CHAPTER 8

EFFECT OF STORAGE ENVIRONMENTS ON STABILITY OF SPRAY DRIED TUNA OIL EMULSION

8.1 Abstract

Omega-3 fatty acids have numerous health benefits but their addition to foods is limited by oxidative rancidity. Spray drying of tuna oil-in-water emulsion droplets that are coated with a lecithin and chitosan multi-layer system could produce emulsion droplet interfacial membranes that are cationic and/or thick, both factors that can help control lipid oxidation. Physicochemical and oxidative stability of the spray dried emulsions were determined as a function of storage temperature and relative humidity (RH). The b value of tristimulus color was used as an index of the color changing in powder during storage. At higher temperature (37°C), the increasing of b-values was higher than low temperature (20°C) ($P<0.05$). The powders stored at low humidity (11 and 33% RH) showed the lower b-values than the powder stored at high humidity (52% RH) during storage ($P<0.05$). Lipid oxidation as indicated by lipid hydroperoxides and TBARS concentration was more rapid during storage at lower relative humidity (11 and 33% compared to 52% RH) ($P<0.05$). The combination of EDTA and mixed tocopherol was able to increase the oxidative stability of spray dried emulsions ($P<0.05$). The mean droplet diameter increased for all samples with the order of increase being 52% > 33% > 11% RH. No change in mean droplet diameter was observed during storage at 20°C, while significant increased at storage temperature of 37°C ($P<0.05$).

8.2 Introduction

Utilization of oils high in omega-3 fatty acids (n-3 FA) in food is limited due to their high susceptibility to oxidation. Lipid oxidation can be reduced by addition of antioxidants and/or by microencapsulation of the oil (Lin *et al*., 1995; Heinzelmann *et al*., 2000; Velasco *et al*., 2000; Kagami *et al*., 2003). The oxidative stability of emulsifiled oil can also be increased by controlling emulsifier type, location and concentration (Donnelly *et al*., 1998; Mancuso *et al*., 1999a; Fomuso *et al*., 2002; Hu *et al*., 2003). From previous work (Chapter 6) when oil-in-water emulsion droplets are surrounded by cationic emulsifiers, prooxidant metals are repelled and lipid oxidation rates decrease. These results are similar to the data from many research (Mei *et al*., 1998a; Mancuso *et al*., 1999a).

Spray dried powdered tuna oil with good physicochemical properties and dispersibility could produce using multilayer membrane emulsions containing corn syrup solids as filler agent (Chapter 7). The results from this work can demonstrated that a novel interfacial engineering technology, based on production of multilayer membranes around oil droplets, is effective for producing spray dried encapsulated tuna oil.

As a constituent of most food systems, water plays a particularly important role in lipid oxidation. Water activity or relative humidity has been used to control lipid oxidation in susceptible food products and to explain the relationship between lipid oxidation rates and moisture content (Nelson and Labuza, 1992). In general, lipid oxidation is lowest at water activity close to the water monolayer, which falls between 0.2 and 0.4 for most food. However, the rate of lipid oxidation increases rapidly when the water activity is either decrease

below or increase above the monolayer (Nelson and Labuza, 1992; Velasco *et al*., 2003). Numerous studies have suggested that this generalized view does apply to a number of systems (Rockland *et al*., 1961; Maloney *et al*., 1966; Quast and Karel, 1972), however, contradicting reports also exist (Kahl *et al*., 1988; Ponginebbi *et al*., 2000; Baik *et al*., 2004).

Physical properties of food powders, e.g. caking, stickiness, crystallization, dispersibility and solubility, can dramatically change upon storage and influence quality depending on temperature and moisture (Roos, 2002; Beristain *et al*., 2003; Thomas *et al*., 2004). The physical changes of the solid matrix of microencapsulated oils may affect the oil distribution with partial release of encapsulated oils and the released oil then may be more exposed and thus susceptible to oxidation (Rosenberg *et al*., 1990; Shimada *et al*., 1991). Maillard reaction products can form during both drying and ambient storage of food powders (Kagami *et al*., 2003; Thomas *et al*., 2004). Chitosan has a large number of free amino groups in its molecule, which could participate in the Maillard reaction (Tanaka *et al*., 1993). As a result of the Maillard reaction, the development of brown color, the formation of desirable or undesirable flavor, a change of texture, and a loss of nutritional value can occur (Tanaka *et al*., 1993; Thomas *et a*l., 2004). However, the Maillard reaction products have been reported act as antioxidants (Elizalde *et al*., 1991; Friedman, 1996). In the present study, the effects of relative humidity (RH) and temperature on dispersibility, color, and oxidative stability of spray dried microencapsulated tuna oil were investigated.

8.3 Materials and Methods

8.3.1 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). Mixed tocopherol (MT) homologs (Covi-ox® T-70, 14% α-tocopherol, 2% β-tocopherol, 60% γ-tocopherol and 24% δ-tocopherol) was obtained from Cognis Corp. (Cincinnati, OH, USA). Disodium ethylenetetraacetic acid (EDTA) was 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.

8.3.2 Methods

8.3.2.1 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 dispersing 3.53 wt% lecithin into the 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 and/or NaOH, and then the solution was stirred for about 1 h to ensure complete dispersion of the emulsifier. A chitosan solution was prepared by dissolving 1.5 wt% powdered chitosan into the stock buffer solution. A corn syrup solids stock solution was prepared by dispersing 50 wt% corn syrup solids into the stock buffer solution.

8.3.2.2 Oil-In-Water 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 solution (3.53 wt% lecithin) using a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland), followed by three passes at 5,000 psi through a single-stage valve homogenizer (APV-Gaulin, Model Mini-Lab 8.30H, Wilmington, MA). This primary emulsion was diluted with aqueous chitosan solution to form a secondary emulsion (10 wt% tuna oil, 2 wt% lecithin and 0.4 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). Secondary emulsions containing corn syrup solids were prepared by mixing the initial secondary emulsions with corn syrup solids solutions. Tuna oil-in-water emulsions contained 5 wt% tuna oil, 1 wt% lecithin, 0.2 wt% chitosan and 20 wt% corn syrup solid. The emulsions were stored at 4 °C prior to spray drying. For evaluation of the effectiveness of antioxidants in the emulsions, the secondary (lecithin-chitosan coated emulsion droplets) emulsions with 500 mixed tocopherol (tocopherols were added to the tuna oil prior emulsion preparation) and 12 μ M EDTA (added directly to the secondary emulsions after emulsion preparation) were prepared.

