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# Freeze–thaw stability of emulsions with soy protein isolate through interfacial engineering

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## ABSTRACT

In this paper, we examined to the influence of interfacial composition on freeze–thaw stability of oil in water emulsions. An electrostatic layer-by-layer deposition method was used to create the multilayered interfacial membranes with different compositions of primary emulsion (Soy protein Isolate); secondary emulsion (Soy protein Isolate – octenyl-succinate starch); tertiary emulsion (Soy protein Isolate – octenyl-succinate starch – Chitosan). The primary, secondary and tertiary emulsions were subjected to from one to two freeze–thaw cycles (–20 °C for 24 h, +25 °C for 18 h) and then their stability was assessed by  $\zeta$ -potential, particle size, microstructure and creaming stability measurements. The crystallization behaviour of emulsions was studied by differential scanning calorimetry (DSC). Primary and secondary emulsions were unstable to droplet flocculation when the water phase crystallized, whereas tertiary emulsions were stable, which was attributed to the relatively thick biopolymer layer surrounding the oil droplets. These results showed the interfacial engineering technology used in the study could therefore lead to the creation of food emulsions with improved stability to freezing and thawing.

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# Stabilité de la congélation-décongélation d'émulsions avec de l'isolat de protéine de soja par ingénierie interfaciale

Mots clés : Chitosane ; Cycle de congélation-décongélation ; Emulsion à multicouches ; Amidon OSA ; Isolat de protéine de soja ; Stabilité

## 1. Introduction

The food industry is one of many industries that heavily rely on the use of emulsions and emulsifiers. Products such as salad dressings, mayonnaise, soups, dips, sauces, desserts and beverages consist of oil-in-water emulsions that are prepared by homogenization. After homogenization, these

products may undergo a variety of processing operations such as mixing, shearing, heating, chilling, freezing and dehydration (Mun et al., 2008). Freezing storage is one of the most important preservation methods for maintaining microbiological and chemical stability and extending the shelf life of food products. There are many potential applications for oil-in-water emulsions that can be frozen and

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### Nomenclature

DSC	Differential scanning calorimetry
L b L	layer- by- layer
SPI	Soy protein isolate
OSA starch	Octenyl-succinate starch
M	molar
mm	millimeter
$d_{43}$	De Brouckere mean diameter
PSD	particle size distributions

then thawed prior to use, e.g. refrigerated and frozen food or pharmaceutical products (Moreno et al., 2015; Thanasukarn et al., 2006). The freezing often adversely affects on the stability emulsion through various physical-chemical mechanisms, including water crystallization, freeze concentration, biopolymer conformational changes and interfacial phase transitions that these make the emulsions become unstable after thawing, which limits their use in food products (Dons et al., 2011; Ghosh and Coupland, 2008; Mun et al., 2008). Thus, Selection of an appropriate emulsifier is one of the most important means available to food manufacturers to reduce or avoid emulsion instability problems. On the other hand, the food industry is attempting to formulate products using natural “label friendly” ingredients, which limits the range of ingredients that can be used. At present, there is a lack of natural emulsifiers that can be used to stabilize emulsified food products against a range of common environmental stresses such freezing and chilling (Mun et al., 2008; Thanasukarn et al., 2006). One strategy that has proved is a layer-by- layer (L b L) electrostatic deposition technique, which creates a multilayer coating around oil droplets. In this technology, oil droplets of emulsion surrounded by a multilayer interfacial coating. Proteins and polysaccharides can use to produce a multilayer emulsion. Therefore, by engineering the properties of interfacial membranes can improve the stability of emulsions to be environmental stresses (Aoki et al., 2005; Benjamin et al., 2012; Caliskan et al., 2015; Evans et al., 2013; Fioramonti et al., 2014; McClements, 2012; Ogawa et al., 2004; Salminen and Weiss, 2014; Surh et al., 2005). In this study, we examined the freeze–thaw stability of oil in water emulsions containing oil droplets surrounded by interfacial membranes consisting of soy protein isolate (SPI), octenyl-succinate starch (OSA starch) and chitosan. These biopolymers choose because they are plentiful natural biopolymers with opposite charges. Soy protein isolates (SPI) used as an emulsifier in food emulsions because of the surface-active properties of their constitutive proteins, the storage globulin 7S ( $\beta$  – conglycinin) and 11S (glycinin) and it has positively charged at pHs below its isoelectric point ( $pI \approx 4.6$ ) (Huang et al., 2012; Keerati-u-rai and Corredig, 2009; Palazolo et al., 2011). The OSA starch contains a negatively charged carboxylic acid part and could absorb to the positive charged. Also, OSA-starch can act as a viscosity improves, but its thickening capacity limited compared to the capacity of other macromolecules used as thickeners (Krstoni et al., 2012; Ljubica et al., 2012; Nilsson and Bergensthl, 2007; Tesch et al., 2002). Chitosan, a linear

