

## Application of modified breadfruit (*Artocarpus altilis*) starch by Octenyl Succinic Anhydride (OSA) to stabilize fish and microalgae oil emulsions

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### Abstract

This research was aimed to examine the ability of breadfruit OSA starch (BOSA) to stabilize fish and microalgae oil emulsions. Fifteen percent of BOSA or pre-heated BOSA (7.5%) and maltodextrin (7.5%), or mixture of unheated BOSA (7.5%) and maltodextrin (7.5%) were used to stabilize 10% fish and microalgae oils. Characterization of native starch and BOSA included measurement of moisture, starch and amylose content, degree of substitution (DS), emulsification capacity, starch microstructure and thermal properties. The emulsion stability was monitored by emulsification index (EI), mean droplet size ( $D_{32}$ ), microstructure using photomicroscope and Peroxide Value (PV). Modification of starch (DS, 0.0243) caused decreasing in starch and amylose content to 75.34% and 27.62%, respectively. Starch thermal properties also decreased the onset temperature to 71.23°C (BOSA). Emulsions prepared with pre-heated mixture of BOSA and maltodextrin both in fish and microalgae oils have the smallest droplet size ( $D_{32}$  4.45 ± 0.09 μm and 3.54 ± 0.22 μm, respectively). Microalgae oil emulsions showed significantly ( $p < 0.05$ ) higher in stability towards oxidation compared to fish oil emulsions (PV, 4.65 ± 0.64 meq/kg oil). This research reveals that breadfruit OSA starch is highly recommended as a potential emulsifier to stabilize emulsion of essential oil containing polyunsaturated fatty acids (PUFAs).

### Keywords

Fish oil emulsion  
 Microalgae oil emulsion  
 Modified breadfruit starch  
 OSA starch

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### Introduction

Breadfruit (*Artocarpus Altilis*) is green tropical fruit, large and rounded in shape. The tree is mainly grown in South East Asia and other tropical regions in the world such as Brazil and Africa (Rincón and Padilla, 2004). In Asian Countries since ancient time, breadfruit simply fried for eating and sliced to make breadfruit chips. Nowadays, the starch has been used for texture stabilizer in food because its thickening and gelling properties (Adebowale *et al.*, 2005). However, the usage of breadfruit starch as food stabilizer in food industries is yet a common practice.

Breadfruit starch is reported to have small, irregular shaped and aggregated granules with average size of 2.3 – 8.4 μm. The starch molecules weight ( $M_w$ ), amylose content, and gelatinization temperature are 1.72 x 10<sup>7</sup> g/mol, 20%, and 69.3°C, respectively (Nwokocha and Williams, 2011). The thermal and rheological properties of breadfruit starch behaved like a yield-pseudoplastic fluid, its paste exhibited shear-thinning fluid characteristic, and showed good thermal and pH stability. In addition, the starch gel proved to have both flexibility and viscosity (Wang *et al.*, 2011). Amylose contributes to

the gelling property of starch whereas amylopectin contributes high viscosity. Flexible starch gels/films probably due to amylose crystallization (van Soest *et al.*, 1996; Myllärinen *et al.*, 2002).

Although the starch has higher ability to withstand viscosity breakdown compared to white yam starch, Nwokocha and Williams (2011) suggested that the breadfruit starch should be modified to improve its properties particularly the water binding capacity, clarity of the paste, as well as to reduce retrogradation. Among several modification methods, starches modified with octenyl succinic anhydride (OSA) have been used widely in food industries.

OSA starches have unique characteristics because the hydrophilic sites gain hydrophobic elements in the form of octenyl groups, developing in whole molecules to have amphiphilic character. OSA starch offers combination of OSA's hydrophobic and steric contribution as well as highly branched macromolecular structure which are useful for stabilizing, encapsulating, interfacial, thermal, nutritional and rheological properties (Sweedman *et al.*, 2013).

Modified OSA starches are obtained from the esterification reaction between starch hydroxyl group

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and octenyl succinic anhydride. Commonly used synthesis pathway is a reaction in aqueous solution under mild alkaline condition. Reaction occurs when the starch in its granular form and hydrogen bonding between starch chains are reduced by the formation of alkoxide functionalities with the starch hydroxyl (OH) groups. Accordingly, the starch granules are swollen thus allow the OSA molecules to diffuse within the starch granules (Sweedman *et al.*, 2013).

Researches that applied OSA starch as emulsions' emulsifier are increasing significantly. It has been used in food, cosmetics, and pharmaceutical industries either as emulsifiers and stabilizers. Emulsions made with OSA starch were more stable compared to whey protein (Charoen *et al.*, 2011). Other study also reported the superiority of OSA starch to stabilize oil-in-water emulsions when used alone rather than in combination with an effective surfactant, sodium dodecyl sulfate (SDS) (Krstonošić *et al.*, 2011).