8.3.2.3 Spray Dried Emulsion Preparation

Spray drying was performed at a feed rate of 2.2 L/h at 180°C inlet temperature using Niro spray dryer with a centrifugal atomizer (Nerco-Niro, Nicolas & Research Engineering Corporation, Copenhagen, Denmark). The powders were vacuumed and stored in a hermetically sealed laminated pouch at -40°C until analysis. The moisture content of the spray dried powders were 1.6 ± 0.2% (g water/100 g powder) in all samples. The final composition (dry basis) of the spray dried tuna oil powders was as follows: tuna oil 19.1%, corn syrup solid (DE 36) 76.3%, lecithin 3.8%, and chitosan 0.8%.

8.3.2.4 Storage Studies

*S*pray dried tuna oil powders (3 g each) were placed in open glass beakers (20 mL, 3.5 cm diameter) and the beakers were placed in desiccators with relative humidity (RH) of 11, 33 or 52%, prepared using saturated LiCl, $MgCl_2$ and $Mg(NO_3)_2$ solutions (Rahman, 1995). To study the effect of relative humidity which below and above the water monolayer value (33% RH), which is shown to obtain the lowest level of lipid oxidation on stability of spray dried tuna oil, the relative humidity at 11% and 52% were selected. All desiccators were then incubated at 37°C (temperature close to room temperature of tropical zone). For studies on the effect of storage temperature, some samples were incubated at 20°C (room temperature of cold weather zone) compared to samples stored at 37°C in 11%RH. Samples were withdrawn at frequent time intervals for analyses.

8.3.2.5 Characterization of Spray Dried Powders

Spray dried tuna oil powders were reconstituted by dissolving 0.5 g powder in 4.5 mL of acetate buffer at pH 3.0 (Hardas *et al*., 2000) . One day after reconstitution, the

emulsion was analyzed for oil droplet diameter distribution 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 double-distilled water prior to analysis so the droplet concentration was less than 0.02 wt%.

Dispersibility of the spray dried emulsions was determined by adding ~0.3 mg powder/mL of acetate buffer 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 ($d_{4,3}$) and obscuration (the fraction of light lost from the main laser beam when the sample was introduced) as a function of time.

The reflectance spectra of spray dried emulsions were measured using a UV-visible spectrophotometer (UV-2101PC, Shimadzu Scientific Instruments, Columbia, MD) equipped with an integrating sphere (ISR-260, Shimadzu Scientific Instruments, Columbia, MD). The dried emulsions were placed in a 0.5 cm path length measurement cell with a black background. Spectra were obtained over the wavelength range 380-780 nm using a scanning speed of 700 nm min^{-1}. The spectral reflectance of the dried emulsions was measured relative to a barium sulfate ($BaSO_4$) standard. The color of samples was reported in term of the *L, a, b* color system. The *L, a, b* values of dried emulsions were calculated from its spectral reflectance using color matching functions (McClements *et al*., 1998).

Lipid hydroperoxides were measured by a modifiled method of Mancuso *et al*. (1999) after an extraction step in which 0.3 mL of reconstituted emulsion (100 mg of emulsion powder in 0.3 mL of acetate buffer) was added to 1.5 mL of

isooctane-2-propanal (3:1 v:v) followed by vortexing three times for 10 s each and centrifuging for 2 min at 3400 g (Centrific^{TM} Centrifuge, Fisher Scientific, Fairlawn, NJ). Next, organic phase (0.2 mL total volume containing 0.015 to 0.2 mL of lipid extract depending on the oxidation state of the lipid) was added to 2.8 mL of methanol-butanol (2:1 v:v), followed by 15 μL of thiocyanate solution (3.94 M) and 15 μL of ferrous iron solution (prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$ in acidic solution). The solution was vortexed, and the absorbance at 510 nm was measured after 20 min. Lipid hydroperoxide concentrations were determined using a cumene hydroperoxide standard curve.

Thiobarbituric acid reactive substances (TBARS) were measured according to Mei *et al*. (1998b). A thiobarbituric acid (TBA) solution was prepared by mixing 15 g trichoroacetic acid, 0.375 g TBA, 1.76 mL of 12 N HCl, and 82.9 mL H_2O. One hundred milliliters of TBA solution was mixed with 3 mL of 2% butylated hydroxytoluene in ethanol and 2 mL of this solution was mixed with 1 mL of reconstituted emulsion (5 mg of emulsions powder in 1.0 mL of acetate buffer). The mixture was vortexed and heated in a boiling water bath for 15 min, cooled to room temperature, and centrifuged at 3400 g for 25 min. Absorbance was measured at 532 nm. Concentrations of TBARS were determined from standard curves prepared using 1,1,3,3-tetraethoxypropane.

8.3.2.6 Statistical Analysis

All data represent the mean of six measurements of two different trials and results are reported as the mean and standard derivation of these measurements. The data were subjected to the analysis of variance (ANOVA). Comparison of means after the ANOVA test was performed using the Duncan's multiple range test.