copolymer of glucosamine and N-acetyl glucosamine connected through  $\beta$ -(1-4) glucosidic link is a unique cationic polysaccharide and represented as a homopolymer. In addition, Chitosan is a popular natural positively charged polysaccharide with properties and nutritional and physiological (Chuah et al., 2009; Garca-Mrquez et al., 2014; Mun et al., 2006; Yuan et al., 2013). In the present study, we examine the influence of interfacial characteristics on the stability of oil in water emulsions to freezing and thawing, where the interfacial properties varied using the L b L electrostatic deposition method. Specifically, we compare the freeze–thaw stability of emulsions coated by (i) SPI layer (ii) SPI- OSA starch (iii) SPI- OSA starch –chitosan layers.

## 2. Materials and methods

Soybean protein isolate (SPI) of food grade purchased from Golhar Co (Mashhad, Iran). Chitosan (with a molecule weight of about 300 kDa and a 90% degree of deacetylation) purchased from Biobasic Inc (Canada). National Starch and Chemicals GmbH, Germany supplied powdered OSA-starch (E1450). Sunflower oil purchased from a local supermarket and used without further purification. Analytical grade sodium chloride, hydrochloric acid, sodium hydroxide, acetic acid and sodium azide purchased from Sigma Chemical Company. Distilled and deionized water used for prepare all solutions.

### 2.1. Solution preparation

The stock solution of SPI prepared by dispersing SPI powder in deionized water and stirred for 5 min then the solution exposed to ultrasonic 500 W (Schaper model Unique USC 25 kHz) for 5 min at 50 °C and then centrifuged at 5000 g for 10 min (Huang et al., 2012). As for the OSA starch stock solution, OSA starch powder suspended in Na-acetate buffer (10 mM, pH 6). The solution placed in a boiling water bath under stirring for 10 min (Nilsson and Bergensthl, 2007). Medium molecular weight chitosan solution prepared by dispersing weighted amount of the powdered material in to 10 mM Na-acetate (pH 4) (Chuah et al., 2009). Sodium azide stock of 0.1 wt% prepared. These solutions stored at room temperature for 24 h to ensure complete dissolution of these materials.

### 2.2. Emulsions preparation

Preliminary studies were carried out to define the optimum SPI, OSA starch and chitosan concentration needed to create the primary, secondary and tertiary emulsions. Primary emulsions prepared by homogenizing 5 wt% sunflower oil with 95 wt% aqueous emulsifier solution (1% w/v SPI and 0.1% wt sodium azide) in high-speed blender (UltraTurrax T-25, IKA Instruments, Germany) followed by sonication for 2 min at a frequency of 20 kHz, amplitude of 60% (VCX 750, Sonics & Materials, Inc., USA). This emulsion adjusted back to pH 3.5 using 1 M HCl. A secondary emulsion prepared by mixing the primary emulsion with OSA-starch so the OSA starch concentration in the secondary emulsion was 0.8% w/

v. The emulsion sonicated for 1 min at frequency of 20 kHz, amplitude 60% to disrupt any flocs formed during the mixing. The secondary emulsions adjusted back to pH 3.5 using 1 M HCl. Tertiary emulsions formed by diluting the secondary emulsions with chitosan solution so the chitosan concentration in the tertiary emulsion was 0.5% w/v and then adjusted back to pH 3.5 using 1 M HCl. It should note the oil phase fraction kept at 5% w/w in both the secondary and tertiary emulsions by changing the ratios of emulsion, biopolymer and deionized water. It should be noted for producing tertiary emulsion, sonication is used twice, this leads to tertiary emulsion particle size is smaller than primary and secondary emulsions.

### 2.3. Freeze–thaw protocol

The primary, secondary and tertiary emulsions stored in vertical plastic containers (internal diameter = 30 mm) for 24 h at  $-20\text{ }^{\circ}\text{C}$  in a temperature – controlled refrigerator. After incubation, the emulsion samples thawed by incubating them in water bath at  $25\text{ }^{\circ}\text{C}$  for 18 h. This freeze–thaw cycle repeated from 0 to 2 times and its influence on emulsion properties measured after each cycle.