Application of breadfruit modified starch with OSA as food stabilizer has never been reported in literature. This research was designed to examine the ability of breadfruit OSA starch (BOSA) to stabilize fish and microalgae oil emulsions and measured the emulsion stability against oxidation. Fifteen percent of BOSA or pre-heated BOSA and maltodextrin, or mixture of unheated BOSA and maltodextrin were used to stabilize 10% fish and microalgae oils, respectively. Emulsions' stability was monitored by evaluating the development of flocculation and sedimentation, measuring the emulsification index, examining the droplet size distribution using Mastersizer and photomicroscope, as well as analyzing the Peroxide Value. The physicochemical characterization of native and BOSA starch was also observed by evaluating the moisture, starch, and amylose content, degree of substitution (DS) and their emulsification capacity. The microstructure of native and BOSA starch granules and their thermal properties were also determined.

## Materials and Methods

Menhaden fish oil and 2-Octen-1-ylsuccinic anhydride were purchased from Sigma-Aldrich (Singapore), Schizochytrium DHA-Rich Algae Oil was purchased from Source-Omega (Mason, OH-USA), maltodextrin with Dextrose Equivalent (DE) < 20% (Himedia, Mumbai-India). Breadfruit starch was extracted from local breadfruits grown in Aceh Province, Indonesia. Other chemicals were of analytical grade. Double distilled water was used to prepare all emulsions in this study.

### Breadfruit starch extraction

The starch extraction is started by peeling off the breadfruits, cutting them into small pieces, washing and blending to become porridge. Water was added into starch porridge with 2:1 ratio and the mixture left overnight. The starch slurries were decanted then dried in the oven for 7 hours at 50°C and sieved.

### Breadfruit starch modification by octenyl succinic anhydride (OSA)

One hundred and twenty five gram of breadfruit starch was dissolved in 475 ml distilled water and pH was adjusted to become 8.5 by addition of Sodium Hydroxide (NaOH) solution. The mixture was continuously stirred using magnetic stirrer while the OSA solution (97%) was added slowly. Modification process was done for two hours mixing and stopped by decreasing pH with addition of Hydrochloric Acid (HCl) solution. The mixture then washed thrice and dried at 40°C for 24 hours (Bhosale and Singhal, 2006).

### Modified starch analysis

The analysis of starch after modification with OSA is determined to characterize: (1) starch physical properties, including moisture content, thermal properties, the shape of starch granules before and after modification using Scanning Electron Microscopy (SEM) and (2) chemical properties: amylose content, starch content, DS value (degree of substitution), and emulsification capacity.

### Starch moisture content

Water (moisture) content of native and OSA modified starched was measured gravimetrically in conventional oven following the method written by Wrolstad *et al.* (2004).

### Total starch content

Total starch content was measured by the method of acid hydrolysis in alcohol as described by Lin *et al.* (2003) with modification. Briefly, 5 g of starch was added into 50 ml of 80% alcohol and stirred for 1 hour, then washed and filtered. The residue was washed with 200 ml water followed by addition of 20 ml of 25% hydrochloric acid. The mixture was refluxed for 2.5 hours, cooled and neutralized with 45% NaOH before diluted to become 500 ml. The reducing sugar from the filtrate was determined and multiplied by factor of 0.9 for the calculation of starch content.

### Determination of amylose content

The amylose content in both native starch and

BOSA was determined by iodine-based colorimetry method. The procedural analysis followed the procedure that used hot NaOH (Mahmood *et al.*, 2007). Briefly, 5 mg sample were weighed into pre-weighed tube. For each 1 mg of starch, 15  $\mu$ L of 95% ethanol and 90  $\mu$ L of 1 M aqueous NaOH were added. The tube was heated for 30 min at 105°C, cooled, weighed and diluted with distilled water to give 1 mg starch per 200  $\mu$ L. Aliquot of 200  $\mu$ L then neutralised with 1 mL of 0.05 M aqueous citric acid, stained with 800  $\mu$ L iodine solution and diluted with 10 mL distilled water to a final volume of 12 mL and the absorbance read at 610 nm.

#### *Characterization of starch granules by scanning electron microscope*

Scanning electron microscopy to investigate the microstructural properties of the native starch and BOSA was performed using SEM JEOL JSM 6510LA (Japan). The samples were placed on the double-sided adhesive carbon tabs, mounted on SEM tubs and coated with gold in a sputter coater. The coated samples were then analyzed using the SEM operating at an accelerating voltage of 20 kV with 500x, 1000x, 2000x magnification (Anwar *et al.*, 2010).