8.4 Results and Discussion

8.4.1 Chemical Changes in the Spray Dried Powders during Storage

The spray dried powders in these experiments contain free amino group (chitosan and phospholipid) and reducing sugars (corn syrup solids), which signifies the possible participation in the Maillard reaction (Tanaka *et al*., 1993). Therefore, tristimulus color value L-, a- and b- were used as an index of the color changing in powder during storage at various temperature and relative humidity (RH). The b-value of the powder stored at 20°C at 11% RH did not change significantly over 25 days ($P \geq 0.05$, Figure 32). At higher temperature (37° C), the b-values increased significantly 100% from 0 to 25 days of storage ($P < 0.05$). Figure 33 shows the change in b-value as a function of relative humidity during storage at 37°C. The powders stored at low humidity (11 and 33% RH) showed the lower b-values than the powder stored at high humidity (52% RH) during 25 days of storage. No differences in b-values were observed between the samples storage at 11% RH and 33% RH ($P \geq 0.05$). For L-(lightness) values, the powders stored at low humidity (11 and 33 % RH, L-values of 95.3 ± 0.1 and 93.8 ± 0.1, respectively) showed higher L-values than the powders stored at high humidity (52% RH, L-values of 56.8 ± 1.1) after 25 days of storage ($P < 0.05$). The a-values of the powders stored at 11 and 33% RH did not change significantly within 25 days of storage (a-value ≈ 0.3 ± 0.2). At 52% RH, the a-value increased from 0.2 ± 0.1 at 0 day to 7.2 ± 3.2 after 25 days of storage ($P < 0.05$).

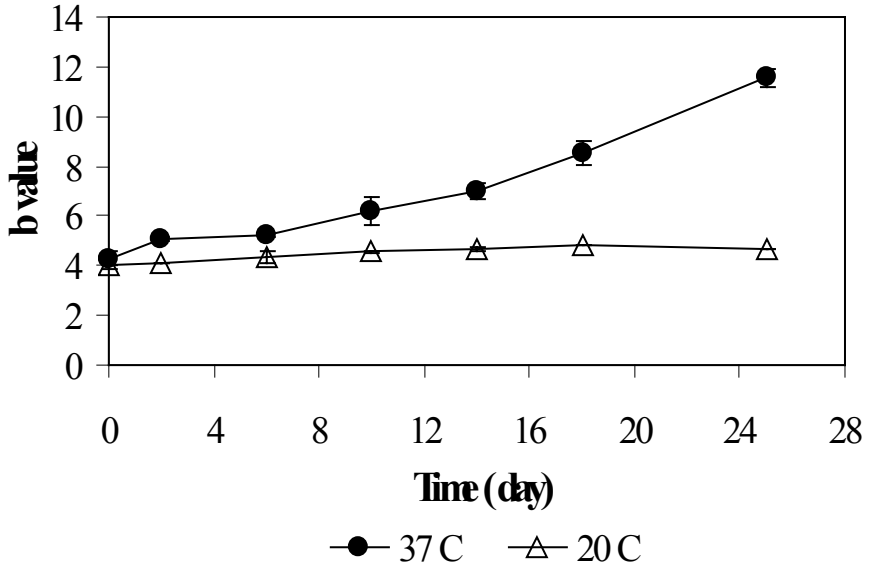


Figure 32 Development of brown color in spray dried powders (19.1% tuna oil, 76.3% corn syrup solids (DE 36), 3.8% lecithin and 0.8% chitosan) during storage in 11% RH at 20 and 37°C.

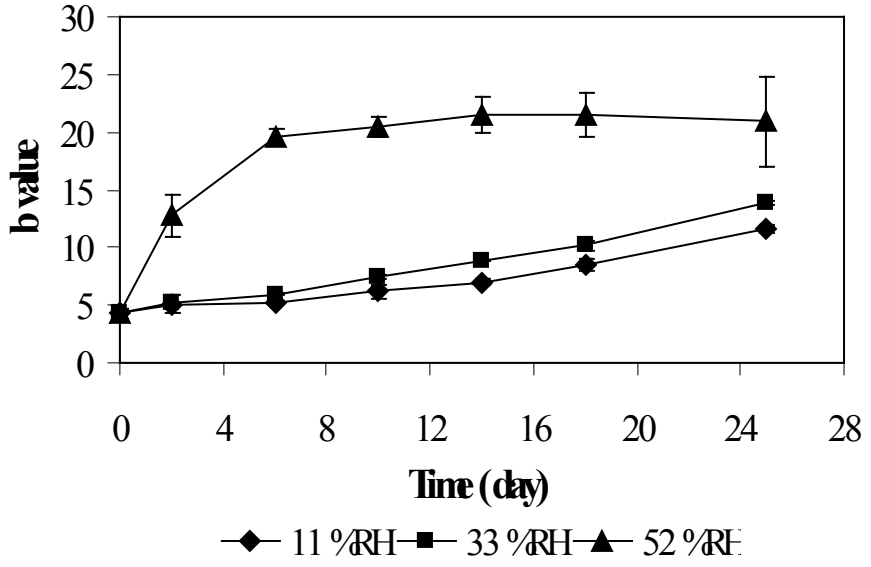


Figure 33 Development of brown color in spray dried powders (19.1% tuna oil, 76.3 % corn syrup solids (DE 36), 3.8

% lecithin and 0.8% chitosan) during storage at 37°C in various humidity.

When the Maillard reaction has taken place between chitosan or phospholipids and reducing sugars (CSS), the amount of free amino groups at the surface of the emulsion droplets would be expected to decrease. Therefore, the electrical charge (ζ-potential) of emulsion droplets reconstituted acetate (buffer pH 3.0) was measured (Figure 34). The ζ-potential of reconstituted emulsions from the powder decreased with the increasing of storage time and relative humidity. At high humidity (52 %RH), the ζ-potential of reconstituted spray dried powder decreased from 62.0 ± 0.7 mV at 0 day to 48.2 ± 1.1 mV at 45 days of storage ($P<0.05$). Similar results were reported by Tanaka *et al*. (1993), who found that amount of free amino groups of chitosan decreased during Maillard reaction at 65°C.