### 2.4. Emulsion droplet size analysis

Size distribution of oil droplets measured by a laser diffraction particle sizer (Fritsch Analysette 22, Germany) relating to scatter laser beam to the size of droplets as described previously (Guzey and McClements, 2006). The particle size was expressed as the mean volumetric size  $d_{43}$  (De Brouckere mean diameter), which is the mean diameter of a sphere with the same volume, and is generally used to characterize a particle.

### 2.5. $\zeta$ -potential measurements

The  $\zeta$ -potential of emulsions determined using a particle electrophoresis instrument (Zetasizer Nano ZS, Malvern

Instrument, UK). Emulsions diluted to a droplet concentration of approximately 0.001 wt% using buffer solutions of the appropriate pH to avoid multiple scattering effects.

### 2.6. Optical microscopy

The microstructure of the emulsions observed using an optical microscope (Olympus BX41, Japan). Before observation, the sample gently mixed. An aliquot of emulsion placed on a microscope slide, covered with a cover slide. An image of the sample acquired using digital image software and stored on a personal computer.

### 2.7. Creaming stability measurements

Emulsions (10 g) were poured in to glass tubes and then stored for 7 days at room temperature. After storage, the heights of any separated layers measured manually using a ruler.

### 2.8. Differential scanning calorimetry

Samples (10–15 mg) placed in aluminum pans, which were then sealed. The thermal behavior of samples characterized using DSC (Mettler Toledo DSC 822, Switzerland). An empty aluminum pan used as the reference pan. The samples cycled from  $20$  to  $-20\text{ }^{\circ}\text{C}$  and vice versa at  $5\text{ }^{\circ}\text{C min}^{-1}$  in the DSC. This temperature cycle repeated up to two times.

### 2.9. Statistical analysis

Experiments performed twice using freshly prepared samples. Averages and standard deviations calculated from these duplicate measurements.

## 3. Results and discussion

In this work, sunflower oil used as the dispersed phase because Magnusson et al. (2011), in the study of the

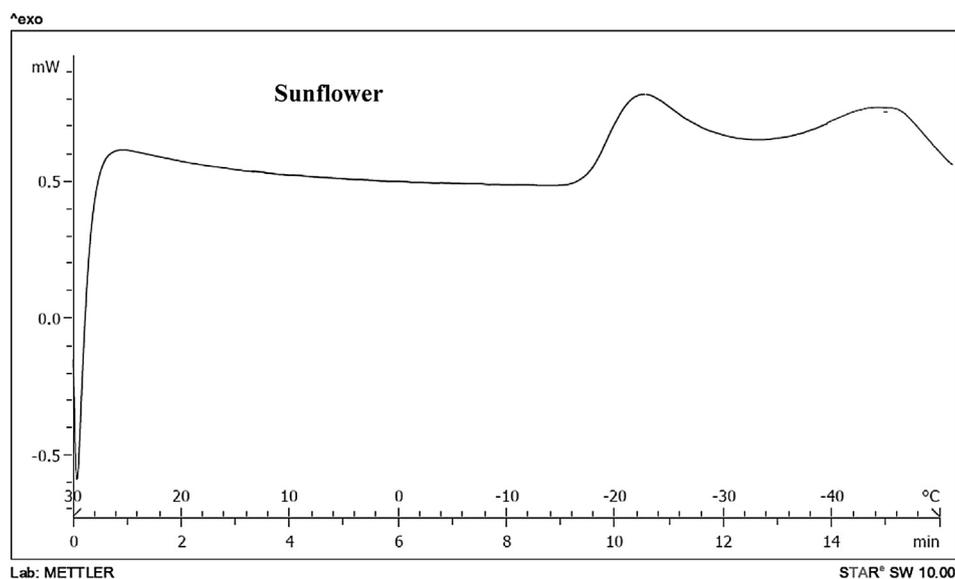


Fig. 1 – DSC curve of sunflower oil.