#### *Thermal properties of starch*

The gelatinization properties of native and OSA modified breadfruit (BOSA) starches were studied using Diamond-1 DSC (Perkin-Elmer Corp., Norwalk, CT, USA) with a slight modification. Each sample was prepared according to 1:3 dry starch: water ratios and then heated from 40°C to 100°C at a rate of 10°C/min (Nor Nadiha *et al.*, 2010).

#### *Emulsification process*

The emulsions were prepared following the method written by Anwar *et al.* (2010) with modification. Three combinations of single and mixed matrices (starch and maltodextrin) were used for emulsification processes: 1) OSA modified breadfruit (BOSA) starch only (M1), 2) mixture of BOSA and maltodextrin which was previously heated to 60-62°C (M2), and 3) mixture of BOSA and maltodextrin without heating (M3). Matrices (25% solid content) were dissolved into distilled water and homogenised using T25 digital Ultra Turrax homogenizer for 1 minute at 8000 rpm. Fish oil or microalgae oil (10 g) was then added into starch solution and homogenisation was continued for 3 minutes at 13000 rpm. Resulted emulsions were kept in screw cap glass bottle and analysed at room temperature.

#### *Emulsion analysis*

Emulsion stability was analysed by monitoring the droplet size distribution and concentration during storage and measurement the emulsification index. Observations were done every day for 7 days storage at room temperature. Emulsion droplet size was analyzed using static multi-angle light scattering (Mastersizer Instrument) according to Yusoff and Murray (2011) and measuring droplet size by photomicroscope. The ability of modified breadfruit starch and maltodextrin to minimize the oxidation of omega-3 fatty acids in emulsion was investigated by monitoring the hydroperoxide formation by measuring Peroxides Value (PV) during storage period.

#### *Emulsification index (EI)*

The experiment was conducted to monitor the visible boundaries during one week storage. The emulsifying capacity of the BOSA and maltodextrin and the emulsions' stability were expressed as the volume of the cream layer to the total volume of the sample, usually called as the emulsification index (EI) and calculated as follows:

$$EI = \frac{\text{volume of cream layer}}{\text{total volume of emulsion}} \quad (1)$$

This method also determined the amount of remaining matrices at the bottom of the test tube which considered as sediment fractions (ratio of sediment volume to added starch or  $\text{mm}^3/\text{mg}$  (Timgren *et al.*, 2013).

#### *Droplet sizing via light scattering*

BOSA fish oil and microalgae oil emulsions droplet-size distributions were measured by static multi-angle light scattering method by Yusoff and Murray (2011) using a Mastersizer Hydro 2000 (Malvern Instrument, Malvern, UK). Average droplet sizes were characterized in terms of the surface area mean diameter defined by:

$$d_{ij} = 1/n_i (n_i d_j) \quad (2)$$

where  $n_i$  is the number of the droplets of diameter  $d_i$ . All measurements were made at room temperature on at least two freshly prepared samples. The -D32 parameter is the sum of the surface area ratio of droplets in each size-class multiplied by the mid-point diameter of the size-class. The refractive indices of water and fish/microalgae oil were taken as 1.330 and 1.467, respectively.

#### *Droplet imaging via photomicroscope*

Images of droplet distribution and size were

captured using Olympus BX41TF photomicroscope (Tokyo, Japan) equipped with camera (Olympus DP12). An emulsion drop was placed onto the microscope slide and carefully covered. The photomicrographs were taken with 400x magnification.

#### *Analysis of hydroperoxides' formation*

Formation of hydroperoxide was performed by measuring the Peroxide Value (PV) by iodometric titration method following the method written by Wrolstad *et al.* (2004).

#### *Statistical analysis*

All experiments were done in two replications. The starch characteristic and thermal properties data were compared using independent t-test. The significant differences for droplet size ( $D_{32}$ ) were analyzed using two ways analysis of variance (ANOVA) and statistical analysis were conducted by SPSS version 20 (SPSS Inc., Illinois, USA). PV data were analyzed using one way ANOVA with Duncan's pos hoc comparison tests. Level of significance was set at  $p < 0.05$ .

