CHAPTER 5

EMULSIFICATION OF TUNA OIL IN TWO-LAYERED INTERFACIAL MEMBRANES

5.1 Abstract

Tuna oil-in-water emulsions (5 wt% tuna oil, 100 mM acetate buffer, pH 3.0) containing droplets stabilized either by lecithin membranes (primary emulsions) or by lecithin-chitosan membranes (secondary emulsions) were produced. The secondary emulsions were prepared using a layer-by-layer electrostatic deposition method that involved adsorbing cationic chitosan onto the surface of anionic lecithin-stabilized droplets. Primary and secondary emulsions were prepared in the absence and presence of corn syrup solids (a carbohydrate widely used in the microencapsulation of oils) and then their stability to environmental stresses was monitored. The secondary emulsions had better stability to droplet aggregation than primary emulsions exposed to thermal processing (30 to 90 °C for 30 minutes), freeze-thaw cycling (-18°C for 22 hours/30°C for 2 hours), high sodium chloride contents (200 mM NaCl) and freeze-drying. The addition of corn syrup solids decreased the stability of primary emulsions, but increased the stability of secondary emulsions. The interfacial engineering technology used in this study could lead to the creation of food emulsions with novel properties or improved stability to environmental stresses.

5.2 Introduction

Tuna oil is a good source of n-3 polyunsaturated fatty acids (n-3 PUFAs), especially EPA (eicosapentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3), which have been shown to be important for the maintenance of good

health and the prevention of a range of human diseases and disorders (Shibasaki *et al*., 1999; Uauy and Valenzuela, 2000; Harris, 2001; 2004). Long-chain PUFAs in tuna oil are highly unsaturated and therefore are highly susceptible to oxidation. Lipid oxidation can be reduced by addition of antioxidants to the oil or by microencapsulation of the oil (Lin *et al*., 1995; Heinzelmann *et al*., 2000; Velasco *et al*., 2000; Kagami *et al*., 2003).

Microencapsulation is a process whereby particles of sensitive or bioactive materials are covered with a thin film of a coating material (Dziezak, 1988). The encapsulated substance (*e.g.,* fats, oils, aromas, flavors) is usually referred to as the "core" material, whereas the film surrounding the core is usually called the "wall" material (Dziezak, 1988; Sheu and Rosenberg, 1995; Dian *et al*., 1996). The wall material is usually composed of relatively low molecular weight carbohydrates, such as corn syrup solids and/or maltodextrin (Lin *et al*., 1995; Sheu and Rosenberg, 1998; Pauletti and Amestoy, 1999; Hardas *et al*., 2000; Heinzelmann *et al*., 2000). Nevertheless, corn syrup solids and maltodextrin can not be used in isolation because they are not effective at forming and stabilizing emulsified oil. Instead, hydrophobic core material is usually homogenized in the presence of an aqueous solution containing an emulsifier (*e.g.,* surfactant, phospholipid or biopolymer) that forms a protective coating around the oil droplets, and then corn syrup solids and/or maltodextrin are mixed with the resulting emulsion (Sheu and Rosenberg, 1995; Hogan *et al*., 2001a; 2001b; Kagami *et al*., 2003). The emulsion is then dried to remove the water (*e.g.,* by spray or freeze drying), which leads to the formation of oil droplets surrounded by emulsifier molecules that are entrapped within a carbohydrate matrix. Many researchers have used mixtures of maltodextrin or corn syrup solids with proteins, such as whey protein and sodium caseinate for microencapsulation (Sheu and Rosenberg, 1998; Pauletti and Amestoy, 1999; Hardas *et al*., 2000). On the contrary, there is

much less information about the mixing of maltodextrins or corn syrup solids with other biopolymers. Because a stable emulsion containing small droplets is critical for microencapsulation, it is important to select an appropriate emulsifying system, as well as the conditions required to obtain a stable emulsion before the drying process.

Recently, the research has shown that oil-in-water emulsions with improved stability to environmental stresses can be produced using an electrostatic layer-by-layer deposition technique that produces oil droplets that are coated by multiple-layered interfacial membranes (Ogawa *et al*., 2003a; 2003b). A *primary* emulsion containing small anionic droplets coated with a lecithin membrane is produced by homogenizing oil and water together in the presence of lecithin, a low molecular weight emulsifier that rapidly adsorbs to the surface of oil droplets during homogenization. A *secondary* emulsion containing cationic droplets coated with a lecithin-chitosan membrane is then produced by adding chitosan to the primary emulsion. Any flocs formed during the preparation of the secondary emulsion are then broken down by the application of disruptive energy, *e.g.,* blending, homogenization or sonication. The production of emulsions containing droplets surrounded by multiple-layered interfacial membranes may prove to be an effective means of improving the stability of microencapsulated oils. The objective of the current study was therefore to examine the effect of corn syrup solids on the stability of emulsions stabilized by lecithin alone or by lecithin-chitosan membranes. The effect of environmental stresses, such as pH, ionic strength, thermal treatment, freeze-thaw cycling and freeze drying were examined. The results of this study may lead to a novel method of improving the properties of encapsulated oils in the food industry.

5.3 Materials and Methods

5.3.1 Materials

Powdered chitosan ("medium molecular weight", ≈ 250 kDa) was purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO). As stated by the manufacturer the properties of this chitosan were: viscosity of 1 wt% solution in 1 wt% acetic acid = 200-800 Cps; degree of deacetylation = 75%-85%; maximum moisture content = 10 wt%; maximum ash content = 0.5 wt%. Powdered lecithin (Ultralec P; acetone insolubles, 97%; moisture, 1.0 wt%, consist primarily of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol) was donated by ADM-Lecithin (Decatur, IL). Corn syrup solids (DRI SWEET®36, Code 335249; dextrose equivalent, 36; molecular weight, 0.5 kDa; total solids, 97.2 wt%; moisture, 2.8 wt%; ash, 0.2 wt%) was obtained from Roquette America. Inc. (Keokuk, IA). Degummed, bleached and deodorized tuna oil was obtained from Maruha Co. (Utsunomiya, Japan; 16 wt% EPA; 14.1 wt% DHA; PV, 0.35± 0.01 mmol/kg oil; TBARS, 0.12±0.01 mmol/kg oil; no tocopherol). Analytical grade sodium choride (NaCl), sodium azide (NaN_3), sodium acetate (CH_3COONa), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade or better. Distilled and deionized water was used for the preparation of all solutions.