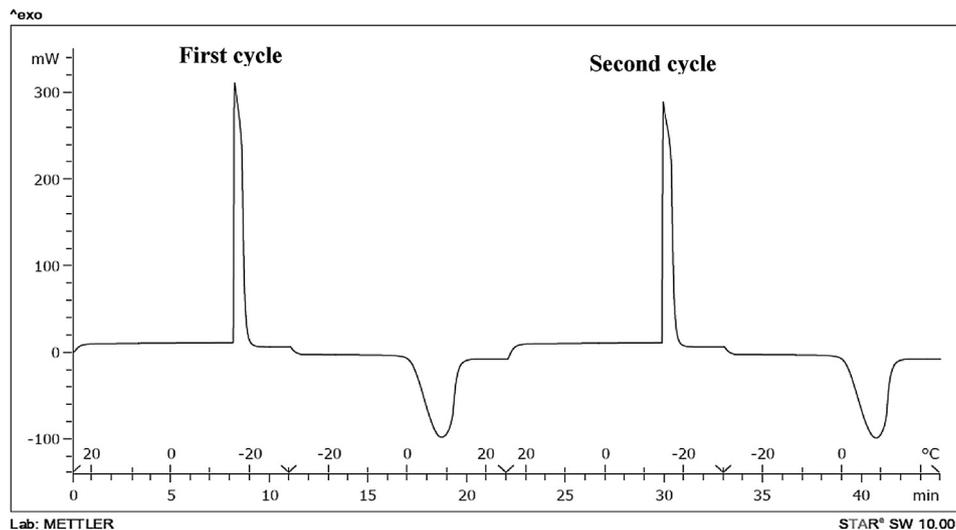


Fig. 2 – DSC curve of primary emulsion.

freeze–thawing of emulsions prepared with different vegetable oils, reported that emulsions prepared with sunflower oil were the most coalescence stable (least increase in droplet size) after repeated freeze–thaw cycles (Magnusson et al., 2011). DSC used to study the crystallization behaviour of primary, secondary and tertiary emulsions. Before analyzing the emulsion samples by DSC, we have made calorimetric measurements on the. DSC test showed to crystallize bulk oil started at  $\sim -18^\circ\text{C}$  (Fig 1) (Magnusson et al., 2011; Palazolo et al., 2011). For the oil phase remains almost in liquid state, emulsions subjected to freezing at  $-20^\circ\text{C}$  (only  $2^\circ\text{C}$  below the onset of crystallization of sunflower oil) for 24 h. Fig. 2 shows the thermograms got on repeated cooling of primary emulsion. On freezing, all three emulsions gave one peak at the temperature ascribed to the continuous phase (water). In addition, during thawing, the heating curve of frozen emulsions showed only an endothermic peak corresponding to melting to aqueous phase (Fig. 3) (Palazolo et al., 2011). Based on these observations, we can remark that oil do not crystallizes in emulsion droplets during the frozen storage at  $-20^\circ\text{C}$

for 24 h. So, the destabilization induced by freeze–thaw in o/w emulsions would mainly attribute to the destabilizing effect of ice formation during frozen storage (Palazolo et al., 2011). When oil-in- water emulsions cooled to temperatures where the water crystallizes, several destabilizing effects can occur in emulsion in the freeze state. First, the dispersed emulsions droplets became concentrated and therefore forced closer together in any unfrozen aqueous phase when ice crystals form, which may cause disruption of the interfacial layer and promote droplet aggregation. Second, to form a freeze-concentrated unfrozen aqueous phase leads to the ionic strength of the aqueous phase increases, and pH decreases. It screened the electrostatic repulsion among droplets may occur in high concentration of salts that make easier to force into the close. Third, ice crystals may physically penetrate oil droplets and disrupt their interface layer, but it making them more prone to coalescence once they thawed (Cortes-Munoz et al., 2009; Ghosh and Coupland, 2008; Mun et al., 2008; Palazolo et al., 2011; Thanasukarn et al., 2006). Therefore, to form multilayer around the oil droplets may have increased

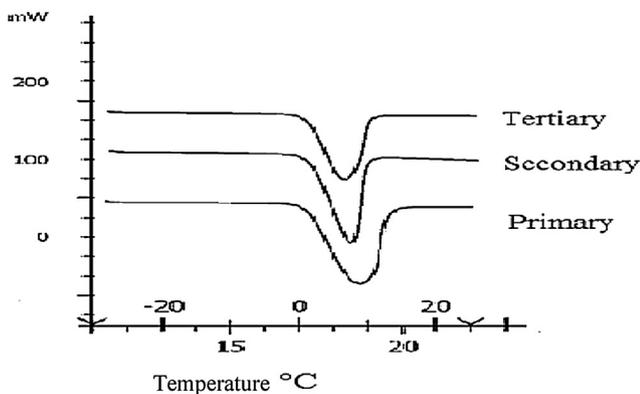


Fig. 3 – Schematic heating DSC thermograms of primary, secondary and tertiary emulsions from  $-20^\circ\text{C}$  to  $20^\circ\text{C}$  at rate  $5^\circ\text{C min}^{-1}$ .