## **Results and Discussion**

#### *Starch characteristics*

Characterization of starches includes the examination of starch granules in their native form and after modification. The esterification of Breadfruit starch with OSA resulted in DS value of  $0.0243 \pm 0.01$ . As can be seen in Table 1, the moisture contents increased from  $9.05 \pm 0.41\%$  (native starch) to  $12.73 \pm 0.37\%$  (OSA-modified starch). However, the starch and amylose content decreased significantly ( $p < 0.05$ ) from  $82.19 \pm 0.23\%$  to  $75.34 \pm 0.18\%$  and  $34.35 \pm 0.48\%$  to  $27.62 \pm 0.29\%$ , respectively. From the result, there was a significant decrease in amylose content in treated starch. OSA modification under alkali treatments may have hydrolyzed the small amylose and amylopectin chains, or caused rearrangement of the starch molecules (Simsek *et al.*, 2015). The reduction of amylose content of alkali-treated starch could be attributed to the disruption of the amorphous region that contains amylose chains (Karim *et al.*, 2008). In addition, the alkali probably affects the amylose rather than the amylopectin molecules and/or regions of the granules (Lai *et al.*, 2004). Lai *et al.* (2004) suggested that the ions in alkali solution diffuse into the amylose-rich amorphous regions of the granules, break intermolecular bonds, and cause the granules to swell to a higher degree, with a concomitantly higher exudation of amylose.

As amorphous region is easily accessible, OS group might distribute and disrupt at this region more than in crystalline domains, which explain the reduction of amylose content in esterified starches (Nor Nadiha *et al.*, 2010). Decreasing amylose content in modified starch was also found by Nor Nadiha *et al.* (2010).

Emulsification capacity increased significantly from  $80 \pm 0.71$  to  $130 \pm 1.41$  g oil/g of starch in native and OSA-modified starch, respectively. The emulsification ability of OSA-modified breadfruit starch is quite high compare to 3% OSA-modified amaranth starch ( $80.56 \pm 3.16$  g oil/g starch) and 3% OSA-modified waxy corn starch ( $79.21 \pm 2.96$  g oil/g starch) as reported by Bholase and Singhal (2006). Starch modification with OSA imparts hydrophobic groups in the naturally hydrophilic starch allows the improvement in emulsification properties (Segura-Campos *et al.*, 2008).

Scanning Electron Microscope (SEM) of native starch granules show irregular-rounded shape, similar to the report written by Rincon and Padilla (2004). The granules of OSA-modified starch are slightly bigger than the native ones and aggregated granules structure is dominant (figures are not shown).

#### *Starch thermal properties*

Starch thermal properties were illustrated by the patterns of transition in endothermic heat flow over increasing temperatures. This study revealed that the native breadfruit starch has the onset temperature ( $T_o$ ) of  $73.53 \pm 0.26^\circ\text{C}$  similarly to the result obtained by Rincón and Padilla, (2004) i.e.  $73.3^\circ\text{C}$ . Measurement of starch thermal properties by differential scanning calorimeter (DSC) confirmed that OSA modification decreased the onset temperature from  $73.53^\circ\text{C}$  to  $71.23 \pm 0.07^\circ\text{C}$ . Peak ( $T_p$ ) and conclusion ( $T_c$ ) temperatures as well as the enthalpy ( $\Delta H_g$ ) of OSA breadfruit starch were lower than the native starch (Table 1). These findings are in agreement with Bao *et al.* (2003). The gelatinization enthalpy ( $\Delta H_g$ ) indicates the energy required to disrupt the starch granule structure and is obtained directly as the area under the endotherm. Generally, gelatinization temperatures have been related to degree of perfection of crystallites in the starch granules, and gelatinization enthalpies to the degree of crystallinity (Gudmundsson and Eliasson, 2006). The lower gelatinization temperature and enthalpy were due to the weakening of hydrogen bonding by the hydrophobic alkenyl groups, helping starch to swell at lower temperature and hence gradually decreasing the enthalpy of all OSA starches (Bao *et al.*, 2003).

Addition of bulky groups into starch backbone increase the structural flexibility of the biopolymer

Table 1. Characteristic of Native and Breadfruit OSA Starch

Sample	Moisture Content (%)	Starch Content (%)	Amylose Content (%)	Degree of Substitution (DS)	Emulsification Capacity (g oil/g of starch)	Temp Onset (T <sub>o</sub> )(°C)	Temp Peak (T <sub>p</sub> ) (°C)	Temp Conclusion (T <sub>c</sub> ) (°C)	Enthalpy ΔH <sub>g</sub> (J/g)
Native Breadfruit Starch	9.05 ± 0.41 <sup>a</sup>	82.19 ± 0.23 <sup>a</sup>	34.35 ± 0.48 <sup>a</sup>	-	80 ± 0.71 <sup>a</sup>	73.53 ± 0.26 <sup>a</sup>	75.29 ± 0.27 <sup>a</sup>	77.19 ± 0.25 <sup>a</sup>	13.51 ± 0.41 <sup>a</sup>
Breadfruit OSA Starch (BOSA)	12.73 ± 0.37 <sup>a</sup>	75.34 ± 0.18 <sup>b</sup>	27.62 ± 0.29 <sup>b</sup>	0.0243 ± 0.01	130 ± 1.41 <sup>a</sup>	71.23 ± 0.07 <sup>b</sup>	73.37 ± 0.38 <sup>b</sup>	75.93 ± 0.37 <sup>b</sup>	11.42 ± 0.28 <sup>b</sup>

