hydrophilic heads, which adsorb onto the water or the dispersed phase. An increase in the BOSA starch concentration also significantly affected the emulsion viscosity. A previous research underlined the stability of menhaden oil-in-water emulsion can be improved by increasing the emulsifiers content which lead to the increased viscosity of the aqueous phase and counteracting flocculation of dispersed phase (Sun and Gunasekaran, 2009). Despite facilitates effective immobilization of dispersed oil droplets, emulsion with high viscosity also inhibiting gravity-induced creaming and serum separation during storage period (Dickinson, 2018).

3.2.2. Creaming index

This method is useful for evaluating and predicting the emulsion shelf life by indicating the coarse emulsion phase separation. The CI is an

emulsion stability tool, where a high CI indicates low emulsion stability during storage. Figure 4 presents the CI results, where both the variables observed, significantly affect the CI. Centrifugation accelerated the coarse emulsion separation and forced the oil droplets to agglomerate and produce larger droplets. This subsequently resulted in an increased CI. In this research, an increase in the oil and OSA starch concentrations was observed to decrease the CI. A coarse emulsion prepared with 40% oil and stabilized using a mixture of 2% OSA and Tween 80 exhibited the lowest CI. Several studies reported that increasing the volume of dispersed phase also resulting for improved emulsion stability. Sun and Gunasekaran (2009) found that increasing the oil phase from 5-50% causing delayed in the creaming process mainly because of the greater packing of oil droplets in a volumetric unit. As the consequences, the emulsion viscosity increased and emulsion droplets migration retarded. This is also in agreement with Dickinson and Golding (1997). They indicated that the emulsion creaming was determined by the oil-phase volume fraction. Increasing the volume fraction of oil droplets can lowered the creaming rate due to the enhanced of emulsion viscosity.

Although centrifugation accelerated the separation, CI remained low in this study for coarse emulsions prepared using 2% BOSA. This indicated that most of the oil droplets were kept stable against separation forces using sufficient quantities of stabilizers, which, in this case, was a mixture of the surfactant (Tween 80) and the heated BOSA. The synergistic effects of high oil concentration and a mixture of Tween 80 and BOSA played an important role to stabilize the emulsion. It has proved that a thicker continuous phase creates more drag on the dispersed phase.

The roles of Tween 80 and biopolymers interaction in stabilizing food emulsion were also underlined by many others. Incorporation of skim milk powder + Tween 80 produced walnut oil emulsion with smallest droplets size. This was mainly caused by its better packing of small amphiphiles thus reduced the surface tension and interfacial free energy of

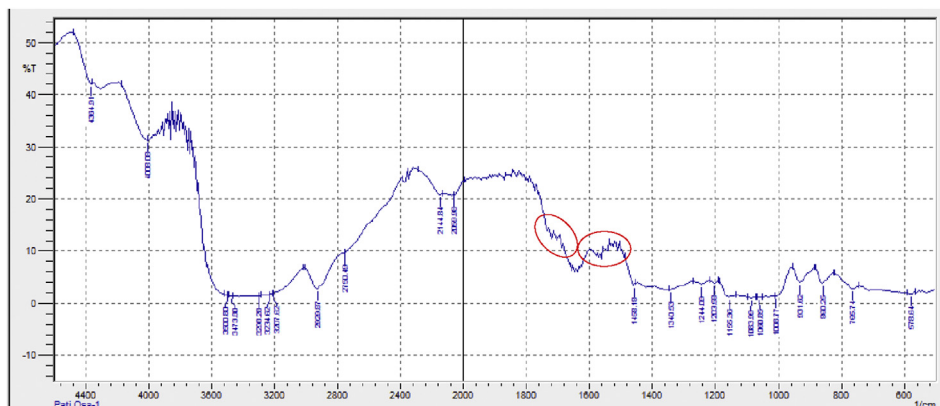


Figure 2. The Fourier transform infrared spectroscopy spectra of the modified breadfruit octenyl succinic anhydride starch (BOSA).