5.3.2 Methods

5.3.2.1 Solution Preparation

A stock buffer solution was prepared by dispersing 2 mM of sodium acetate and 98 mM of acetic acid in water and then adjusting the pH to 3.0. An emulsifier solution was prepared by dissolving 3.53 wt% lecithin powders into stock buffer solution. The emulsifier solution was sonicated for 1 min at a frequency of 20 kHz, amplitude of 70% and duty cycle of 0.5 s (Model 500, sonic dismembrator, Fisher Scientific,

Pittsburgh, PA) to disperse the emulsifier. The pH of the solution was adjusted to 3.0 using HCl or NaOH, and then the solution was stirred for about 1 h to ensure complete dissolution of the emulsifier. A chitosan solution was prepared by dissolving 1.5 wt% powdered chitosan in sodium acetate-acetic acid buffer solution. A corn syrup solids solution was prepared by dispersing 50 wt% corn syrup solids in sodium acetate-acetic acid buffer solution.

5.3.2.2 Emulsion Preparation

A concentrated tuna oil-in-water emulsion (15 wt% oil, 3 wt% lecithin) was made by blending 15 wt% tuna oil with 85 wt% aqueous emulsifier (lecithin) solution using a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland), followed by three passes at 5,000 psi through a two-stage high-pressure valve homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA). This primary emulsion was diluted with aqueous chitosan solution (containing sodium azide as a preservative) to form a secondary emulsion (5 wt% tuna oil, 1 wt% lecithin, 0.02 wt% NaN_3 and 0.2 wt% chitosan). Any flocs formed in the secondary emulsion were disrupted by passing it once through a high-pressure valve homogenizer at a pressure of 4,000 psi (Ogawa *et al*., 2003a). The electrical charge on the droplets changed from negative (~ -52 mV) for the primary emulsion to positive (~ + 57 mV) for the secondary emulsion when the chitosan was present, which indicates that the cationic chitosan molecules adsorbed to the surface of the anionic lecithin-coated emulsion droplets (Magdassi *et al*., 1997; Ogawa *et al*., 2003a). Primary and secondary emulsions containing varying concentrations of corn syrup solids (0-25 wt%) were prepared by mixing the initial primary and secondary emulsions with corn syrup solids solutions. The pH of the final emulsions was adjusted back to 3.0 using HCl or NaOH.

5.3.2.3 Evironmental Stresses Study

The influence of environmental stresses on the properties of primary and secondary emulsions in the absence and presence of corn syrup solids with the same oil concentration (5.0 wt%) was studied:

pH. All emulsion samples were initilally prepared at pH 3.0 and then the pH of the emulsions was adjusted to the desired pH (3-8) by adding NaOH solution. The emulsions (10 mL) were transferred into glass test tubes (internal diameter = 15 mm, height = 125 mm), which were then stored at room temperature prior to analysis.

NaCl. Emulsions containing NaCl (200 mM) were prepared by adding NaCl solution to primary and secondary emulsions. Emulsions sample (10 mL) were then transferred into glass test tubes (internal diameter = 15 mm, height = 125 mm), and stored at room temperature prior to analysis.

Thermal Treatment. Emulsion samples (10 mL) were transferred into glass test tubes (internal diameter = 15 mm, height = 125 mm), which were then stored in a water bath for 30 min at a fixed temperature (30, 60 and 90°C). The emulsions were then placed immediately into a cold water bath for 10 min and stored at room temperature prior to analysis.

Freeze-Thaw Cycling Stability. Emulsion samples (10 mL) were transferred into plastic test tubes (internal diameter = 10 mm, height = 95 mm), which were frozen by placing them in a -18°C freezer for 22 h and then thawed by placing them in a water bath at 30°C for 2 h. This freeze-thaw cycle was repeated two times.

Freeze Drying. The emulsion samples (50 mL) were transferred into Petri dishes (internal diameter = 90 mm, height = 20 mm), which were frozen by placing them over night in a -80°C freezer. A laboratory scale freeze-drying device (Virtis, the Virtis Company, Gardiner, NY) was used to dry the frozen emulsions. The freeze-drying was performed at a vacuum

pressure of 1 atm for 48 h with a condenser temperature around -55°C.

5.3.2.4 Emulsion Characterization

After the emulsions had been subjected to environmental stress their physicochemical properties were characterized. The apparent viscosity of aqueous solution (η_S) and emulsions (η_E) was measured at 25°C using a dynamic shear rheometer (Constant Stress Rheometer, CS10, Bohlin Instruments, Cranbury, NJ). The samples were contained in a concentric cylinder cell (the diameter of the inner cylinder was 25 mm, and the diameter of the outer cylinder was 27.5 mm) with a thin layer of mineral oil on top of the sample to prevent water evaporation. The relative viscosity was calculated from η_E/η_S.

The creaming stability of emulsion samples (10 ml or 67 mm) was monitored at room temperature by visual observation of the height of the serum layer formed at the bottom of glass tubes (H_S), expressed as a percentage of the total height of the emulsions in the tubes (H_E): Creaming Index = 100 × (H_S/H_E).

The particle size distribution of the emulsions was measured using a static light scattering instrument (Malvern Mastersizer Model 3.01, Malvern Instruments, Worcs., UK). To prevent multiple scattering effects the emulsions were diluted with pH-adjusted acetate buffer prior to analysis so the droplet concentration was less than 0.02 wt%.