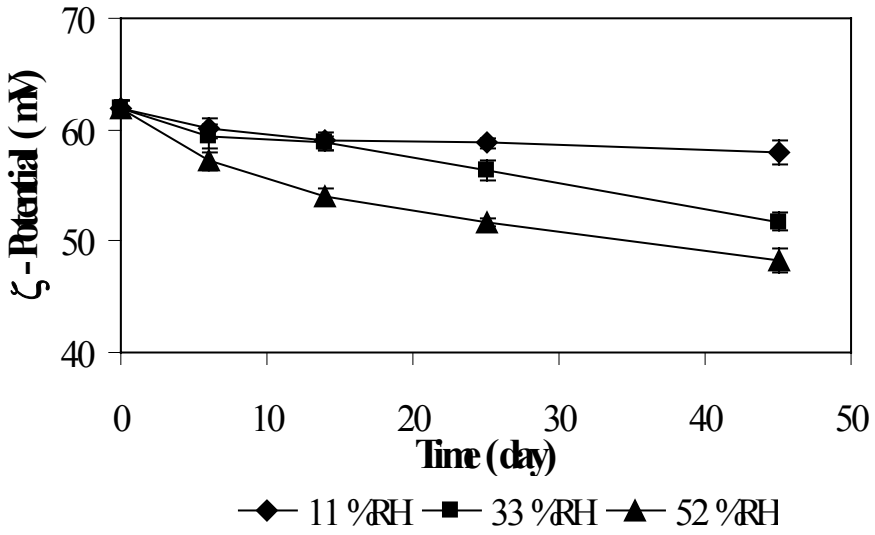


Figure 34 Influence of storage humidity environments on electrical charge (ζ-potential) of reconstituted emulsion

of spray dried powdered (19.1% tuna oil, 76.3 % corn syrup solids (DE 36), 3.8 % lecithin and 0.8% chitosan).

8.4.2 Oxidative Stability

Previous work has shown that lipid oxidation in spray dried powders does not correlate well with free or extractable fat (Hogan *et al*., 2003). Therefore, the oxidative stability of dried powder in this study was determined based on total lipid. The multilayer interfacial membrane on the dried emulsion droplets dramatically improved the oxidative stability of tuna oil when compared to bulk oil ($P<0.05$) as indicated by both lipid hydroperoxide and TBARS formation (Figure 35a and b, respectively). These results are similar to those reported by Kagami *et al*. (2003), microcapsules of the sodium caseinate-highly branched cyclic dextrin wall system improved oxidative stability of fish oil. Addition of mixed tocopherol (500 ppm) to bulk oil decreased lipid hydroperoxide and TBARS concentrations at 18 days of storage ($P<0.05$, Figure 35). However, encapsulation of tuna oil with the multilayer interfacial membrane was more effective in retarding oxidation than the mixed tocopherol in the bulk oil. Due to, the fact that mixed tocopherol can inhibit lipid oxidation only by scavenging free radicals, which donate hydrogen and form stable antioxidant radical. But encapsulation can inhibit lipid oxidation through both physical (inhibition of oxygen diffusion into the lipid and metal chelation) and chemical (free radical scavenging of carbohydrate encapsulating agents) mechanisms (Decker, 1998). Therefore, microencapsulated tuna oil was more stable to lipid oxidation than mixed tocopherol treated tuna oil.

Figure 36 shows lipid hydroperoxide and TBARS formation as a function of RH in the spray dried tuna oil powders. In the control samples, lipid hydroperoxide formation was in the order of 33% RH $\geq$ 11% RH > 52% RH (Figure 36a). A similar trend was observed in the development of TBARS during storage although TBARS in the samples at 11 and 33% RH became similar after 25 days of storage (Figure 36b). Several investigators have suggested that lipid oxidation in foods is lowest at water activities close to the water monolayer, which falls between 0.2 and 0.3 for many foods, due to a decrease of the catalytic effect of transition metals, quenching of free radicals and singlet oxygen and/or retardation of hydroperoxide decomposition (Ponginebbi *et al*., 2000; Velasco *et al*., 2003). However, other studies have suggested that this generalized view does not apply to a number of other foods. For instance, the encapsulated orange oil and methyl linoleate have been found to be more oxidatively stable in very dry (0% RH) and very moist (> 75% RH) states, whereas more rapid oxidation was found in the intermediate range (Kahl *et al*., 1988; Nelson and Labuza, 1992). For this study, the oxidative stability of microencapsulated tuna oil powders decreased when the RH increased from 11 to 33%. These results are similar to the data from Minemoto *et al*. (1997), they found that oxidation of encapsulated linoleic acid being higher as RH increased. However, the low oxidation in the microencapsulated tuna oil powders at 52% RH was observed in this study probably due to several chemical and physical changes in the powders during storage. First, lipid oxidation products may polymerize to produce brown-colored oxypolymers in the presence of other compounds such as amines, amino acids and antioxidants. These polymerization reactions can lead to inactivation of free radicals, a pathway that can influence oxidation ratse and produce siginicant color formation in powders (Zamora and

Hidalgo, 2005). Second, Maillard reaction products are well documented to reduce lipid oxidation (Elizalde *et al*., 1991; McGookin and Augustin, 1991; Wijewickreme *et al*., 1999). Wijewickreme *et al*. (1999) reacted L-lysine with D-glucose, D-fructose, and D-ribose under different conditions and produced a Maillard reaction products with a potent antioxidant activity in the linoleic acid emulsions. For the present work, brown color formation (as seen from increase in b-value, Figure 34) was observed in the powder storage at 52% RH. The formation of brown pigments could explain why lipid oxidation was slower at the in powders stored at high relative humidity. Third, when the powder was stored at a temperature higher than glass transition temperature (Tg) of corn syrup solids (Tg of corn syrup solids with DE = 36 at 52% RH is ~ 6°C), the sugars can crystallize causing the powder particles to collapse (Thomas *et al*., 2004). The collapse of powders is linked to a decrease in porosity, thus, lipid oxidation could be lower due to a decrease in the diffusivity of O_2 (Thomas *et al*., 2004).