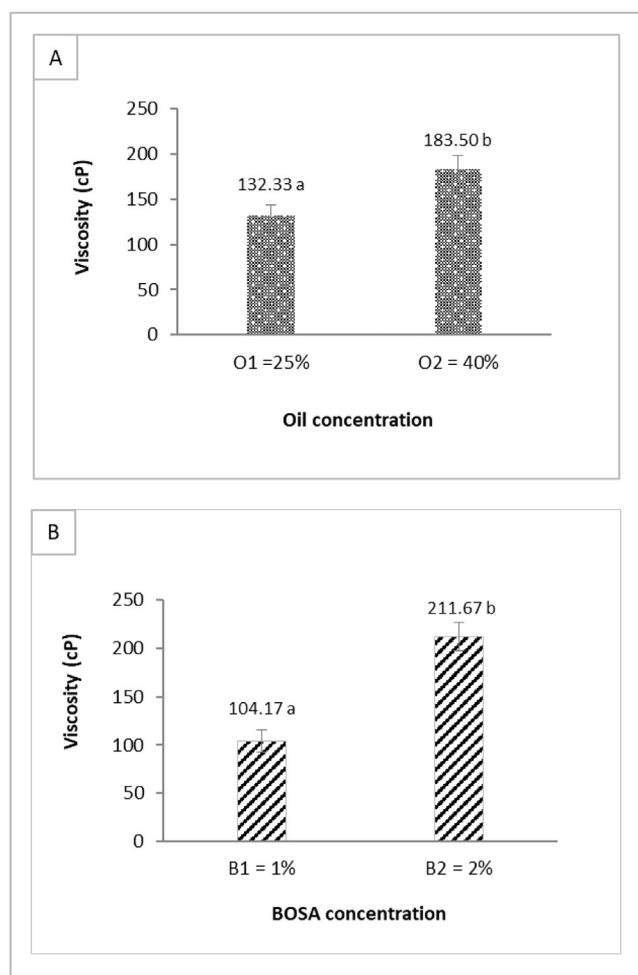


Figure 3. The effects of the oil and BOSA concentrations on the coarse emulsion viscosity: A) 1–2 % BOSA; B) 25–40% Oil. Each bar with different letters a or b are significantly different ($p < 0.05$).

the emulsion (Shamaei et al., 2017). Another study revealed that Tween 80 was also able to interact with gelatin based on the molecular structure. Interaction of branched Tween 80 molecules with gelatin caused changes in surface properties and emulsion viscosity (Sovilj et al., 2013).

3.2.3. Emulsifying activity

This measurement was conducted to observe the coarse emulsion separation by monitoring the visible boundary formation. The height of the emulsified layer (cream) or the remaining layer (serum) in the tube during storage was noted. The stability was expressed as the EA, as depicted in Figure 5.

As shown in Figure 5, reducing the concentrations of the BOSA starch and oil decreased the EA. However, the highest combined content of both the variables (2% BOSA starch and 40% oil) increased the EA. The decreasing EA observed in Figure 5 could be explained by the lower viscosity of emulsion prepared with lower concentration of oil (25%) and BOSA (1%). This condition allowed higher mobility of the dispersed phase, causing coalescence or flocculation, increased the probability of droplet aggregation thereby lead to emulsion instability.

The highest EA was 95.3%, which was observed on the first day of storage. Although a decreasing trend was observed, no significant decline ($p > 0.05$) could be observed up to seven days of storage. The EA slightly decreased to 85.3% at the end of storage (10 days). Similar patterns were perceived for the remaining two combinations (1% OSA starch and 40% oil; 2% OSA starch and 25% oil). The EA values for these combinations were almost identical throughout the storage period, and no significant decline ($p > 0.05$) could be observed among them.

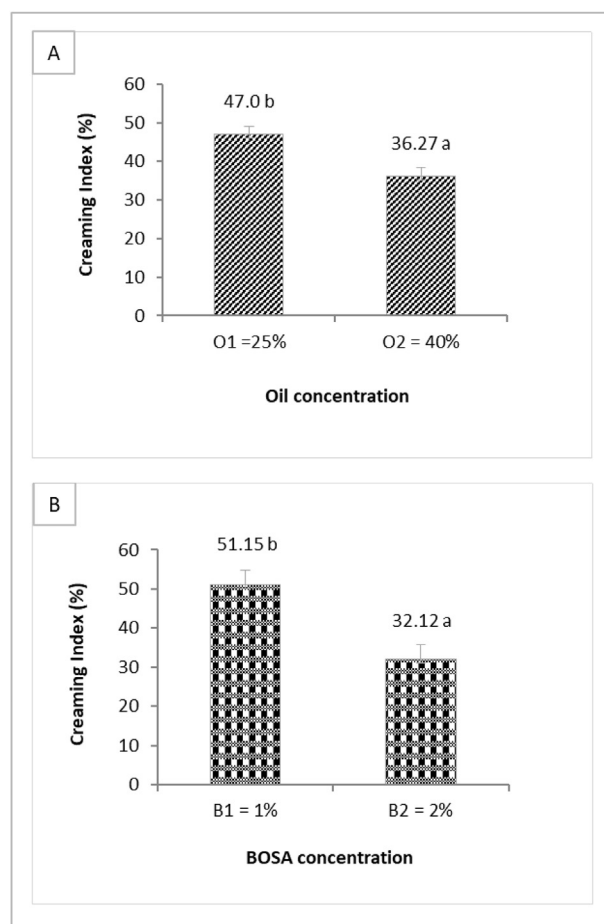


Figure 4. The effects of the oil and BOSA concentrations on the creaming index of the coarse emulsions: A) 1–2 % BOSA; B) 25–40% Oil. Each bar with different letters a or b are significantly different ($p < 0.05$).

3.2.4. Emulsion droplet distribution

The emulsion droplet distribution was measured to evaluate the size and distribution of emulsion droplets using a light microscope with 40× magnification. The results are presented in Figure 6, which illustrates polydisperse droplets with various sizes that partially coalesced to form large droplets. Figure 6c and d denotes the distribution of emulsion droplets with an oil concentration of 40%. It can be observed that the number of droplets is higher than that observed in the previous Figure (6a and b). Adding 2% OSA starch results in a uniform droplet size; coalescence can be minimized even though some droplet aggregation cannot be avoided.