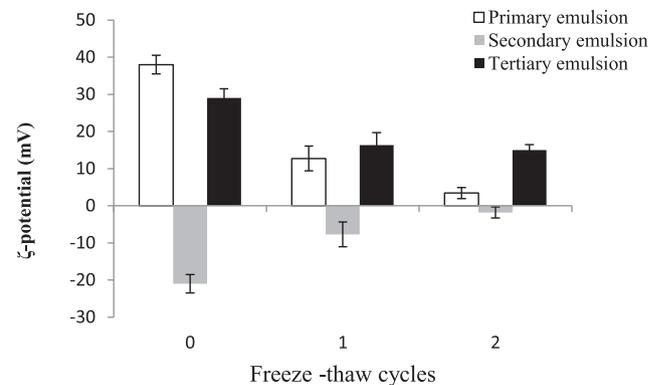
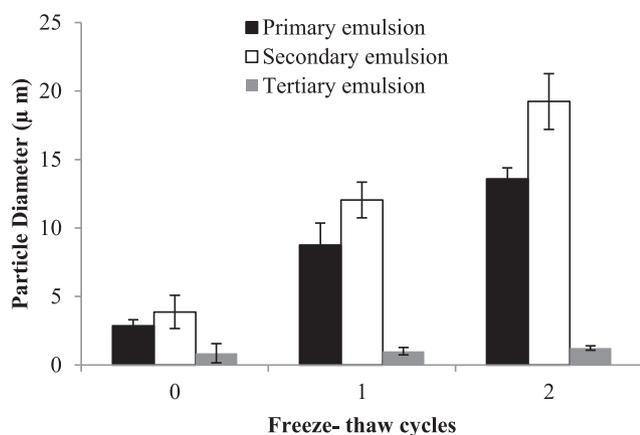


Fig. 4 – Dependence of particle electrical charge ( $\zeta$ -potential) of primary, secondary and tertiary emulsions on the freeze–thaw cycles.



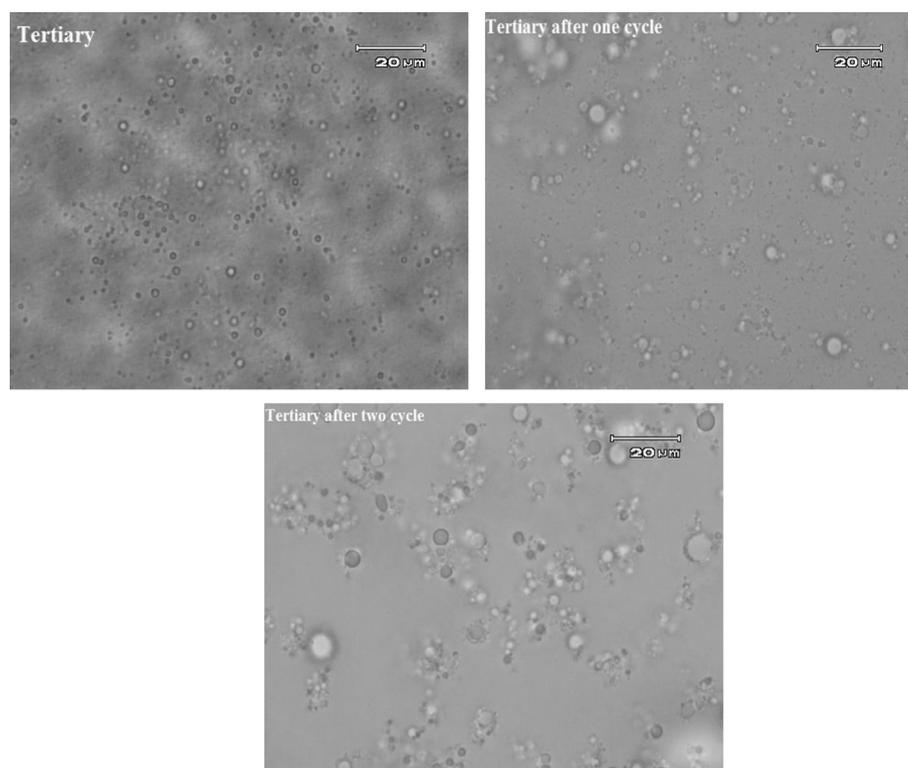
**Fig. 5 – Dependence of particle diameter ( $D_{4,3}$ ) of primary, secondary and tertiary emulsions on number of freeze–thaw cycles.**

the stability of the emulsions to ice crystallization by altering these physic–chemical processes (Dons et al., 2011; Thanasukarn et al., 2006).