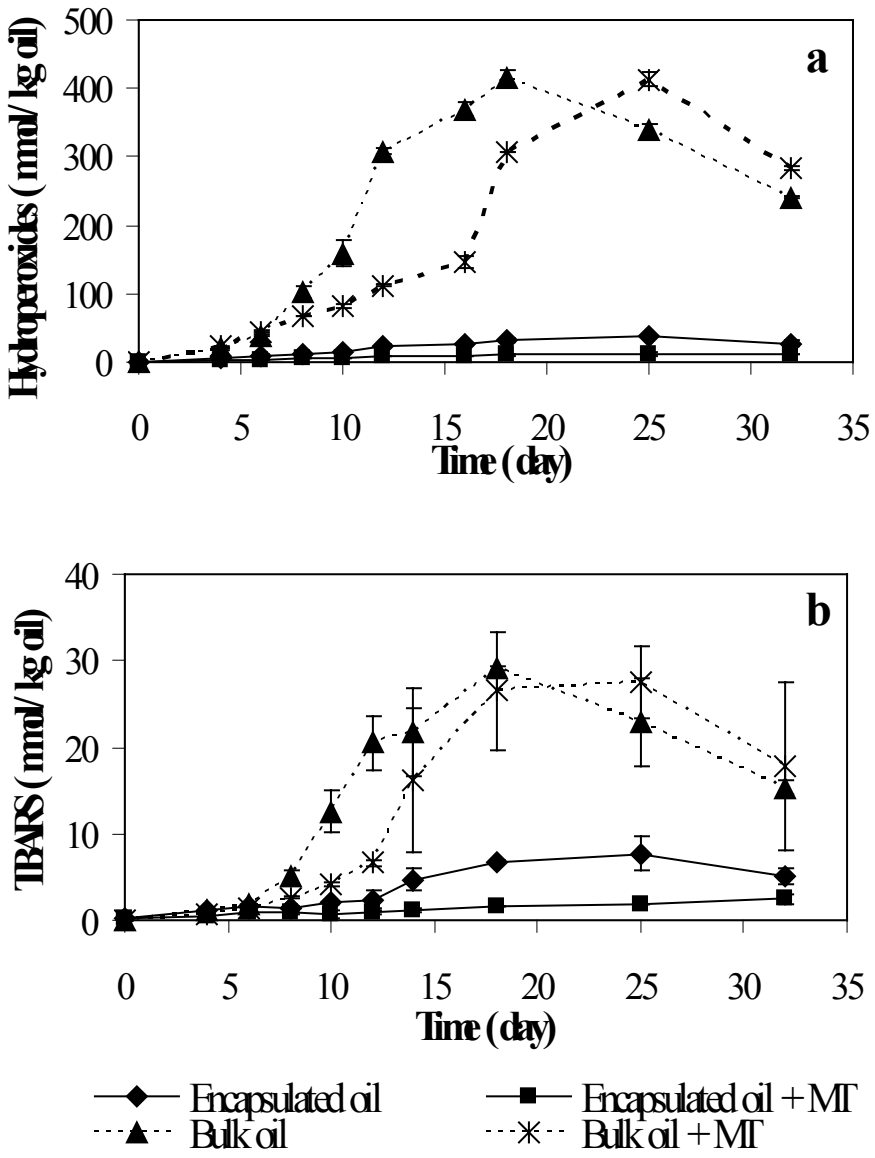


Figure 35 Formation of lipid hydroperoxides (a) and TBARS (b) in bulk oil and encapsulated oil (19.1% tuna oil, 76.3 % corn syrup solids (DE 36), 3.8% lecithin and 0.8% chitosan) in the absence and presence of 500 ppm mixed tocopherol (MT) during storage at 37°C in 33% RH.