The electrical charge (ζ-potential) of oil droplets in the emulsions was determined using a particle electrophoresis instrument (ZEM5003, Zetamaster, Malvern Instruments, Worcs., UK). The emulsions were diluted to a droplet concentration of approximately 0.008 wt% with pH-adjusted double-distilled water prior to analysis to avoid multiple scattering effects.

The microstructure of selected emulsions was determined using optical microscopy (Nikon microscope Eclipse E400, Nikon Corporation, Japan). Emulsions were gently agitated in a glass test tube before measurement to ensure that they were homogeneous. A drop of emulsion was then placed on a glass side and observed at an objective magnification of 20x. An image of the emulsion was acquired using digital image-processing software (Micro Video Instruments Inc., Avon, MA) and stored on a personal computer.

5.3.2.5 Statistical Analysis

All experiments were carried out in at least duplicate using freshly prepared samples and results are reported as the mean and standard derivation of these measurements.

5.4 Results and Discussion

5.4.1 Influence of Corn Syrup Solids Concentration on Secondary Emulsion Characteristics

The purpose of these experiments was to examine the influence of corn syrup solids concentration on the properties of emulsions containing lecithin-chitosan-coated droplets (secondary emulsions). A series of secondary emulsions with different corn syrup solids concentrations was prepared (5 wt% tuna oil, 1 wt% lecithin, 0.2 wt% chitosan, 100 mM acetate buffer, pH 3.0, 0-25 wt% corn syrup solids). The emulsions were then stored at room temperature and the mean particle diameters, creaming stability, electrical charge (ζ-potential) and apparent viscosity were measured.

Table 7 Influence of corn syrup solids concentration on electrical charge (ζ-potential), apparent viscosity (η_{APP}) and relative viscosity (η_{REL}) of secondary emulsions consisting of 5 wt% tuna oil, 1 wt% lecithin, 0.2 wt% chitosan, 100 mM acetate buffer (pH 3.0).

Corn syrup solids (wt%)	Measured Properties		
	ζ-potential (mV)	η_{APP} (mPa s)	η_{REL}
0	58.0 ± 2.0	7.89 ± 0.0	1.16 ± 0.01
5	57.5 ± 1.8	8.71 ± 0.1	1.16 ± 0.02
10	56.6 ± 1.3	9.90 ± 0.2	1.17 ± 0.03
15	56.5 ± 0.4	11.8 ± 0.3	1.20 ± 0.03
20	56.4 ± 1.0	14.4 ± 0.3	1.18 ± 0.03
25	56.5 ± 1.2	18.3 ± 0.1	1.16 ± 0.03

The presence of corn syrup solids had no significant effect ($P<0.05$) on the electrical charge of the droplets in the secondary emulsions (Table 7). These results suggested that the corn syrup solids did not interfere with the adsorption of the chitosan to the droplet surfaces, *e.g.,* by displacing it from the droplet surfaces. This would be expected since corn syrup solids are hydrophilic nonionic oligosaccharides and would therefore not be expected to be particularly surface active or to adsorb to charged surfaces.

The addition of non-adsorbing biopolymers, such as corn syrup solids, to the aqueous phase of oil-in-water emulsions induces an attractive "depletion" interaction between the oil droplets due to an osmotic effect (McClements, 2000). This attractive force increases as the concentration of non-adsorbing biopolymer increases until eventually it may become large enough to overcome the repulsive interactions between the droplets and cause them to flocculate. Recently, it has been shown that this kind of depletion flocculation may promote droplet coalescence because the droplets are brought into close proximity for extended periods (Ye *et al*., 2004). The lowest concentration required to cause depletion flocculation is referred to as the critical flocculation concentration (CFC), which depends on the dimensions of the biopolymer molecules and the

size of the emulsion droplets (McClements, 1999). Therefore, the propensity of corn syrup solids to promote depletion flocculation or coalescence in the emulsions was examined.

Corn syrup solids (0 to 25 wt%) was no significant effect ($P \geq 0.05$) on the mean droplet diameter (0.26 ± 0.02 μm) or creaming stability (Creaming Index = 0% after 24 hours, data not show) of the secondary emulsions. These results suggested that the corn syrup solids did not promote droplet flocculation or coalescence. Viscosity measurements were also used to confirm that the droplets in the emulsions were not flocculated (Table 7). The viscosity of an emulsion (η) at low droplet concentrations ($\phi < 5\%$) is described by the Einstein equation: $\eta = \eta_0 (1+2.5\phi)$, where η_0 is the viscosity of the liquid surrounding the droplets (continuous phase) and ϕ is the dispersed phase volume fraction (McClements, 1999). This equation assumes that the liquid is Newtonian, the droplets are rigid spheres, and there are no droplet-droplet interactions. For this experiment, the relative viscosity ($\eta_{REL} = \eta/\eta_0$) of the emulsions could be predicted by the Einstein equation which value [$\eta_{REL} = 1+2.5\phi = 1+(2.5)(0.05)$] ~ 1.14-1.15. If droplet flocculation occurred in the emulsions then the relative viscosity of emulsion samples should be appreciably greater than the value given by the Einstein equation. From the results, the relative viscosity of the emulsions measured in the presence of corn syrup solids (η_{REL} = 1.15-1.20) was fairly similar to the value predicted by the Einstein equation, which indicated that flocculation did not occur at the corn syrup solids concentrations used in this experiments ($\leq$25 wt%). In summary, the particle size, creaming and viscosity measurements indicated that corn syrup solids (DE 36) did not promote droplet flocculation in the emulsions when used at concentrations of 25 wt% or less.