Means ± standard deviation within the same columns followed by different superscript a or b are significantly different (p<0.05)

Table 2. Droplet Size (D<sub>32</sub>) of Fish Oil and Microalgae Oil Emulsions

	Fish Oil Emulsions	Microalgae Oil Emulsions
	D <sub>32</sub> (μm)	
Breadfruit OSA Starch (BOSA)	28.77 ± 0.39 <sup>Bb</sup>	41.54 ± 0.23 <sup>Ba</sup>
Heated BOSA and maltodextrin	4.45 ± 0.09 <sup>Ca</sup>	3.54 ± 0.22 <sup>Cb</sup>
Unheated BOSA and maltodextrin	49.53 ± 1.53 <sup>Ab</sup>	56.55 ± 1.11 <sup>Aa</sup>

Means ± standard deviation within the same columns followed by different superscript A, B or C are significantly different (p<0.05)

Means ± standard deviation within the same rows followed by different superscript a, b or c are significantly different (p<0.05)

causing reduction gelatinization temperature (Miller *et al.*, 1991). The magnitude of changes in physical properties of OSA-modified starches depends on the botanical origin of the native starches and the degree of substitution (Bao *et al.*, 2003). Differences in molecular alignment of each starch origin influences the properties of starch after OSA modification (Thirathumthavorn and Charoenrein, 2006).

#### Mean droplet size

As can be seen in Table 2, the smallest droplets size were given by emulsions prepared with pre-heated mixture of BOSA and maltodextrin both in fish (D<sub>32</sub> 4.45 ± 0.09 μm) and microalgae oils and (D<sub>32</sub> 3.54 ± 0.22 μm). Some possible explanation for this finding are: starch granules start to gelatinize during heat treatment at 60°C and break-up the particles into molecules, some types of unmodified native starches start swelling at 55°C, during heating, water is first absorbed in the amorphous space of starch, which leads to a swelling phenomenon. Water then enters via amorphous regions the tightly bound areas of double helical structures of amylopectin. At ambient temperatures these crystalline regions do not allow water to enter. Heat causes such regions to become diffuse, the amylose chains begin to dissolve, to separate into an amorphous form and the number and size of crystalline regions decreases. These phenomena are exposing more hydrophobic groups / regions into the system.

OSA starches are able to adsorb at interfaces, which resulted in the formation of a layer composed of granules and agglomerates located on the periphery

of the drops of oil, favoring stability from the steric hindrance (Tesch *et al.*, 2002; Torres *et al.*, 2007). OSA starches can function as electrosteric stabilizers, though due to the size of the molecules relative to the number of charged groups, their function is primarily steric, which affects their uses. The short octenyl succinate side chains bring OSA starch molecules to the oil-water interface, and the long amylopectin backbone protects the droplets against flocculation by the mechanism of steric stabilization (Dokić *et al.*, 2012). OSA starch forms strong films at the oil-water interface providing to the emulsions resistance to reagglomeration (Bhosale and Singhal, 2006; Dickinson, 2009). Due to amphiphilic nature OSA starch shows strong surface activity (Prochaska *et al.*, 2007). Its macromolecular structure provides capability to modify aqueous viscosity and to stabilize oil-in-water emulsions (Dickinson, 2009). Increasing of the viscosity in combination with the previously described ability of adsorption at interfaces, enables OSA starch to act as an emulsifier and stabilizer of emulsions.

These findings are supported by photomicroscopes in Figures 1b and 1e. The starch granules were observed in black particles while the oil droplets were in blue color. Most of the granules had solubilized as their edge had disappeared due to heating at 60-62°C for 10 min. The unheated OSA-modified breadfruit starch stabilized the oils in the form of starch particles rather than in molecular form. In Figures 1a, 1c, 1d and 1f, the starch granules retained their shape because of no heat treatment for the starch.