3.3. Stability of fine emulsions

3.3.1. Average droplet size in fine emulsions

The average droplet diameter in fine emulsions was measured using a Malvern Zetasizer Nano Instrument after both HPH and LFU were conducted. The results are presented in Figure 7, where the lowest average droplet diameter (765 nm or 0.765 μm) was achieved using an emulsion prepared by LFU with 10% added oil. LFU also produced a micron-size emulsion (1.370 μm) after the incorporation of 40% oil. Although both the aforementioned processes exhibited similar trends, the average droplet diameter increased with increasing oil addition to the LFU emulsions only ($p < 0.05$), however the increments were statistically not significant ($p > 0.05$) in the HPH emulsion. HPH produced a more uniform average droplet size (0.964–1.099 μm), as can be seen in Figure 8a, when compared to that produced by LFU (Figure 8b).

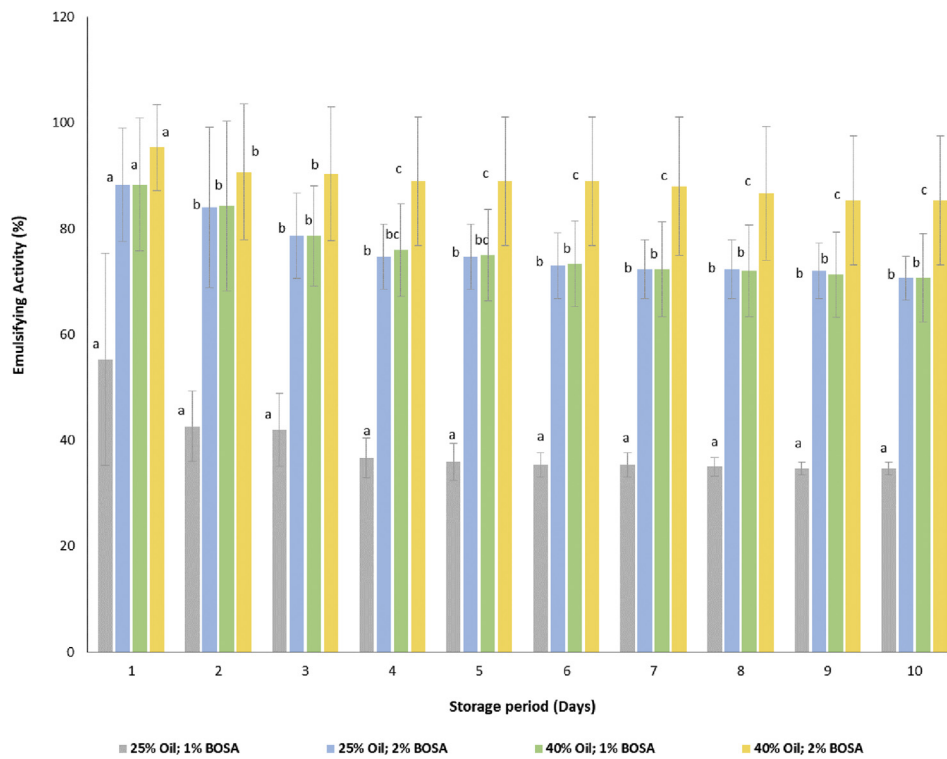


Figure 5. The emulsifying activity of the coarse emulsion during the storage test. The bars in each day of storage (where the samples prepared in different conditions) followed by different letter a, b or c are significantly different ($p < 0.05$).

HPH and LFU exhibit distinct differences. LFU is based on mechanical vibrations, which are amplified and transmitted down the length of a probe or horn to which the tip is attached. During emulsification, rapid tip vibration causes cavitation by which microscopic bubbles are observed to form and collapse (Servant et al., 2001). Tremendous energy is released in the cavitation field when thousands of cavitation bubbles collapse. Further, the fluid mechanism in LFU is determined by the

erosion and shock effects of the collapsing cavitation bubble. Therefore, the primary dispersed oil in an emulsion can rapidly break down into droplets of micron and submicron sizes (Gogate et al., 2011; Abismail et al., 1999).

As depicted in Figure 7, the average droplet diameter increased significantly as the oil percentage increased from 10% to 40% when a fine emulsion was homogenized using LFU (confirmed by particle size