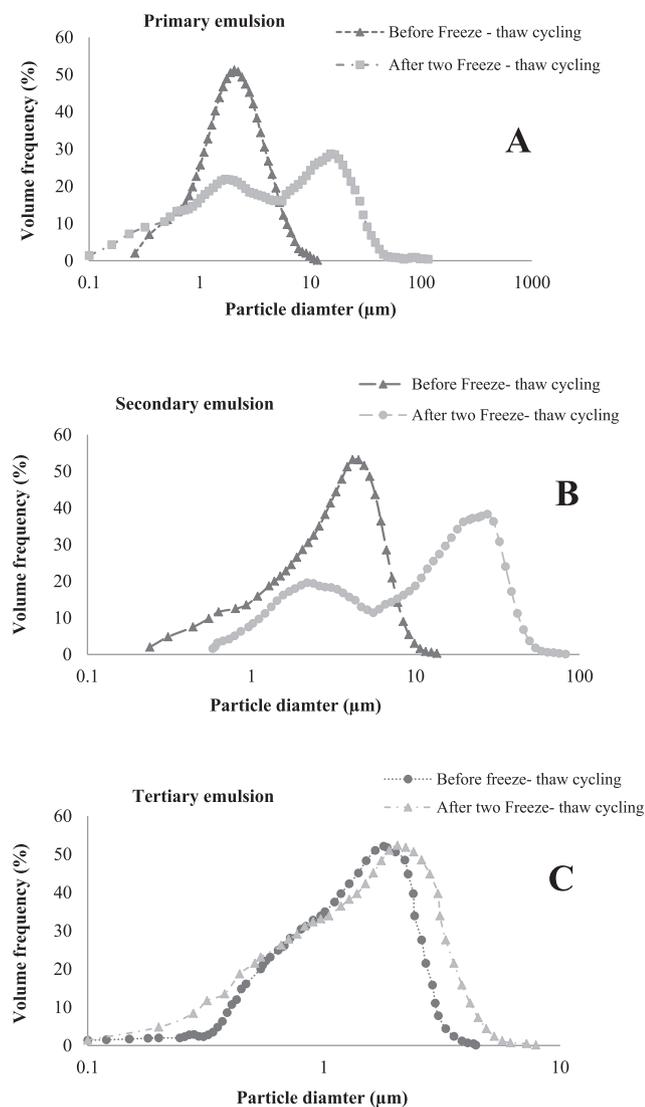
The influence of Freezing–thawing on the stability of primary, secondary and tertiary emulsions examined. The influence of freeze–thaw cycles on the  $\zeta$ -potential, particle diameter and microstructure of primary, secondary and tertiary emulsions measured after they had stored at room temperature for 1-day after preparation. Creaming stability also observed after 7 days of storage at room temperature. We used  $\zeta$ -potential to provide indirect information about the

interface composing primary, secondary and tertiary emulsions. At first, we discuss the measurements made on emulsions that did not subjected to freeze–thaw cycling. The  $\zeta$ -potential of the droplets in the primary emulsions was  $+38 \pm 2.67$  mV, which can attribute to the fact the pH of the solution (pH 3.5) was below the isoelectric point ( $pI \approx 4.6$ ) of the adsorbed SPI molecules. The  $\zeta$ -potential of the droplets in the secondary emulsions was  $-21 \pm 1.49$  mV, which showed that anionic OSA–starch had adsorbed to surfaces of the cationic protein–coated droplets. The  $\zeta$ -potential of the droplets in the tertiary emulsions was  $+29 \pm 2.15$  mV, which showed that cationic chitosan molecules had adsorbed to the surface of the anionic SPI – OSA starch coated droplets. The effect of freeze–thaw cycles on the  $\zeta$ -potential of emulsions showed in Fig. 4. Freeze–thaw cycles caused a decrease in the magnitude of the electric charge on the droplets in the primary and secondary emulsions and valued close to zero while the number of cycles caused no large change in the  $\zeta$ -potential of tertiary emulsion, which suggested the interfacial composition was less altered by these treatments. This reduction may attribute to the loss of solubility of soy proteins at a low temperature (Palazolo et al., 2011). These results showed, electrostatic interactions might be less important in frozen systems because of the increase in ionic strength that occurs when ice crystals form, and solutes are concentrated in the unfrozen aqueous phase (Ghosh and Coupland, 2008; Mun et al., 2008; Thanasukarn et al., 2006).