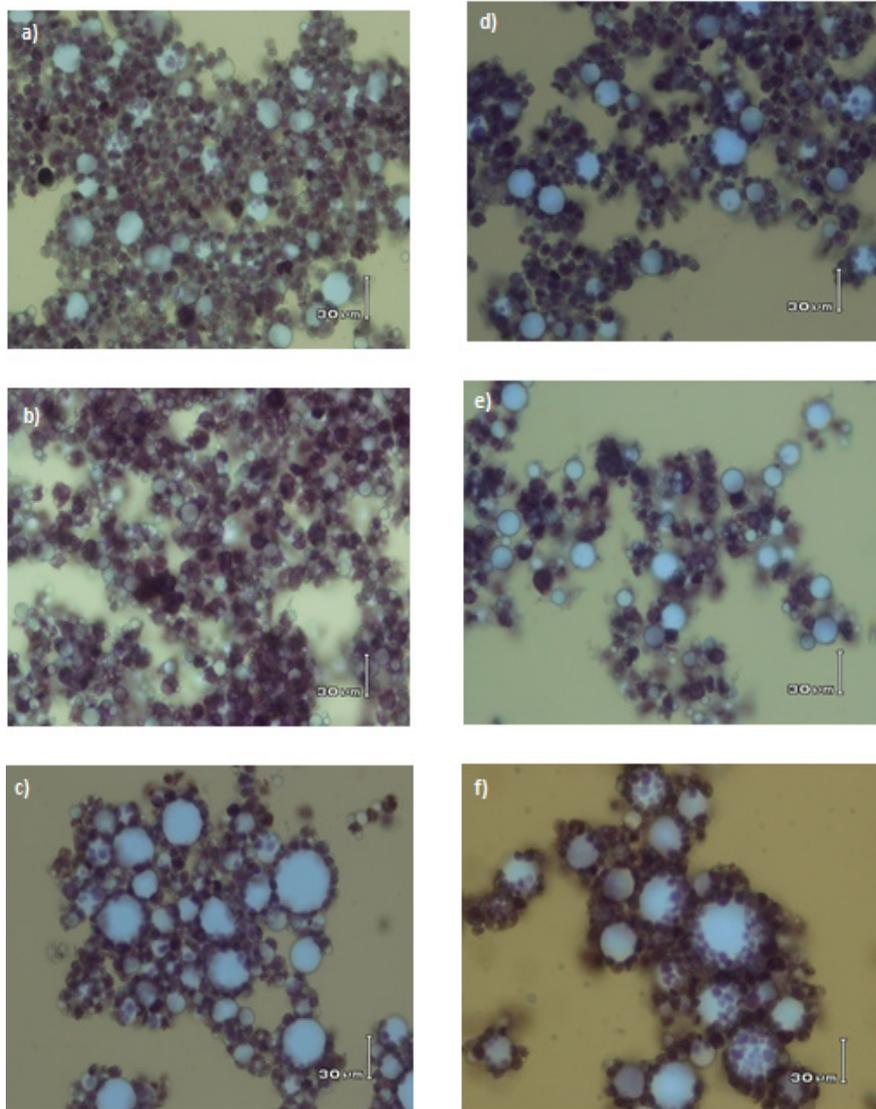


Figure 1. Fish Oil Emulsions prepared from: a) 15% BOSA, b) heated 7.5% BOSA + 7.5% maltodextrin, c) unheated 7.5% BOSA + 7.5% maltodextrin, microalgae Oil Emulsions prepared from : d) 15% BOSA, e) heated 7.5% BOSA + 7.5% maltodextrin, f) unheated 7.5% BOSA + 7.5% maltodextrin

For this kind of system, the shape of starch particles and the way they are arranged at the interface must also be taken into account. Although the BOSA concentration is doubled but the stabilization effect is less than in the heated system. Largest droplet sizes were observed for the third mixture of unheated BOSA and maltodextrin.

Figure 1b also showed that the droplet sizes of heated BOSA and maltodextrin were relatively smaller than droplets of the other two emulsions (Figures 1a and 1c). This finding was also observed for microalgae emulsion (Figure 1e). Both figures also proved that coalescence and flocculation occurred significantly in emulsion prepared by unheated BOSA and maltodextrin (Figures 1c and 1f).

#### *Emulsification index (EI)*

The stability of emulsions was monitored every

day for seven days of storage. In the first 36 hours, fish oil and microalgae oil emulsions were completely stable. There were no visible boundaries layer observed. The boundaries layers were the emulsion separation that visible during storage and can be in the form of serum layer (usually at the bottom of the emulsion) or cream layer (on top) or sedimentation of starch. As can be seen from Table 3, the EI of fish oil emulsions prepared from: 1) Breadfruit OSA starch (BOSA), 2) heated of BOSA and maltodextrin, 3) unheated of BOSA and maltodextrin were decreased gradually after 48 hours up until 6 days storage in room temperature. BOSA only and mixture of heated BOSA and maltodextrin showed better matrices for fish oil emulsion as indicated by the EI values. However, no starch sedimentation monitored for the heated BOSA and maltodextrin until the end of