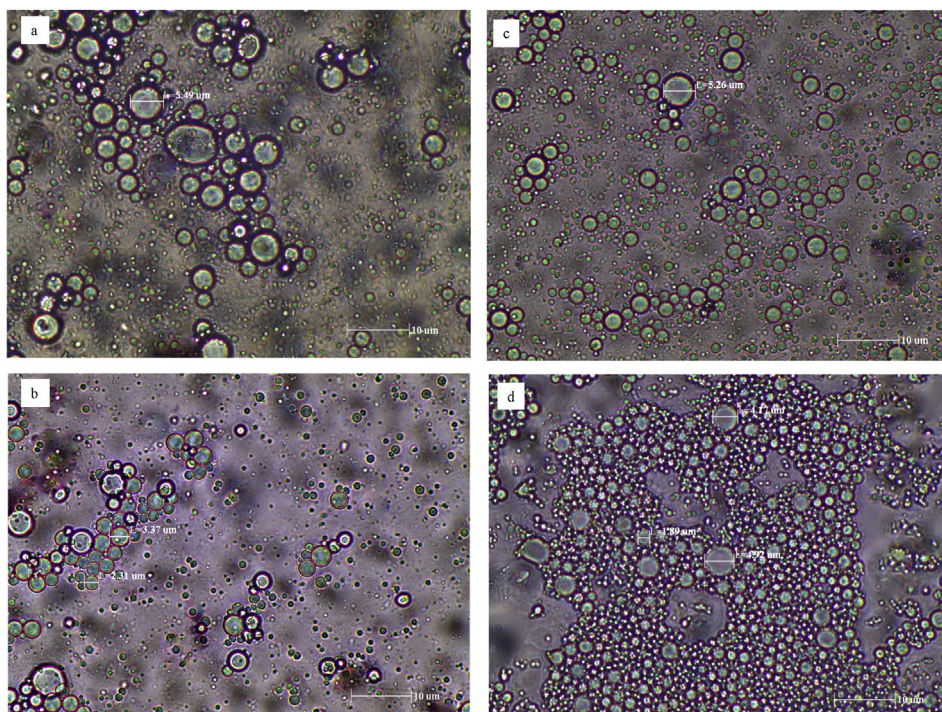


Figure 6. Microscopic images of the coarse emulsion: (a) 25% oil and 1% BOSA, (b) 40% oil and 1% BOSA, (c) 25% oil and 2% BOSA, and (d) 40% oil and 2% BOSA.

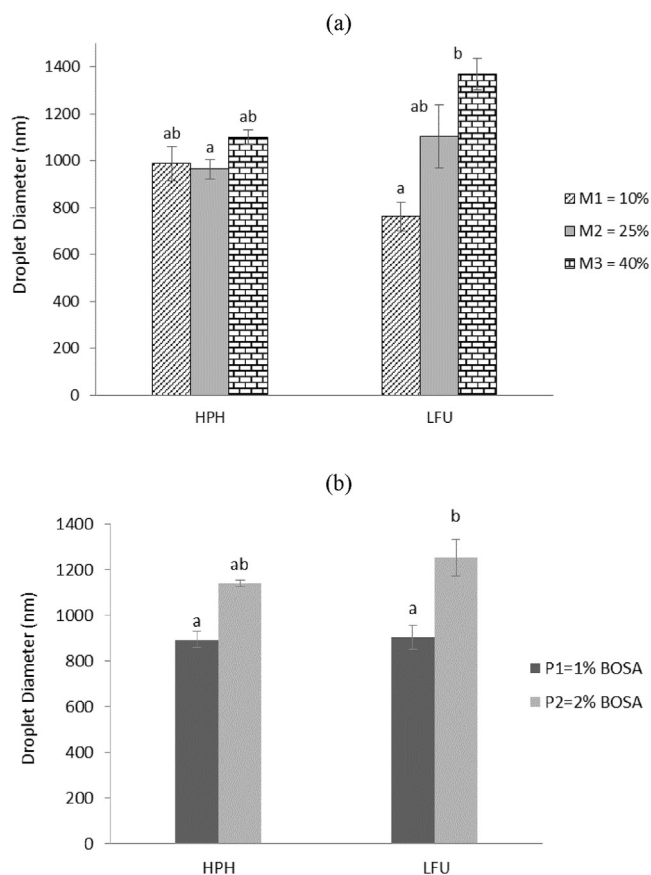


Figure 7. The droplet size of the fine emulsions measured using a particle size analyzer: (a) comparison of HPH and LFU based on oil percentage (25%–40%); (b) comparison of HPH and LFU based on BOSA percentage (1%–2%). Each bar with different letters a or b are significantly different ($p < 0.05$).

distribution in Figure 8b). This indicates that the ultrasonication is efficient when the proportion of dispersed phase in emulsion is lower. The acoustic energy emanating from the probe tip can be absorbed efficiently in this case. Further, the size reduction in LFU is attributed to the high shear forces associated with the ultrasonic cavitation in liquid media (Trujillo and Knoerzer, 2011).

Significant increment in the average droplet diameters when 40% of oil was added can be caused by the increase of dispersed phase volume which required more energy to break the oil into small droplets. In fact, the supplied energy for emulsification process remained the same (either for 10% oil or 40% oil) thus the same level of power dissipation cannot break the droplets efficiently at higher oil volume, and hence, the droplet size increased (Ramisetty et al., 2015).

However, the differences between the average droplet diameters of the fine emulsions prepared using HPH were not statistically significant ($p > 0.05$) as shown in Figure 7a, b and confirmed by Figure 8a. This indicated that the average droplet diameter was not significantly influenced by the percentage of added oil. In this study, the HPH mechanism allows emulsion stabilization with a wide range of oil loads, i.e., 10%, 25%, and 40%. A similar observation was reported by Silva et al. (2015); here, the oil content of the nanoemulsions prepared by HPH did not significantly affect the hydrodynamic diameter (H_d) in statistical terms.