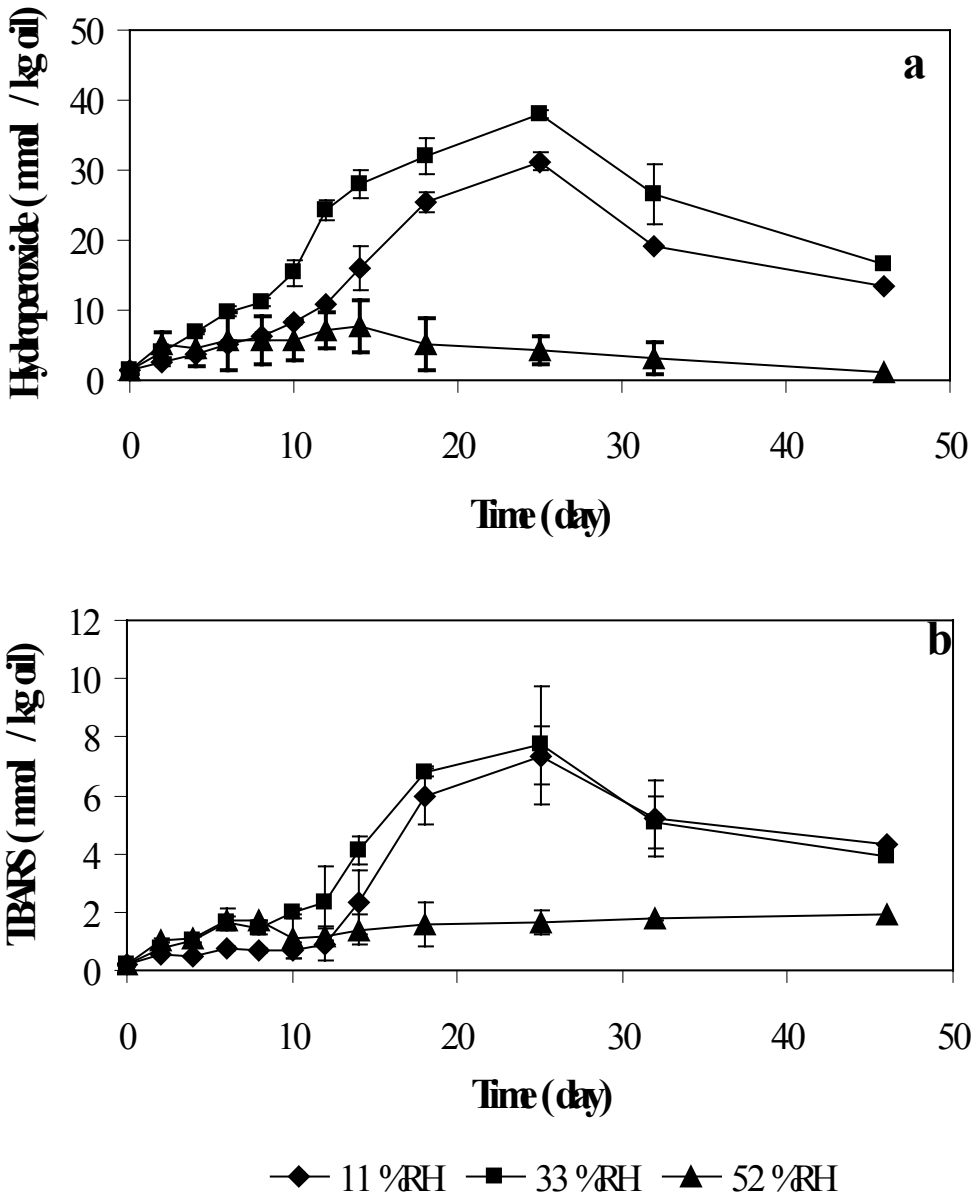


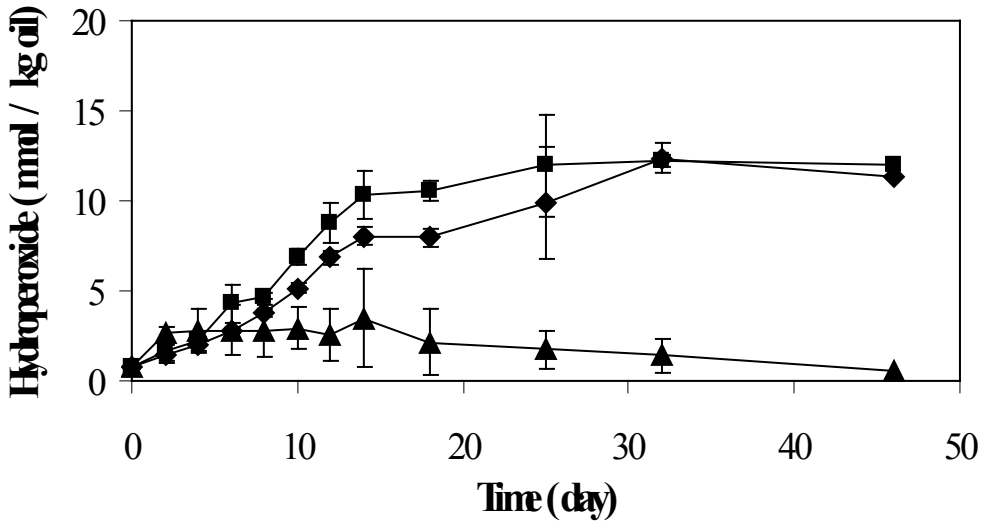
Figure 36 Formation of lipid hydroperoxides (a) and TBARS (b) in control (no antioxidants) spray dried tuna oil powder during storage at 37°C in various humidity.

It was found, from Chapter 6, that the combination of mixed tocopherol and EDTA has been shown to be very effective to inhibit lipid oxidation in oil-in-water emulsions stabilized by lecithin-chitosan membrane. However, the physical nature of dried emulsions could effect the antioxidant activity of tocopherol and EDTA in a different manner than the oil-in-water emulsions (McClements and Decker, 2000). Therefore, the effectiveness of mixed tocopherol and EDTA on lipid oxidation in the spray dried tuna oil powders was reevaluated. Both lipid hydroperoxides (Figure 37a) and TBARS (Figure 37b) formation in the spray dried emulsions containing 500 ppm mixed tocopherol and 12 μM EDTA were lower than samples with no added antioxidants (Figure 36) at all RH studied after 46 days of storage indicating that the antioxidants were also effective in spray dried emulsions ($P < 0.05$). Spray dried tuna oil that stored at high relative humidity (52% RH) showed higher oxidative stability than low relative humidity (11% and 33% RH) both in the presence and absence of antioxidants (500 ppm mixed tocopherol and 12 μM EDTA) (Figure 36 and 37). From this results might be concluded that the Maillard reaction products had higher activity to retard lipid oxidation in spray dried tuna oil than antioxidants. However, more information is needed to explain these phenomena.

8.4.3 Reconstituted Emulsion Droplet Size and Dispersion

The mean particle diameter of the emulsion droplets in spray dried powders was measured after storage at 37°C at various relative humidities for different times after they were reconstituted in acetate buffer (pH 3.0) (Figure 38). The mean particle diameter measured at a particular storage time (D) was normalized relative to the initial mean particle diameter (D_0) for that sample to highlight differences between the samples. The mean particle diameter increased for all samples with the order

of increase being 52% > 33% > 11% RH. One of most important factors that determine the stability of dried powders is the temperature. Figure 38 shows that mean particle diameter of the reconstituted spray dried powders increased during storage at 11% RH and 37°C. No change in mean droplet diameter was observed during storage at 20°C (Figure 39). At storage temperature of 37°C, mean droplet diameter began to increase after 14 days of storage and increased by more than 200% after 18 days of storage. The increase in mean particle diameter observed during storage at 37°C could be attributed to the Maillard reaction, which would lead to the formation of polymers between carbohydrates and amine groups at the emulsion droplet interface.



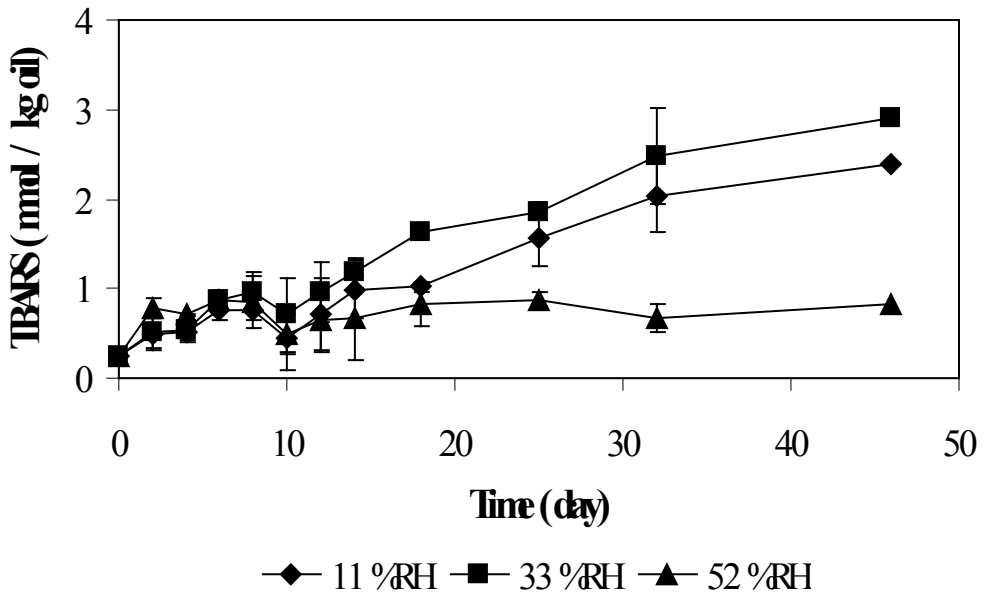


Figure 37 Formation of lipid hydroperoxides (a) and TBARS (b) in antioxidants (500 ppm mixed tocopherol and 12 μM EDTA) treated microencapsulated tuna oil during storage at 37°C in various humidity.