The influence of freeze–thaw cycling on the mean particle diameter ( $D_{4,3}$ ) and microstructure of primary, secondary and tertiary emulsions measured. Fig. 5 shows freeze–thaw cycling caused an appreciable increase in the mean particle size in the primary and secondary emulsions while only a



**Fig. 6 – Optical micrographs of tertiary emulsion A: before B: after one freeze–thaw cycle and C: after two freeze–thaw cycle.**

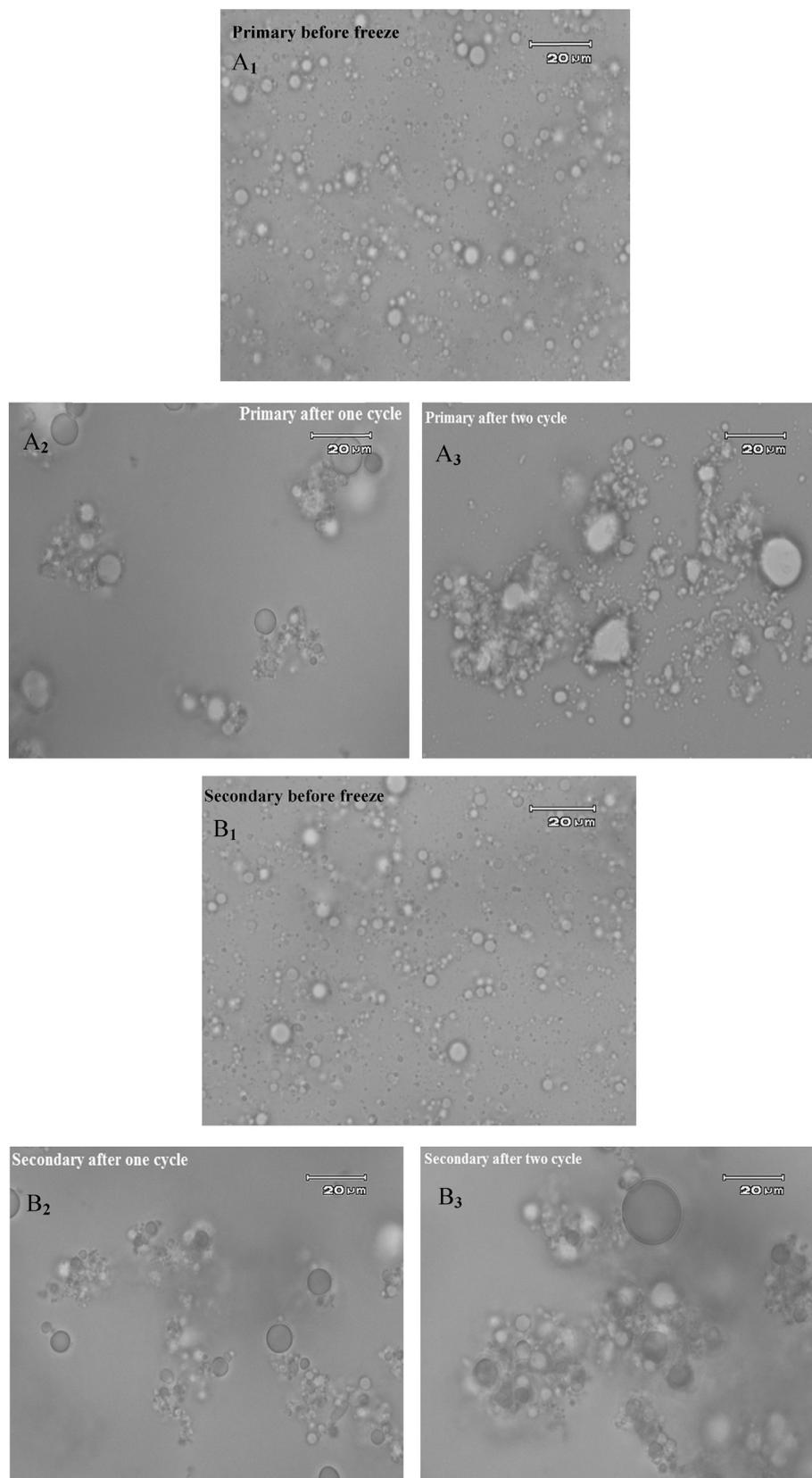


**Fig. 7 – Particle size distributions of A: primary, B: secondary and C: tertiary emulsions after two freeze–thaw cycles.**