5.4.2 Influence of Ionic Strength and pH on Emulsion Stability

The influence of ionic strength (NaCl concentration) and pH on the stability of both lecithin-coated (primary emulsion) and lecithin-chitosan-coated (secondary emulsion) droplets in the absence and presence of corn syrup solids were examined. A fixed corn syrup solids concentration of 20 wt% was used to prepare the primary and secondary emulsions in these studies. A series of dilute emulsions (5 wt% oil) was prepared by dispersing the concentrated primary and secondary emulsions in a variety of aqueous solutions with different pH values (3 to 8) and ionic strengths (0 and 200 mM NaCl). The emulsions were stored at room temperature for 1 week and then the mean particle diameter, electrical charge (ζ-potential) and creaming stability were measured.

In all the emulsions, the magnitude of the ζ-potential decreased appreciably as the NaCl concentration was increased from 0 to 200 mM at pH 3 ($P<0.05$, Table 8), which can be attributed to electrostatic screening effects (McClements, 1999). In addition, all of the emulsions were stable to droplet aggregation at 0 and 200 mM NaCl at pH 3 (Table 8), which indicates that the combined strength of the attractive interactions between the droplets (*e.g.,* van der Waals, depletion and hydrophobic) was insufficient to overcome the combined strength of the repulsive interactions (*e.g.,* electrostatic, steric and thermal fluctuation) even in the presence of salt (McClements, 1999).

The ζ-potential of the lecithin-stabilized droplets in the primary emulsions was negative at all pH values (Figure 17) due to the fact that the pK_a value of the anionic phosphate groups on lecithin are typically less than 3 (Koynova and Caffrey, 1998). The magnitude of the ζ-potential decreased slightly when the pH was raised from 3 to 8, which can be attributed to the electrostatic screening effect associated with the increase in ionic strength that occurs when the pH was increased. The ζ-potential of the lecithin-chitosan stabilized

droplets in the secondary emulsions was positive at low pH values (< pH 7) but became negative at higher values (Figure 17). The cationic groups on chitosan typically have pK_a values around 6.3-7 (Schulz *et al*., 1998), hence the chitosan begins to lose some of its charge around this pH. Consequently, there may have been a weakening in the electrostatic attraction between the chitosan and the lecithin-coated droplets, which may have led to the release of some of the adsorbed chitosan. Nevertheless, the fact that the ζ-potential of the droplets was less negative in the secondary emulsions than in the primary emulsions at the same high pH values suggests that some of the chitosan remained adsorbed to the droplet surfaces.

Table 8 Influence of NaCl (0 or 200 mM) and corn syrup solids, CSS (0 or 20 wt%) on mean particle diameter and electrical charge (ζ-potential) of emulsions consisting of 5 wt% tuna oil, 1 wt% lecithin, 100 mM acetate buffer (pH 3.0), and either 0 wt% chitosan (primary emulsion) or 0.2 wt% chitosan (secondary emulsion).

	NaCl concentration (mM)	
Measured Properties	0	200
Mean Particle Diameter (μm)		
Primary emulsion	0.39 ± 0.06	0.41 ± 0.06
Primary emulsion + CSS	0.38 ± 0.04	0.44 ± 0.08
Secondary emulsion	0.26 ± 0.01	0.26 ± 0.01
Secondary emulsion + CSS	0.27 ± 0.06	0.26 ± 0.04
Electrical Charge (ζ-potential)		
Primary emulsion	-52.8 ± 0.2	-27.1 ± 0.7
Primary emulsion + CSS	-51.8 ± 1.7	-26.5 ± 0.1
Secondary emulsion	55.5 ± 0.1	20.8 ± 1.3
Secondary emulsion +	54.7 ± 1.8	19.3 ± 3.5

CSS

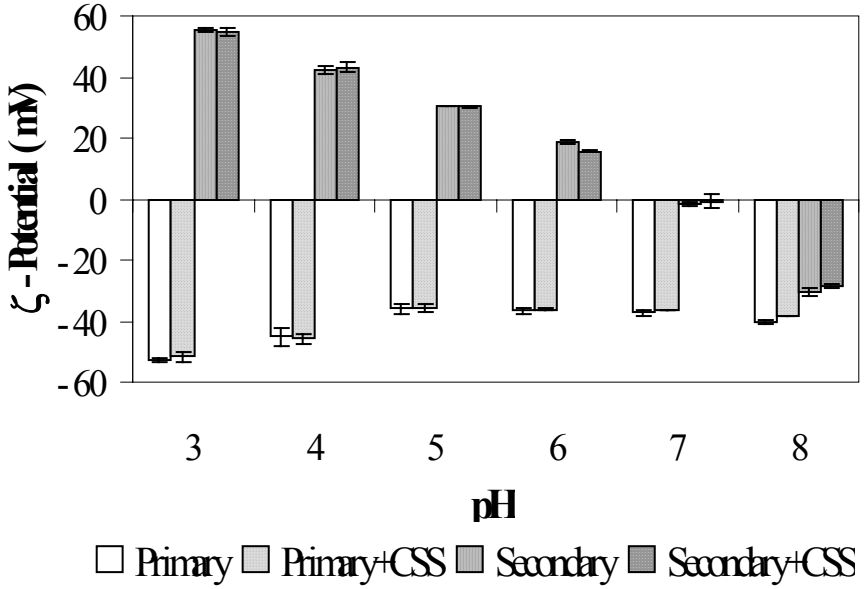


Figure 17 Influence of pH on electrical charge (ζ-potential) of emulsion droplets in primary (5 wt% tuna oil, 1 wt% lecithin, 100 mM acetate buffer, pH 3.0) and secondary emulsions (5 wt% tuna oil, 1 wt% lecithin, 0.2 wt% chitosan, 100 mM acetate, pH 3.0) in the absence and presence of 20 wt% corn syrup solids (CSS).