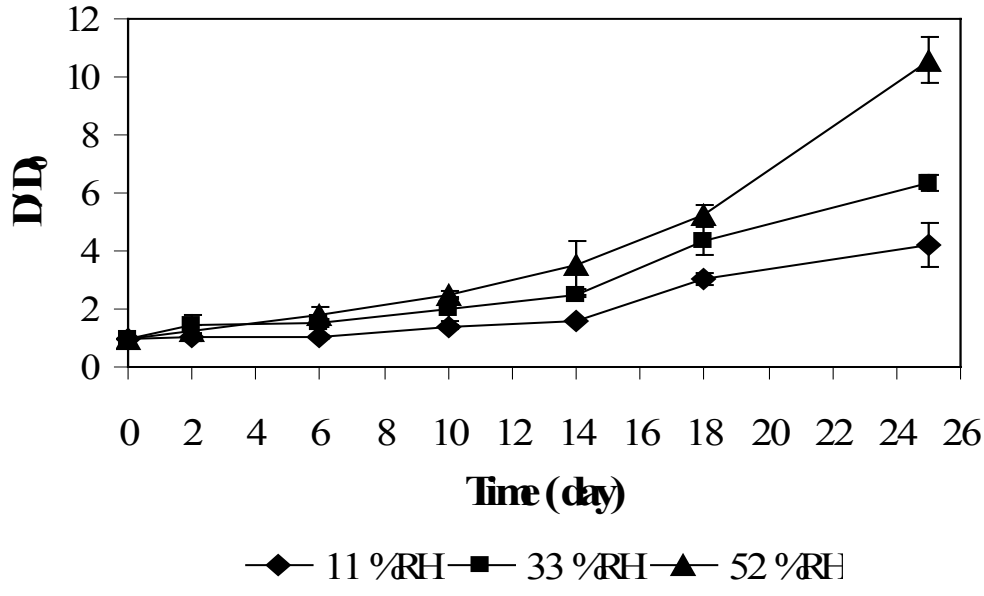


Figure 38 Mean droplet diameter of reconstituted tuna oil emulsion (5 wt% oil, 1 wt% lecithin, 0.2 wt% chitosan

and 20 wt% corn syrup solids) during storage at 37°C in various humidity.

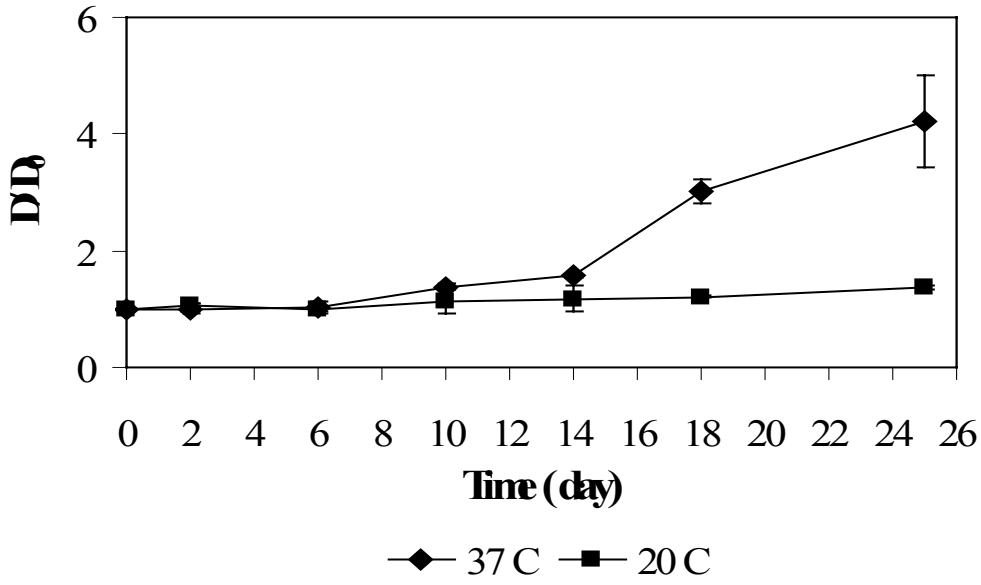


Figure 39 Mean droplet diameter of reconstituted tuna oil emulsion (5 wt% oil, 1 wt% lecithin, 0.2 wt% chitosan and 20 wt% corn syrup solids) during storage at 11% RH and 20 and 37°C.