small increase in mean particle size in the tertiary emulsion. On the other hand, the tertiary emulsion compared with the primary and secondary emulsions was more stable to droplet aggregation (Fig. 6). A large increase in mean particle diameter showed the extensive droplet aggregation occurred in the primary and secondary emulsions after freeze–thaw cycling. The tertiary emulsion was relatively stable to droplet aggregation at all freeze–thaws cycling. Fig. 7 shows comparatively the particle size distributions (PSD) of primary, secondary and tertiary emulsions before and after freeze thawing. Primary emulsion showed bimodal particle size distribution in a wide range of particle diameters after freeze–thawing. This result is attributed to the presence of flocs induced by the freeze–thaw process. In addition, a peak at higher particle size ( $>20 \mu\text{m}$ ) was observed in the primary emulsion, which is consistent with a coalescence destabilization induced by freeze–thawing. Freeze-thawed secondary

emulsions did not resist the stress induced by freeze–thawing and hence, were destabilized by droplet coalescence and forming bimodal distributing of sizes. The presence of these particle populations could be attributed mainly to droplet aggregates occurred in the secondary emulsion after freeze–thaw cycling. In addition, a peak at higher particle size ( $>25 \mu\text{m}$ ) was observed in the secondary emulsion, which is consistent with a coalescence destabilization induced by freeze–thawing. For tertiary emulsion under freeze–thawing, monomodal PSD was observed. The PSD shows a little change in the particle size and the relative stability of the tertiary emulsion. This phenomenon could explain the chitosan adsorbed to SPI-OSA starch could increase the relatively thick interfacial membranes of droplets emulsions. For primary emulsion, to form visible oil in the upper part of the container was the result of the extensive coalescence process. Instability saw in the secondary emulsion after freeze–thaw cycling may be caused by the re-association and re-arrangement of OSA starch molecules between droplet surfaces in close during or after freezing. The factor that may help explain to the instability of primary and secondary emulsions is the high tendency of soy protein isolates to aggregate by effect of low temperature (Palazolo et al., 2011). It known the chilling and freezing of aqueous dispersions of soy protein isolates induce the aggregation of 11S globulin. At sub-zero temperatures, this aggregation involves to forming species of high molecular weight (15S + 15S fractions) by thiol/disulfide exchange reactions. Ice is formed from water molecules both in the bulk phase and interface during freezing that promoting the protein aggregation in both zones (Palazolo et al., 2011). Cortes-Munoz et al. (2009) reported the destabilization of emulsions during freeze–thawing was probably promoted by disruption or collapse of interfacial film because of protein aggregation in bulk phase and at the o/w interface (Cortes-Munoz et al., 2009). In addition, the SPI proteins might have been denatured and thus lost their functionality when the emulsions were subjected to freezing temperature (Palazolo et al., 2011). All of these various mechanisms should contribute to the freeze–thaw instability of primary and secondary emulsions. The optical microscopy results show that after freezing–thawing the droplets found in dense flocs separated by large regions of continuous phase (Fig. 8).

The influence of freeze–thaw cycling on the creaming stability of primary, secondary and tertiary emulsions also measured. Creaming instability observed in the primary emulsion after two freeze–thaw cycles, as shown by the visible observation of a cream layer at the top and a serum layer at the bottom of the emulsion the result of the extensive coalescence process. The secondary emulsion observed to separate into an opaque ‘cream’ layer at the top and a transparent layer at the bottom after one freeze–thaw cycle. Our results showed that primary and secondary emulsions were creaming instability after one freeze–thaw cycle. On the other hand, the tertiary emulsion remained stable to creaming even after two freeze–thaw cycles. The creaming stability measurements showing that droplets coated with three-component interfacial layers were more stable to freeze–thaw cycling than those coated with a one- or two components because these interfacial membranes were rather thick and resistant to disruption.



**Fig. 8 – Optical micrographs of A<sub>1</sub>: primary emulsion before freeze A<sub>2</sub>: primary emulsion after one freeze–thaw cycle and A<sub>3</sub>: primary emulsion after two freeze–thaw cycle, B<sub>1</sub>: secondary emulsion before freeze B<sub>2</sub>: secondary emulsion after one freeze–thaw cycle and B<sub>3</sub>: secondary emulsion after two freeze–thaw cycle.**

#### 4. Conclusion

This study has shown that of layer-by-layer deposition method could produce emulsions that are stable to freeze thaw cycling. The emulsions with oil droplets coated by a three-component interfacial layers (SPI-OSA starch – chitosan) were more stable to freeze–thaw cycling than those coated with either a one (SPI) or two (SPI-OSA starch) component layer. The improved stability of the tertiary emulsions can credit to the relatively thick interfacial membranes provide resistant to disruption. The interfacial engineering technology described in this work may prove to be a useful means of improving the freeze–thaw stability of a variety of food and non-food oil-in-water emulsions.

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