The mean particle diameter of the primary emulsions remained low at all pH values (Figure 18), suggesting that these emulsions were stable to droplet aggregation. The secondary emulsions were stable to droplet aggregation at pH <5.0, but highly unstable at higher pH values, as deduced from the large increase in mean particle diameter ($P<0.05$). The instability of the secondary emulsions at higher pH values was probably because the ζ-potential was relatively low (Figure 17), which reduced the electrostatic repulsion between the droplets, thus leading to extensive droplet flocculation (Ogawa *et al*., 2003a).

In addition, partial desorption of chitosan molecules from the droplet surfaces may have led to some bridging flocculation.

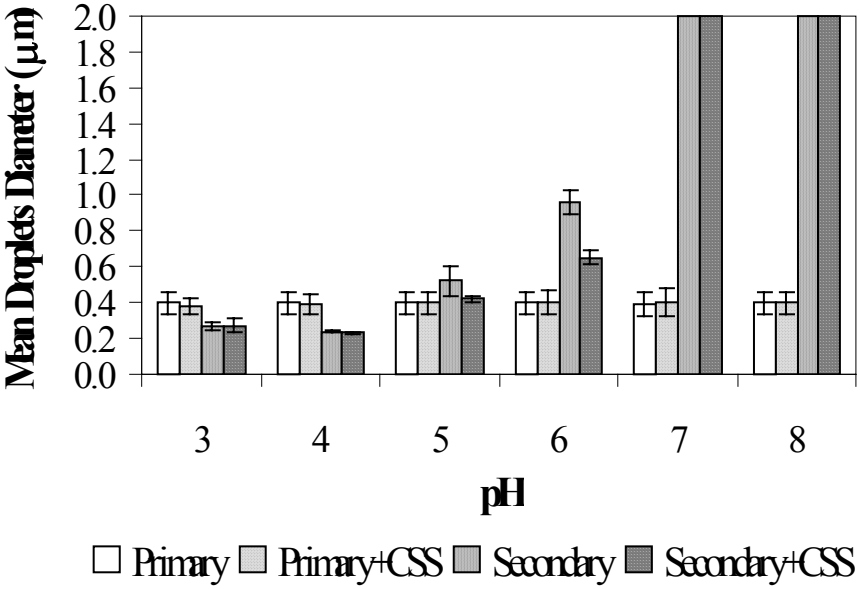


Figure 18 Influence of pH on mean diameter of emulsion droplets in primary (5 wt% tuna oil, 1 wt% lecithin, 100 mM acetate, pH 3.0) and secondary emulsions (5 wt% tuna oil, 1 wt% lecithin, 0.2 wt% chitosan, 100 mM acetate, pH 3.0) in the absence and presence of 20 wt% corn syrup solids (CSS).

The influence of corn syrup solids on the ζ-potential and mean particle diameter of the diluted primary and secondary emulsions at different pH (3-8) and ionic strength (0 or 200 mM NaCl) was also measured (Figures 17 and 18). At all pH and ionic strengths, there was no significant difference between the ζ-potential and mean particle diameter measurements of the primary emulsions in the absence or presence of corn syrup solids ($P \geq 0.05$). In addition, there was no significant effect of corn syrup solids on the ζ-potential of the secondary emulsions at all pH and ionic strengths. Nevertheless, corn syrup solids did have a significant impact on the mean particle diameter of

the secondary emulsions at pH 6 (Figure 18). The mean particle diameter was smaller in the secondary emulsion in the presence of corn syrup solids (0.76 ± 0.06 μm) than in the absence of corn syrup solids (1.19 ± 0.17 μm). This effect might be due to the fact that corn syrup solids increase the viscosity of the aqueous phase, which retards the movement of oil droplets thereby inhibiting their aggregation (McClements, 2000). Nevertheless, the presence of the corn syrup solids could not prevent the extensive droplet aggregation observed in the secondary emulsions at higher pH values.

5.4.3 Influence of Thermal Treatment on Emulsion Stability

The aim of these experiments was to examine the influence of thermal treatment on the stability of primary and secondary emulsions in the absence and presence of corn syrup solids (20 wt%). Emulsions with the same oil concentration (5 wt%) were held at temperatures ranging from 30 to 90°C for 30 min, cooled to room temperature, and then stored for 24 h prior to measuring their mean particle diameter, ζ-potential and microstructure.

There was no significant effect of heating or corn syrup solids on the ζ-potential of either the primary (-51 ± 2 mV) or secondary (57 ± 2 mV) emulsions ($P \geq 0.05$). In addition, there was no significant effect of heating or corn syrup solids on the mean particle diameter of the droplets in the secondary emulsions (0.27 ± 0.03 μm) ($P \geq 0.05$). On the other hand, there was evidence of droplet coalescence in some of the primary emulsions after heating, with d_{32} increasing from 0.40 ± 0.06 μm in the emulsions treated at 30°C (in the presence or absence of corn syrup solids) to 0.56 ± 0.02 μm (no CSS) and 1.54 ± 0.26 μ m (+CSS) in the emulsions treated at 90°C. Thus, corn syrup solids appeared to promote droplet aggregation during heating. This finding was confirmed by optical microscopy, which showed that on average the individual droplets were larger in the primary emulsions containing corn syrup solids than in those

without corn syrup solids (Figure 19). The increase in mean particle diameter in the primary emulsions observed at higher temperatures may have been due to a change in the optimum curvature of the lecithin molecules promoting coalescence, *e.g.,* due to dehydration of the head groups (McClements, 1999; Ogawa *et al*., 2003b). The fact that droplet coalescence was greater in the primary emulsion containing corn syrup solids may have been because some of the lecithin was bound to the corn syrup solids (Wangsakan *et al*., 2001) and was therefore unavailable to stabilize the droplets. Presumably, coalescence was not observed in the secondary emulsions because the droplets were coated with chitosan, which would have prevented the lecithin layers on different droplets from coming into close contact.