Table 3. Emulsification Index and Sediment Fraction of Fish and Microalgae Oil Emulsions

a. Fish Oil Emulsions								
Matrices' Combination	Emulsification Index (EI)				Sediment Fraction (mm <sup>3</sup> /mg)			
	24h	2 d	4 d	6 d	24h	2 d	4 d	6 d
Breadfruit OSA Starch (BOSA)	1.0±0.0	0.93±0.09	0.89±0.15	0.83±0.25	0	0	0.10±0.07	0.16±0.11
Heat treated : BOSA and Maltodextrin	1.0±0.0	0.94±0.07	0.74±0.01	0.67±0.06	0	0	0	0
Unheated : BOSA and Maltodextrin	1.0±0.0	0.70±0.05	0.67±0.01	0.64±0.02	0	0	0	0
b. Microalgae Oil Emulsions								
Matrices' Combination	Emulsification Index (EI)				Sediment Fraction (mm <sup>3</sup> /mg)			
	24h	2 d	4 d	6 d	24h	2 d	4 d	6 d
Breadfruit OSA Starch (BOSA)	1.0±0.0	na	na	na	0	0.39±0.15	0.41±0.09	0.48±0.01
Heat treated : BOSA and Maltodextrin	1.0±0.0	1.0±0.01	0.99±0.02	0.96±0.06	0	0	0	0
Unheated : BOSA and Maltodextrin	1.0±0.0	na	na	na	0	0.23±0.10	0.24±0.08	0.32±0.02

na = not available

storage test.

For the case of microalgae oil emulsions, both BOSA only and unheated BOSA + maltodextrin resulted starch precipitation (due to difference in density between the starch and emulsions system) during storage which were up to  $0.48 \pm 0.01$  and  $0.32 \pm 0.02$  mm<sup>3</sup>/mg (ratio of sediment volume to added starch), respectively. Almost no separation occurred and no sedimentation observed for emulsions from heated BOSA and maltodextrin (Table 3). This was probably due to a small particles or the break-up of particles into molecules which prevented the formation of sedimentation.

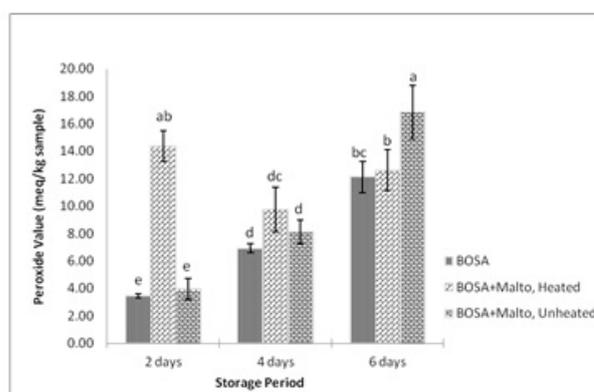
Sedimentation is predicted as a result of free starch or unabsorbed starch granules that remained in the continuous phase. If the excess of granules in continuous phase are more than the amount that needed to stabilize the oil droplets then sedimentation occur. This finding is in agreement with what have been investigated others (Tcholakova *et al.*, 2004; Timgren *et al.*, 2013).

#### Peroxide value (PV)

Monitoring formation of hydroperoxides by PV was conducted to evaluate the ability of BOSA and maltodextrin to protect fish and microalgae oils from oxidation in emulsions. Figure 2a showed increasing tendency of PV for emulsions prepared with BOSA and mixture of unheated BOSA and maltodextrin over the storage period. Unlikely, fish oil stabilized by heated BOSA and maltodextrin exhibited decreasing trend although the PV had reached  $14 \pm 1.11$  meq/kg oil at the second day of storage.

The initial PVs of bulk oils were difficult to be maintained at their lowest level due to high surface area of oil droplets in emulsions. In addition, the emulsification process caused increasing temperature

A



B

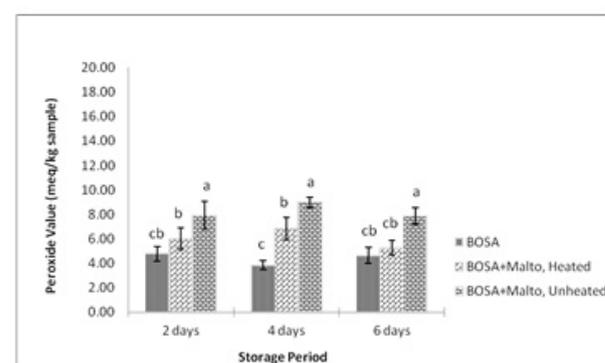


Figure 2. Peroxide Values of: a) fish oil emulsion; b) microalgae oil emulsion, obtained during storage

in emulsion and may initiate the oxidation process. This is in agreement with what have been found by others i.e., the ease to oxidation in oil-in-water emulsion is attributed to the high surface area which increases lipid interactions with aqueous pro-oxidants including oxygen (Chaiyasit *et al.*, 2007).