HPH splits large droplets of a previously prepared coarse emulsion into smaller droplets using high pressure through a narrow gap or a small-diameter hole (Kaci et al., 2017). The emulsion droplet formation by this process is dependent on the droplet break up and coalescence. The droplet break-up is controlled by the amount and type of shear given by the device as well as the droplet resistance to deformation (Tadros et al., 2004). Additionally, the droplet coalescence rate is governed by how

quickly the surfactant can be absorbed into the surface of a newly formed droplet, the type and concentration of the surfactant, and the surface activity of the surfactant (McClements, 2015).

This research used a combination of surfactant (Tween 80), which constituted 1% of the total oil content, with 1% or 2% BOSA. The percentage of BOSA was calculated based on the final emulsion volume. Tween 80 has three polyoxyethylene (POE) chains derived from polyethoxylated sorbitan and oleic acid which is able to reduce the surface tension. Similarly, OSA starch can behave as surface-active molecules (Nilsson and Bergenstahl, 2007) and also called as a new class of polymeric surfactant (Holmberg et al., 2002) which exhibit typical behavior for polyelectrolyte aqueous solution and reduced viscosity at low concentration.

Although the average droplet diameters for the LFU emulsions were statistically significant ($p < 0.05$), the results denoted that the average droplet diameters of HPH emulsions were not statistically significant ($p > 0.05$) regardless of the BOSA percentage used (Figure 7b). This observation is consistent with that of O'Sullivan et al. (2014). They also observed no significant differences between the emulsion droplet sizes at emulsifier concentrations greater than 0.5 wt.% for emulsions fabricated with Tween 80 and for the milk protein isolate prepared by high-pressure valve homogenization.

A minimum stabilizer (surfactant and modified starch combination) concentration is required for stabilizing the emulsion interface. If this condition is fulfilled then a submicron-size emulsion can be obtained. However, once a sufficient concentration is reached, excess stabilizer will remain in the continuous phase (O'Sullivan et al., 2015). The highest average droplet diameter (1.340 μm) was observed for an emulsion prepared by LFU with 40% added oil. This condition can be caused by the recoalescence of the emulsion droplets because of insufficient amount of surfactant and BOSA. The factors involved in the recoalescence phenomena using ultrasonic equipment were also highlighted by Jafari et al. (2007). A combination of the low adsorption rate of emulsifier and the high energy density can be considered to be the most important factor. The former was related to a low emulsifier concentration, whereas the latter was related to ultrasonication, where the amount of droplet collision increases, particularly in the emulsification area close to the probe tip.

3.3.2. Fine emulsion droplet size and shape using transmission electron microscopy

The droplets of a fine emulsion were carefully evaluated using TEM to observe their size and shape. Figure 9a depicts the emulsion droplets at 18,500 \times magnification, where small oil droplets (<100 nm) were observed to aggregate to form large entities. A similar figure was obtained by Silva et al. (2015) in which a nanoemulsion of the MCT oil was observed to be stabilized by anionic, cationic, and nonionic surfactants. Figure 9b and c denote the emulsion droplets at 37,000 \times magnification. These figures show relatively round droplets surrounded by dark layers that are considered to be surfactants and modified starch layers absorbed into the interfacial membrane. Similar findings also reported by Sjöö et al. (2015). They showed the oil droplets surrounded by the barrier layer formed by gelatinization of heat-treated starch at the interface. These starch layers were confirmed from the micrographs as the dark layers at the interface. In another study done by Fernandez-Avila and Trujillo (2016), the dark layers resembled the protein layers surrounding the oil droplets.

3.3.3. Visible boundaries of fine emulsion during storage

Fine emulsions were stored at room temperature, and their stability was monitored for 42 days. Figures 10 and 11 denote the stability of the emulsions prepared by LFU and HPH, respectively. As depicted in Figure 10, all the LFU emulsions were stable for 13 days except for the M2P2 emulsion (a mixture of 25% oil and 2% BOSA starch) in which serum separation had already started 24 h after homogenization and increased slowly until the end of the storage time. Similarly, the same