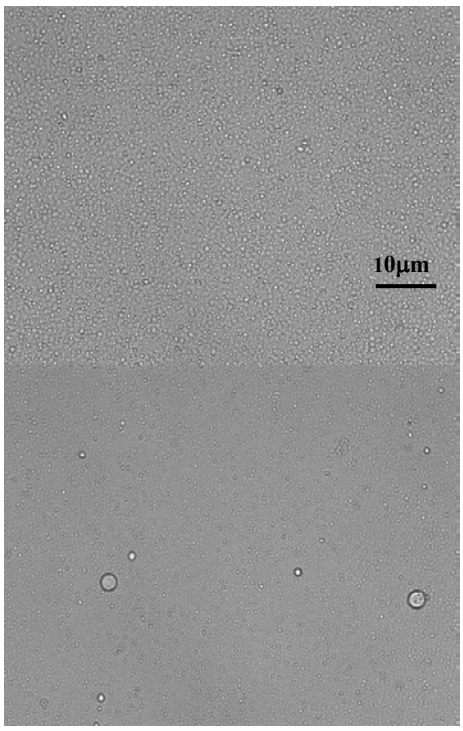
5.4.4 Influence of Freeze-Thaw Processing on Emulsion Stability

The aim of these experiments was to examine the influence of freeze-thaw processing on the stability of primary and secondary emulsions in the absence and presence of corn syrup solids. Emulsions were subject to two freeze-thaw cycles consisting of 22 h at -18°C in a freezer followed by 2 h at 30°C in a water bath. The results indicated that all of the emulsions were unstable to the freeze-thaw process (Table 9). There was a greater than 8-fold increase in mean particle diameter after the first cycle in both primary and secondary emulsions in the absence of corn syrup solids ($P<0.05$). A number of physicochemical mechanisms may be responsible for the extensive droplet aggregation observed in our freeze-thawed emulsions. First, when the emulsions were cooled some of the water crystallized, which caused the droplets to become highly concentrated in the regions of unfrozen aqueous phase separating the ice crystals, thereby promoting increased droplet-droplet interactions (Saito *et al*., 1999). Second, it is possible that ice crystals formed during freezing may have penetrated

into the oil droplets and disrupted their interfacial membranes, thus making them more prone to coalescence (Harada and Yokomizo, 2000). Third, cooling may have caused some of the fat in the emulsion droplets to crystallize, which may have promoted partial coalescence due to penetration of a fat crystal from one droplet through the membrane of another droplet (Vanapalli *et al*., 2002).

The effect of corn syrup solids on the stability of the emulsions during freeze-thaw process was also investigated. There was a 2-fold increase in the mean diameter of the particles in the secondary emulsion containing corn syrup solids, but oiling-off (the formation of a layer of oil on top of the samples) was observed in the primary emulsion in the presence of corn syrup solids. The secondary emulsions were therefore more stable in the presence of corn syrup solids than in its absence (Table 9), with the mean particle diameter decreasing from 4.8 ± 0.01 μm in the absence of corn syrup solids to 0.6 ± 0.06 μm in the presence of corn syrup solids after the first freeze-thaw cycle. The corn syrup solids probably improved the stability of the secondary emulsions by increasing the osmolyte concentration in the aqueous phase, thereby reducing its crystallization temperature and limiting the total amount of ice crystals formed. This phenomenon would have increased the volume fraction of unfrozen aqueous phase available to the oil droplets, thereby causing less droplet-droplet interactions (Komatsu *et al*., 1997; Ozaki and Hayashi, 1997). On the other hand, corn syrup solids actually promoted instability in the primary emulsions, since oiling-off occurred even after the first cycle of the freeze-thaw process. This may have been because some of the lecithin was bound by the corn syrup solids (Wangsakan *et al*., 2001; 2003), which reduced the amount available to stabilize the oil droplets.

30°C **90°C**



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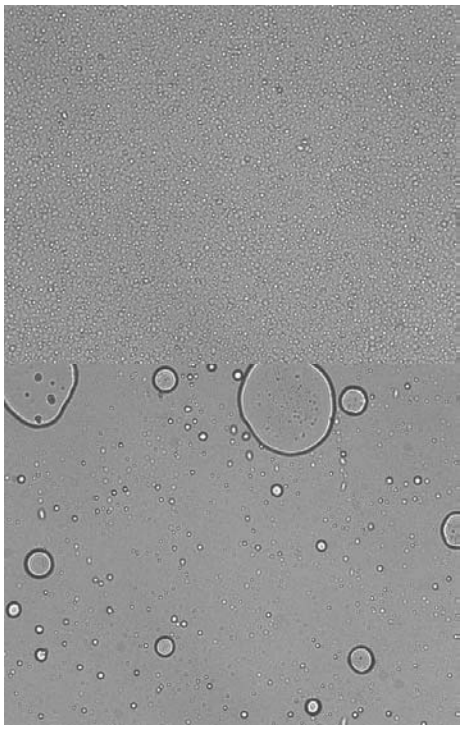

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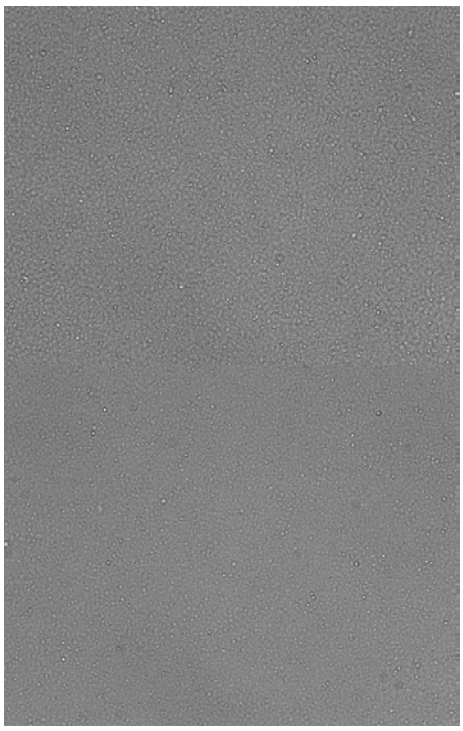

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Figure 19 Dependence of microstructure on isothermal treatment temperature (30 and 90°C for 30 min) for: a) primary emulsion; b) primary emulsion in the presence of 20 wt% corn syrup solids; and c) secondary emulsions.