The rate and efficiency of powder dispersion is also an important property of powdered food ingredients (Freudig *et al*., 1999; Hogekamp and Schubert, 2003). The rate and efficiency of powder dispersion was determined using a laser diffraction technique. A small sample (~0.3 mg/mL of buffer) of the spray dried emulsion powder was added to a continuously stirred buffer solution contained within the measurement 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 droplet obscuration (Figure 40) and mean particle diameter (d_{43}) (Figure 10) of the system as a function of time. The d_{43} value is particularly sensitive to the

presence of large particles in a sample, while the obscuration is sensitive to the total amount of material dispersed in the fluid. When samples were tested on the same day that they were spray dried (0 day), the droplet obscuration (Figure 40) increased steeply with agitation time up to 3 min, after which it reached a fairly constant value (21.6 ± 0.8%). In addition, d_{43} (Figure 41) decreased from over 10 μm at the beginning to less than 1 μm after 1 min stirring. The droplet obscuration and mean particle size remained relatively constant at agitation times longer than 3 min. The large particles size and the lower droplet concentration 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 droplet obscuration indicated that the majority of the powder dispersed rather quickly producing a homogeneous suspension (Raphael and Rohani, 1996). For the powders stored at 20 °C in 11% RH for 45 days, the droplet obscuration took slightly longer (5 minutes) to reach a constant value (22.4 ± 1.1%) than for the fresh powders (3 minutes). In addition, the large particles took longer to fully disappear (3 minutes) in the 45 days old sample than in the 0 day old sample (1 minute). Nevertheless, the final mean particle diameter of the fully redispersed emulsions was similar to the droplet diameter of the original liquid emulsions (d_{32} = 0.34 ± 0.01 μm) for both the 0 and 45 days old samples. The increase in dispersion time for the 45 days old sample may have been caused by the collapse of the particle structure during storage. A decrease in the surface area of powder particles exposed to the surrounding water phase is known to decrease dispersion rates. Moreover, a decrease in particle porosity would limit the access of water to the interior of the powder particles (Thomas *et al*., 2004).

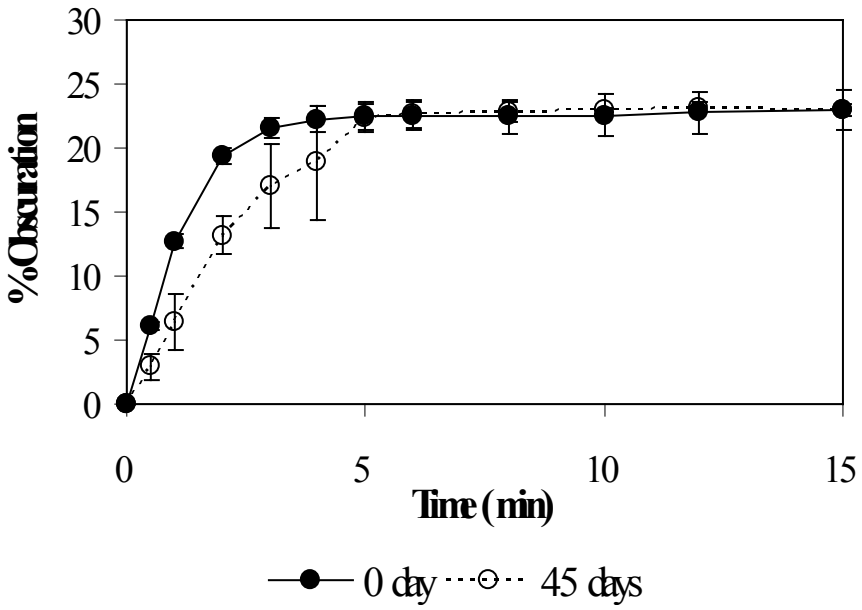


Figure 40 Influence of stirring time on obscuration of reconstituted emulsion after microencapsulated powdered (20°C at 11% RH for 45 days) was added to the stirring cell of laser diffraction instrument.

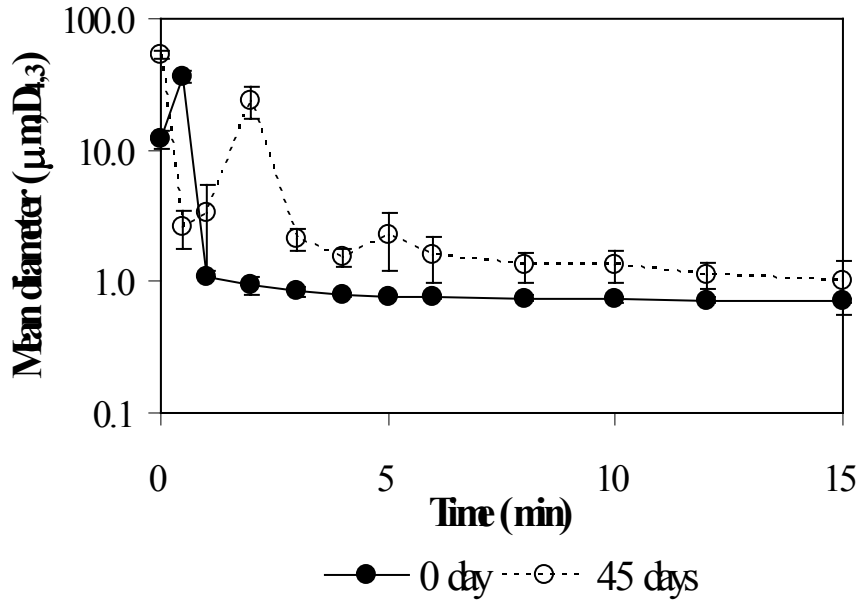


Figure 41 Influence of stirring time on mean particle diameter of reconstituted emulsion after microencapsulated

powdered (20°C at 11% RH for 45 days) was added to the stirring cell of laser diffraction instrument.

8.5 Conclusion

This study has shown that the oxidative stability of dried tuna oil-in-water emulsion droplets coated by a lecithin-chitosan multi-layer system is higher than bulk tuna oil. The combination of mixed tocopherol and EDTA was effective to increasing the oxidative stability of spray dried multi-layer emulsion. In the present study, spray dried tuna oil emulsion did not follow classical oxidation patterns of minimum oxidation at intermediate water activity but instead has minimal oxidation at high water activity. Decreasing oxidation rates at high water activity could be due to physical and chemical changes in the powder including sugar crystallization that could decrease oxygen permeability to the lipid and/or the formation of Maillard reaction products that can act as antioxidants. The oxidative stability of spray dried tuna oil powder stored at high relative humidity was higher than low relative humidity but the physicochemical stabilities were not good *e.g.*, color changing, increasing in droplet diameter and long dispersed time. Therefore, the spray dried tuna oil in this research should be stored at 20°C in 11% RH to maintain both physical and oxidative stability. Overall, these data suggest that spray dried tuna oil-in-water emulsions stabilized by lecithin-chitosan membranes may be used to produce n-3 fatty acids that are more oxidatively stable than bulk oils.