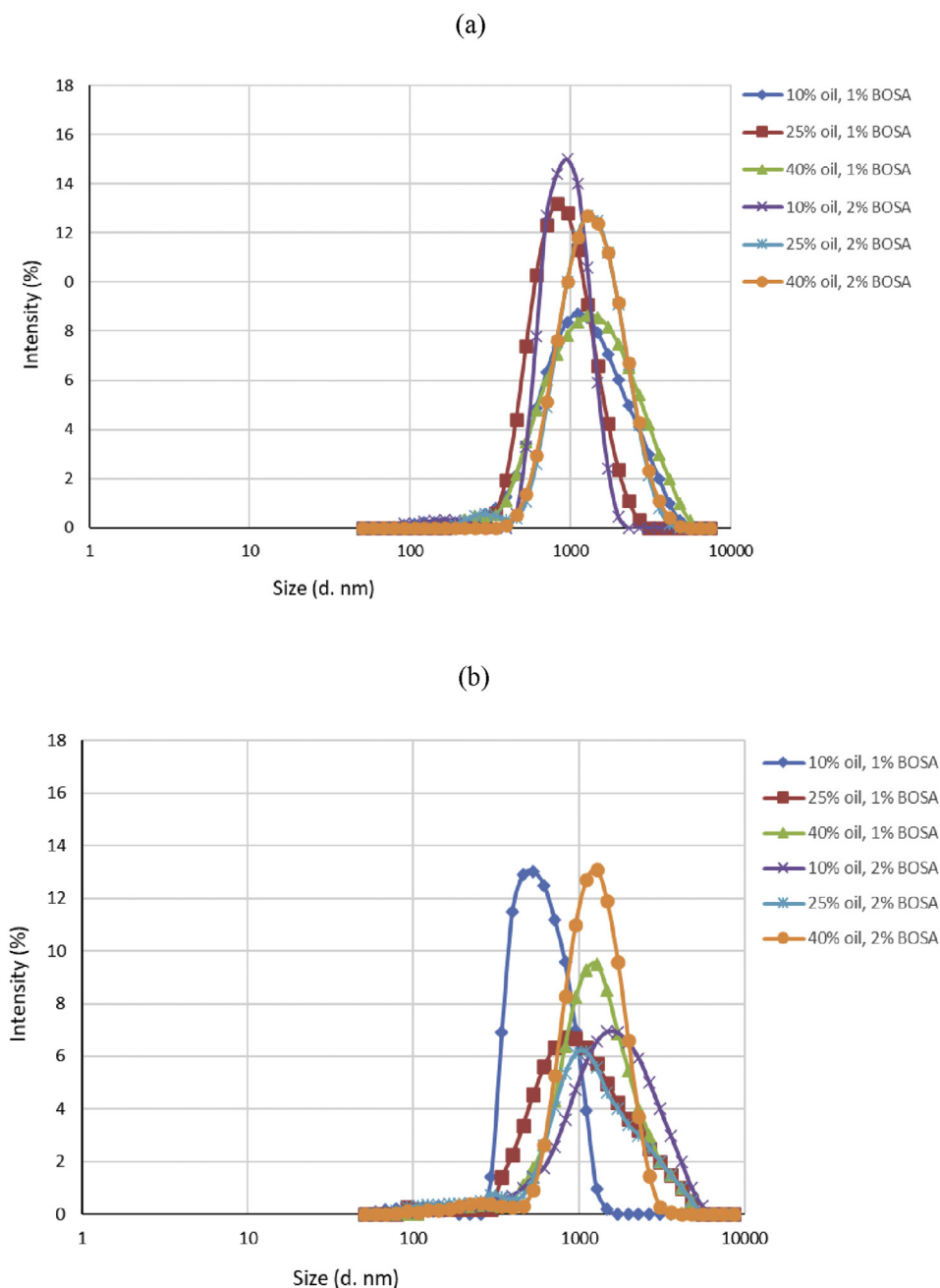


Figure 8. Particle size distribution of emulsion droplets based on intensity produced by: (a) HPH process and (b) LFU process.

formula (M2P2), prepared using HPH, exhibited serum separation two days after emulsification (Figure 11).

The lowest stability was exhibited by the M1P1 emulsions prepared using both HPH and LFU. The M1P1 emulsion prepared by LFU (Figure 10) was stable for only 13 days and started to separate the following day. Over 4 ml of the serum layer was formed after 14 days of storage, and oiling off occurred after 28 days. The oil phase was distinctly observed (2.5 ml) at the end of the storage test (day 42). Similarly, the M1P1 emulsion prepared by HPH exhibited a similar behavior (Figure 11). Separation by oiling off was noticed on day 21 and increased by more than 1 ml at the end of the storage test. The oil separation phenomena in this study were observed for emulsions with the lowest oil concentration, i.e., 10%; these emulsions exhibited the lowest average droplet diameters (Figure 7). The lower stability of emulsions containing 10% oil phase could also be explained by the viscosity of the emulsion where at 10% the emulsion viscosity is lower, which allows higher

mobility of the droplets thus causing coalescence. According to Silva et al. (2011), nanoemulsions with low sizes have a high tendency to aggregate because they exhibit a considerable susceptibility to Brownian motion. This condition may increase the probability of droplet collision, leading to aggregation as a trigger for emulsion instability.

Generally, HPH produced more stable emulsions when compared with those produced by LPU. The addition of 40% oil in each process in combination with 1% and 2% BOSA starch resulted in improved emulsion stability. The most stable emulsions (42 days of storage) were those prepared by 40% oil and 2% BOSA starch (M3P2) using either LPU or HPH.

Starch modification by OSA improves the native starch properties, particularly the solubility in cold water. Its application at high concentration resulted in a low-viscosity emulsion and its emulsifier function relies on short octenyl succinate side chains that drive the OSA molecules to the interface and form a strong film, and amylopectin prevents the