Table 9 Dependence of mean particle diameter (μm) of primary and secondary emulsions on number of freeze-thaw cycles (-18°C for 22 h/30°C for 2 h) in the absence and presence of 20 wt% corn syrup solids (CSS).

	Number of cycles		
Emulsions	0	1	2

Primary emulsion	0.40 ± 0.06	3.18 ± 0.17	Oiling off
Primary emulsion + CSS	0.40 ± 0.06	Oiling off	Oiling off
Secondary emulsion	0.27 ± 0.04	4.79 ± 0.01	6.98 ± 0.44
Secondary emulsion + CSS	0.28 ± 0.05	0.61 ± 0.06	2.94 ± 0.26

5.4.5 Influence of Freeze Drying on Emulsion Stability

The purpose of these experiments was to examine the influence of freeze-drying and corn syrup solids on the stability of primary and secondary emulsions. Emulsions were frozen at -80°C and dried under vacuum in a commercial freeze dryer. After finishing the drying process the dried products were ground using a mechanical device (Handy Chopper, Black & Decker Inc., Shelton, CT). The particle diameter of the particles in the ground products was less than 500 μm (CRC Micro Sieve, The Chemical Rubber Co, Cleveland, Ohio). The dried emulsions were reconstituted by dissolving them in sodium acetate-acetic acid buffer (pH 3.0) to a final oil concentration that was equivalent to that of the original liquid emulsions (5 wt%). The powder and solution were mixed using a high-speed blender (Type 37600 Mixer, Barnstead/Thermolyne, Dubuque, Iowa) until the droplets were completely dispersed and then kept at room temperature for 24 h. The mean particle diameter and the microstructure of the emulsions were then determined (Figure 20).

The primary emulsions were highly unstable to the freeze drying process. In the absence of corn syrup solids, primary emulsions were completely destabilized by the freeze-drying process, with evidence of a distinct oil layer on top of the samples. In the presence of corn syrup solids, a dispersible powder could be produced from the primary emulsion, but there was distinct evidence of droplet coalescence after its

reconstitution (Figure 20a). In the absence of corn syrup solids, there was a significant increase in the mean diameter of the particles in the secondary emulsions, with $d_{3,2}$ increasing from 0.25 ± 0.01 μm before freeze drying to 5.94 ± 0.02 μm after reconstitution. Optical microscopy suggested that the origin of this increase was extensive droplet flocculation and coalescence that occurred in the secondary emulsions after reconstitution (Figure 20b). In the presence of corn syrup solids, there was no significant difference between the mean particle diameters of the secondary emulsions measured before freeze drying and after reconstitution (0.26 ± 0.03 μm), which was confirmed by optical microscopy (Figure 20c). These results clearly indicated that the stability of lecithin-stabilized emulsions (primary emulsion) to freeze drying could be improved by coating them with a chitosan layer (secondary emulsion). Moreover droplet aggregation in the secondary emulsions could be prevented by addition of corn syrup solids.

Droplet aggregation after freeze drying of the emulsions might have occurred during the freezing step and/or the drying step. Possible physicochemical mechanisms responsible for droplet aggregation during the freezing step were discussed in the previous section. Possible physicochemical mechanisms responsible for droplet aggregation during the drying step include: (i) when the water is removed the droplets come into closer proximity thereby facilitating coalescence; (ii) dehydration of the emulsifier molecules promotes interactions between emulsifier molecules adsorbed onto different droplets. The coalescence stability can often be improved by adding relatively high concentrations of carbohydrates to the system prior to drying. These molecules may prevent droplet aggregation by forming a glassy phase around the droplets that prevents them from coming into close proximity, or by forming hydrogen bonds with the adsorbed emulsifier molecules thereby preventing interactions between different membranes (Allison *et al*., 1998; Allison *et al*., 1999).

It should be noted that this study did not investigate the influence of freezing rate on the stability of the emulsions to freeze drying. The emulsions used in this work were cooled at a relatively slow rate compared to typical industrial practices, and slow cooling often promotes droplet coalescence. It would therefore be interesting to investigate the influence of freezing rate on emulsion stability in future studies.

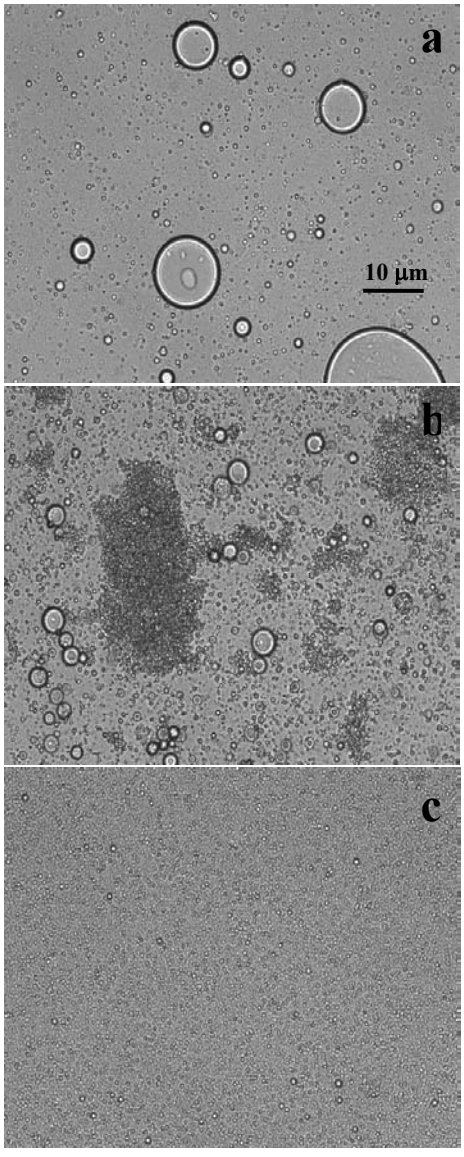

Figure 20 Microstructure of reconstituted emulsions for: a) primary emulsion; b) and c) for secondary emulsions in the absence and presence of 20 wt% corn syrup solids, respectively.