The rate of oxidation in microalgae emulsions was lower than in fish oil emulsions (Figure 2b). The maximum PV was  $8.99 \pm 0.44$  meq/kg oil, measured

in unheated BOSA and maltodextrin emulsion while hydroperoxides formation in unheated BOSA and heated BOSA and maltodextrin were relatively low with average PV of below 7.0 meq/kg oil. PV discrepancies among the two emulsions may be caused by differences in the type and quantity of fatty acids contained in fish and microalgae oils. According to Certificate of Analysis provided by Nutrasource Diagnostic Inc., Guelph-Ontario, Canada, for Source-Omega LLC, Chapel Hill, NC-USA, microalgae oil was extracted from *Chromista* (*Schizochytrium* sp) and rich in omega-3 fatty acids, mainly Docosahexaenoic Acid (DHA) (454.67 mg / g oil or 56%). Meanwhile, the fish oil used contain lower amount of these fatty acids and higher in palmitic (15-20%) and palmitoleic acids (9-14%) (Sigma Product Information, Product Number F 8020).

Miyashita *et al.* (1995) reported that the oxidative stability of PUFAs in an aqueous solution was reversed from that in bulk phase and in the emulsion form in which DHA (22:6n-3) was found to have the highest oxidative stability followed by Eicosapentaenoic Acid (EPA, 20:5n-3),  $\alpha$ -Linolenic Acid (ALA, 18:3n-3),  $\gamma$ -Linolenic Acid (GLA, 18:3n-6), and LA (18:2n-6). The oxidative stability increased with increasing degree of unsaturation (Miyashita *et al.*, 1995). PUFAs have a tight pack conformation in an aqueous solution (Kato *et al.*, 1992) and it may cause difficulties for free radicals and/or oxygen to attack the substrates in such conformation.

Despite composition of fatty acids, the amount of antioxidant present in the products must also take into account. Most of essential oils with high amount of PUFAs contained mixed tocopherols (TOHs) and the activity is related very much to their concentration upon addition, particularly  $\alpha$ -tocopherol (Kulås and Ackman, 2001; Zuta *et al.*, 2007). According to the product information, menhaden fish oil used was the standard refined fish oil without addition of antioxidant. On the other hand, the microalgae oil was stabilized by green tea as antioxidant and had initial PV of 1.3 meq / kg oil.

Green tea was used as antioxidant in microalgae oil used in this research. A study reported that dechlorophyllized green tea extract (DGTE) exhibited excellent antioxidant activity in sea blubber oil and menhaden fish oil (Wanasundara and Shahidi, 1998). Addition of green tea as antioxidant in microalgae oil showed that the oil was well protected and stable until the experiments started although the DHA content was quite high (454.67 mg/g oil). Therefore, it is presumed that significant oxidation did not occur in bulk oil, and that any changes in oxidation

were caused by emulsification process and storage conditions.

## Conclusion

It has been demonstrated that breadfruit starch that has been modified with octenyl succinic anhydride (BOSA) can act as emulsifiers for oil-in-water emulsions. Emulsions prepared with pre-heated mixture of BOSA and maltodextrin both in fish and microalgae oils showed the smallest droplet sizes. Heat-treatment of BOSA at 60°C had gelatinized the starch granules and break-up the particles into molecules thus exposing more hydrophobic regions into the colloidal system. Smaller molecular weight/size of heated surface active starches will move/migrate faster into the droplet interface thus covering droplets that just have been formed by homogenization. No separation and sedimentation were observed for heated BOSA and maltodextrin stabilized emulsions during storage. Small size of starch granules/particles or the break-up of starch granules/particles into molecules might also prevented the formation of sedimentation.

Microalgae oil emulsions appear to be more stable towards oxidation compared to fish oil emulsions. PV discrepancies among the two emulsions may be caused by differences in the type and quantity of fatty acids contained in fish and microalgae oils beside the type and amount of antioxidant present in the products.

This research reveals that breadfruit OSA starch is highly potential to be used as food emulsifier. In particular, BOSA are able to stabilize emulsion of essential oils containing high amount of polyunsaturated fatty acids. These results may be of great interest to researchers investigating the enhancement of hydrophobicity of modified underutilized starches in order to broaden the application and usability of starch in food, pharmaceutical and cosmetic industries. Further work on the possibility of these hydrophobic starches could interact with other surface active components such as proteins can also be further explored.

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