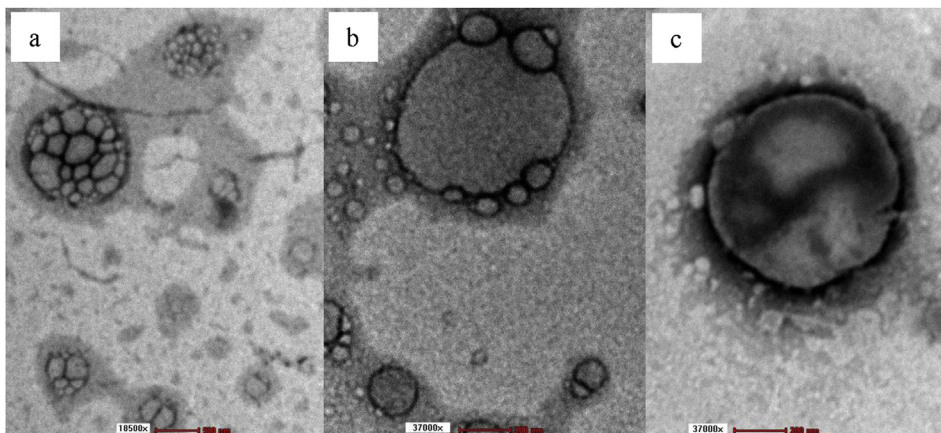


Figure 9. The fine emulsion droplet size and shape determined by transmission electron microscopy at: (a) 18,500× magnification, (b) and (c) 37,000× magnification.

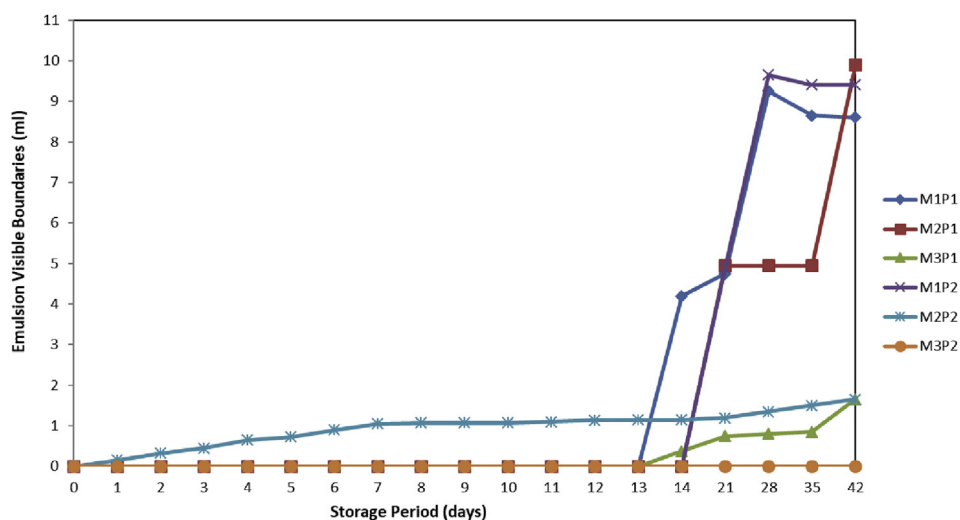


Figure 10. The stability test on fine emulsions prepared by LFU process based on the formation of visible boundaries during storage at room temperature.

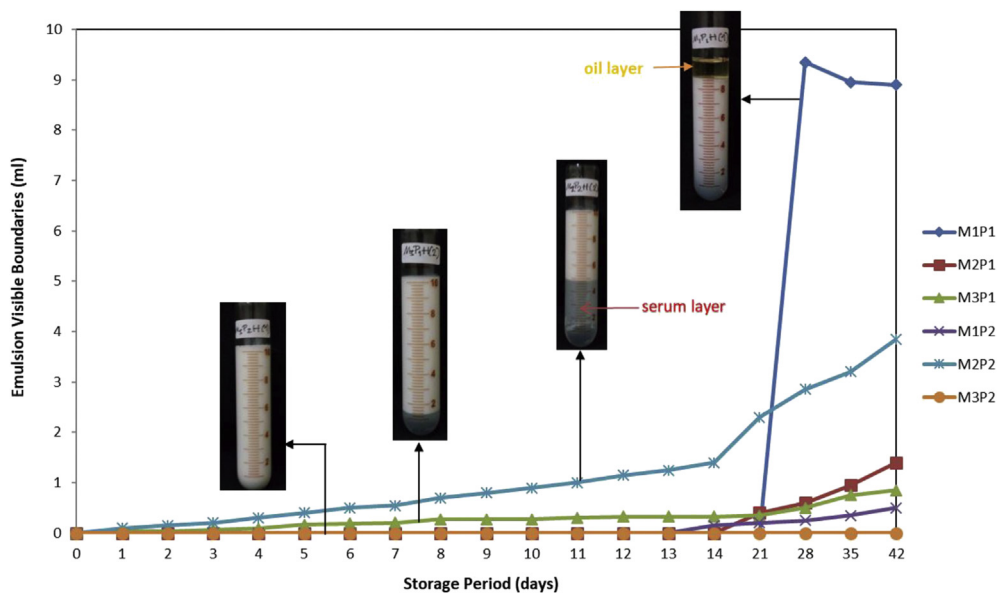


Figure 11. The stability test on fine emulsions prepared by HPH process based on the formation of visible boundaries during storage at room temperature.

droplet agglomeration (Dickinson, 2009). These observations are also presented in previous studies (Dokić et al., 2012; Tesch et al., 2002), where OSA starch produced stable emulsions at low concentrations (1%–2%).