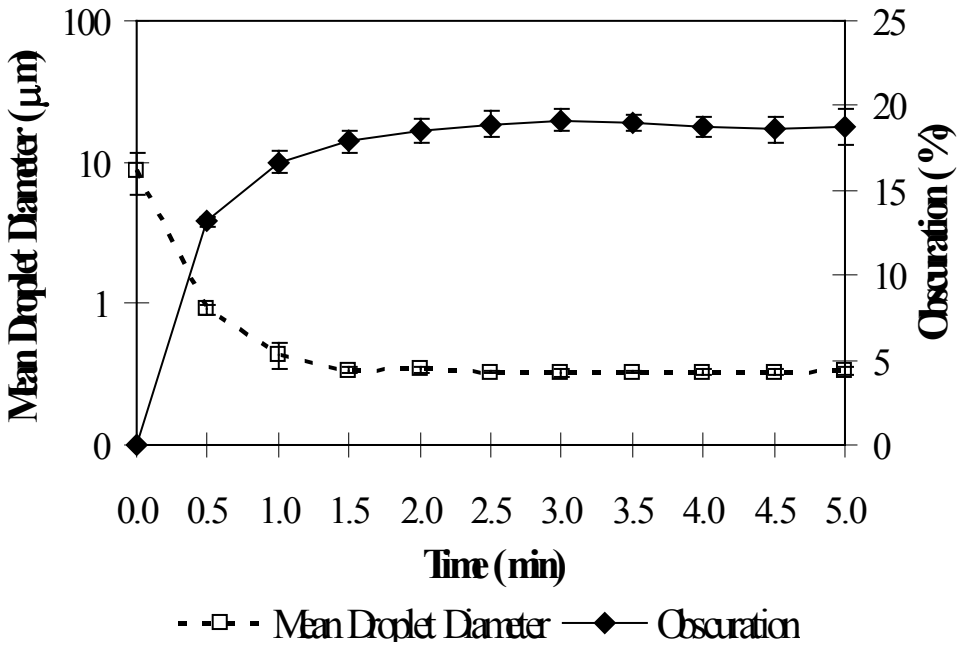

Figure 21 Time dependence of the mean particle diameter and obscuration of powdered secondary emulsions containing 20 wt% corn syrup solids that were dispersed into an aqueous solution in the measurement cell of a light scattering instrument.

The rate and efficiency of powder dispersion is particularly important in the application of powdered food ingredients (Freudig *et al*., 1999; Hogekamp and Schubert,

2003). For this reason, we used a laser diffraction technique to provide information about the rate and efficiency of the dispersion of the freeze-dried emulsions. A small sample (~0.3 mg/mL of buffer) of the emulsion powder that gave the best stability (*i.e.,* the secondary emulsion containing corn syrup solid) was added to a continuously stirred buffer solution contained within the stirring chamber of a laser diffraction instrument (Malvern Mastersizer Model 3.01, Malvern Instruments, Worcs., UK). The dispersibility of the powdered emulsion was then assessed by measuring the change in mean particle diameter and obscuration (the fraction of light lost from the main laser beam when the sample was introduced) of the system as a function of time (Figure 21). The obscuration increased with agitation time up to 2 min (18.5 ± 0.7%) after which it reached a constant value. On the other hand, the mean particle diameter decreased from 8.7 ± 2.8 μm at the beginning to 0.34 ± 0.02 μm after 2 min stirring. The obscuration and mean particle size remained relatively constant at agitation times longer than 2 min. The large particles size and the lower obscuration observed at the beginning of the measurement indicates considerable clumping of the emulsion powder (Freudig *et al*., 1999). The rapid decrease in particle size and increase in obscuration indicated that the majority of the powder dissolved rather quickly giving a homogeneous suspension (Raphael and Rohani, 1996).

In this study, the effectiveness of two layer membranes consisting of lecithin-chitosan (secondary emulsions) was compared to one layer membranes consisting of lecithin (primary emulsions) for improving emulsion stability to freeze-drying. In future studies, it would be informative to compare the performance of multilayer membranes to those of one layer membranes created from conventional surface-active food biopolymers, such as proteins or polysaccharides.

5.5 Conclusion

This study has shown that stable tuna oil-in-water emulsions containing droplets stabilized by lecithin-chitosan membranes can be produced using an electrostatic deposition method, which involves adding a positively charged biopolymer to an emulsion containing negatively charged droplets. These emulsions remain stable to droplet flocculation and coalescence in the presence of quite high levels of corn syrup solids (<25 wt%), which is commonly used in the microencapsulation of oils. The multilayered emulsions have better stability to thermal processing, freeze-thaw cycling and drying than primary emulsions. The interfacial engineering technology used in this study could lead to the creation of food emulsions with improved stability to environmental stresses.

5.6 Concision of Further Study

Tuna oil-in-water emulsions with improved stability to environmental stresses can be produced using an electrostatic layer-by-layer deposition technique producing oil droplets coated by multiple-layers of emulsifiers. Due to n-3 fatty acids in tuna oil susceptible to oxidation so the oxidative stability of tuna oil emulsions coated by lecithin alone or by lecithin-chitosan before and after drying needed to be determined. The ability of the antioxidants, mixed tocopherol and EDTA, on the stability of the tuna oil emulsions was also examined.