This investigation used BOSA dispersion in water, which was heated to 60 °C and hold for 10 min. Oil was added to the continuous phase at room temperature, which was followed by continuous mixing. According to our previous observation, the gelatinization temperature of BOSA starch was ± 71 °C (Anwar et al., 2017). Heating BOSA to the pre-gelatinization temperature (60 °C) caused the starch granules to swell. Swollen granules may be disrupted during emulsification and release starch molecules. Therefore, the oil droplets in the current study may be stabilized by the starch molecules at the oil–water interface. This is consistent with the observation from our earlier investigation (Anwar et al., 2017). Sjöo et al. (2015) explained that heating starch before or after emulsification induced a different stabilization mechanism. Stabilization by intact starch granules can be observed when the emulsion was not heated. However, when starch was heated prior emulsification then the emulsion stabilized by the starch molecules leaching from starch granules or fragments of gelatinized starch disrupted during the emulsification process.

This research revealed that a higher oil content was stabilized by only 2% BOSA starch to produce a stable emulsion (both coarse and fine emulsions) when compared with the amount of stabilization by 1% OSA starch. High percentage of oil produce dense emulsion droplets. The addition of BOSA starch to such systems increased the emulsion viscosity. A viscous continuous phase ensured stability by minimizing the droplets movement in the system and preventing droplets aggregation and coalescence. The presence of Tween 80 as a surfactant might be doubled the droplet protection by forming a thin layer surrounding the oil droplets; according to Porter (1994), this also reduced the interfacial tension.

Pichot (2010) explained the manner in which the low-molecular-weight surfactant, including Tween 80, protects the oil droplets. Immediately after homogenization, the droplet size was observed to drastically decrease, whereas the “naked” interfacial area rapidly increased. Under these conditions, Tween 80 quickly assembled at the newly formed interface, promoting further break-up by reducing the interfacial tension and stabilizing the emulsion.

This study explored the stabilization mechanism by adding a mixed emulsifier (BOSA starch and low-molecular-weight surfactant) to the O/W emulsion. The stability provided by a mixed emulsifier system can be explained by the aforementioned statement from Pichot (2010). Additionally, the BOSA starch plays an important role not limited to thickening the continuous phase because it also functions as an emulsifier (polymeric surfactant). Therefore, all the available oil–water interfaces can be coated with double layers formed by both the surfactant and OSA starch.

Tween 80 is an O/W surfactant, and this type of surfactant emulsion microstructure is dependent on the surfactant concentration. The displacement of colloidal particles (OSA starch) from the interface is possible when the surfactant concentration increases because of strong competition among the particles for absorption at the interface and the interaction between the surfactant and the particles (Pichot, 2010).

In this study, the synergistic effect of Tween 80 and BOSA on surface tension could be a result of surface-active complex formation of mixture stabilizers on emulsion interface. Chanamai and McClements (2002); Charoen et al. (2011) and Dickinson (2018) stated that droplets coated with OSA starch remain highly stable with respect to coalescence and flocculation once the state of surface saturation has been reached. According to Nilsson et al. (2007), amylopectin fraction in OSA starch is responsible for interfacial functionality and during emulsification process these big molecules are quickly moved to the newly created oil–water interfaces. The proportion of amylopectin in BOSA starch was dominant (72.38%) compared to only 27.62% of amylose (Anwar et al., 2016). The amylopectin structural attributes that contribute to the steric stabilization effectiveness are its large molecule size, its high backbone rigidity

and its extensive chain branching (Sweedman et al., 2013; Dokić et al., 2008).

4. Conclusions

This study proved that the BOSA starch is a potential stabilizer for the O/W emulsions. The concentrations of the BOSA starch and oil significantly affected the emulsion viscosity, CI, and EA. Increasing the BOSA starch and oil concentrations improved the emulsions stability. A coarse emulsion that was prepared by adding 40% of oil and 2% of BOSA starch was observed to be the most stable, exhibited the lowest CI with EA of 85.3% after 10 days of storage. Increased the volume fraction of oil droplets enhanced emulsion viscosity thus created more drag on the dispersed phase. The same formula also produced fine emulsions that remained stable for more than 42 days, regardless of the homogenization method. HPH produced a more uniform average emulsion droplet size when compared to that produced by LFU. HPH mechanism allows emulsion stabilization with a wide range of oil loads whereas LFU process is efficient when the proportion of dispersed phase in emulsion is lower. The oil droplets were kept stable against separation forces using sufficient quantities of stabilizers, which was a mixture of Tween 80 and the heated BOSA. The heated BOSA resulted stabilization by the starch molecules at the oil–water interface. BOSA starch plays important roles not limited to thickening the continuous phase but also functions as an emulsifier (polymeric surfactant). We assume that all the available oil–water interfaces may be coated with double layers formed by both the surfactant and BOSA starch. Future research should be done to investigate other methods to modify the breadfruit starch to meet certain criteria so that it is applicable not only in food application but also in related fields.

Declarations

Author contribution statement

Sri H. Anwar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Dian Hasni: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Syarifah Rohaya, Miranda Antasari: Performed the experiments; Analyzed and interpreted the data.

Christina Winarti: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Funding statement

This work was supported by The Ministry of Research, Technology and Higher Education of Republic of Indonesia [025/SP2H/LT/DRPM/II/2016 (IPTEK)].

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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