CHAPTER 6

INCREASING THE OXIDATIVE STABILITY OF LIQUID AND DRIED TUNA OIL-IN WATER EMULSIONS

6.1 Abstract

Omega-3 fatty acids have numerous health benefits but their addition to foods is limited by oxidative rancidity. Engineering the interfacial membrane of oil-in-water emulsion droplets to produce a cationic and/or thick interface is an effective method to control lipid oxidation. The oxidative stability of tuna oil in emulsions coated by lecithin alone or by lecithin-chitosan before and after freeze-drying was studied. The ability of the antioxidants mixed-tocopherol (100, 500 and 1000 ppm) and/or EDTA (12, 60 and 120 μM) on the stability of the emulsions was also examined. Both liquid and dried tuna oil-in-water emulsions droplets coated by lecithin and chitosan are more oxidatively stable than emulsion coated by lecithin alone as indicated both lipid hydroperoxide and TBARS concentration ($P<0.05$). No difference in effectiveness in the ability of 500 and 1000 ppm mixed tocopherols ($P\geq0.005$) to inhibit lipid oxidation and their inhibiting lipid oxidation more effectively ($P<0.05$) than 100 ppm. The combination of mixed tocopherols and EDTA was more effective than mixed tocopherol alone ($P<0.05$). Production of emulsions droplets coated with lecithin and chitosan could be an excellent technology for stabilization of oxidative unstable lipids for use in a variety of food products.

6.2 Introduction

Utilization of oils high in n-3 fatty acid in food is limited due to their high susceptibility to oxidation. Most

functional foods would contain n-3 fatty acid as dispersed lipids. Therefore it is important to understand the mechanisms of oxidation of emulsified n-3 fatty acid so that effective antioxidant technologies can be developed. The most commonly used method of retarding lipid oxidation in foods is by the addition of antioxidants (Nawar, 1996). In emulsions, antioxidants behavior is different from bulk oil system. The apparent activity of chain breaking antioxidants in multiphasic food systems such as emulsions is dependent on their effective concentrations in the physical location where lipid oxidation is most prevalent (*e.g.* lipid vs. water). In bulk oils, hydrophilic antioxidants preferentially locate at the oil-air interfaces and reverse micelles where lipid oxidation rates are high. Therefore, hydrophilic antioxidants are more effective at protecting bulk lipids from oxidation than lipophilic antioxidants that are dispersed throughout the oil phase. In oil-in-water emulsion, lipophilic antioxidants would concentrate in the oil droplets or at the oil-water interfaces and inhibit lipid oxidation more effectively than hydrophilic antioxidants that can partition into the water phase (Frankel *et al*., 1994).

In addition to chain breaking antioxidants, lipid oxidation in oil-in-water emulsions can be inhibited by many other mechanisms. Metal chelators such as EDTA are effective at inhibiting lipid oxidation in oil-in-water emulsions when present at concentrations above the concentration of prooxidant metals (Mancuso *et al*., 1999; Frankel *et al*., 2002). The oxidative stability of emulsifiled oil can also be increased by controlling emulsifier type, location and concentration (Donnelly *et al*., 1998; Mancuso *et al*., 1999; Fomuso *et al*., 2002; Hu *et al*., 2003). For example, when oil-in-water emulsion droplets are surrounded by cationic emulsifiers, prooxidant metals are repelled and lipid oxidation rates decrease (Mei *et al*., 1998a; Mancuso *et al*., 1999). An additional method

to inhibit lipid oxidation in oil-in-water emulsion is to produce thick interfacial emulsion droplet membranes that hinder interactions between water-soluble prooxidants and lipids inside the emulsion droplet (Silvestre *et al*., 2000).

The previous work (Chapter 5) 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-layers of emulsifiers. The production of this emulsions system may prove to be an effective means of improving the oxidation stability of emulsified fish oil since both the emulsion droplet charge and thickness can be controlled. Because n-3 fatty acids could find utilization in functional foods as both liquid and dried ingredients, the objective of this study was to examine the oxidative stability of tuna oil emulsions coated by lecithin alone or by lecithin-chitosan before and after drying. The ability of the antioxidants mixed-tocopherol and EDTA on the stability of the emulsions was also examined.

6.3 Materials and Methods

6.3.1 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.

6.3.2 Methods

6.3.2.1 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 disembrator, 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.

6.3.2.2 Emulsion Preparation

A coarse tuna oil-in-water emulsion was made by blending 15 wt% tuna oil with 85 wt% emulsifier (lecithin) solution using a high-speed blender (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland). To produce the primary emulsion, the coarse emulsion was sonicated for 2 min at a frequency of 20 kHz, amplitude of 70% and duty cycle of 0.5 (Model 500, sonic dismembrator, Fisher Scientific, Pittsburgh, PA). This primary emulsion was mixed with the chitosan and buffer solution to form a secondary emulsion with a final concentration of 5 wt% tuna oil, 1 wt% lecithin, and 0.2 wt% chitosan. Any flocs formed in the secondary emulsion were disrupted sonication for 2 min at a frequency of 20 kHz, amplitude of 70% and duty cycle of 0.5 (Ogawa *et al*., 2003a). The electrical charge on the droplets (determined by zeta potential) 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). Corn syrup solids were added to the primary and secondary emulsions by mixing with the corn syrup solids stock solution to obtain a final concentration of 5 wt% tuna oil, 1 wt% lecithin, 0.2 wt% chitosan and 20% corn syrup solids**.** The pH of the final emulsions was adjusted to 3.0 using HCl or NaOH if necessary.

For antioxidant evaluation, three kinds of secondary (lecithin-chitosan coated emulsion droplets) emulsions were prepared: (i) control (without antioxidants); (ii) mixed tocopherol (100, 500 and 1000 ppm) and (iii) with mixed tocopherol plus EDTA (500 ppm mixed tocopherol and 12, 60, or 120 μM, 90, 448 or 896 ppm, respectively EDTA). In samples with mixed-tocopherols, the antioxidant was added to the tuna oil prior emulsion preparation whereas EDTA was

added directly to the secondary emulsions after emulsion preparation.

6.3.2.3 Freeze Drying

The emulsion samples (1 mL) were transferred into microcentrifuge tubes, 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 at room temperature using a vacuum pressure of 1 atm for 48 hr.

6.3.2.4 Lipid Oxidation Measurement

To monitor lipid oxidation during storage, emulsions (10 mL) were placed in lightly sealed screw-cap test tubes or microcentrifuge tubes and allowed to oxidize at 37 or 55°C in the dark. Oxidative stability was evaluated by measuring lipid hydroperoxide and thiobarbituric acid reactive substances (TBARS). Lipid hydroperoxide was measured by a modifiled method of Mancuso *et al*. (1999). Lipids in 0.3 mL of emulsion were extracted by mixing with 1.5 mL of isooctane-2-propanal (3:1 v:v) and vortexing three times for 10 s each followed by centrifugation for 2 min at 3400 g (Centrific^{TM} Centrifuge, Fisher Scientific, Fairlawn, NJ). Next, the 0.2 mL of organic phase 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.

TBARS were measured according to Mei *et al*. (1998b). A 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 emulsion diluted 30 times in water. 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.

For measurement of oxidative stability in freeze-dried emulsions, the dried emulsions were reconstituted with 1 mL of acetate buffer and then the lipid hydroperoxide and TBARS values were carried out as described above.

6.3.2.5 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.

6.4 Results and Discussion

6.4.1 Oxidative Stability of n-3 Fatty Acids in Oil-In-Water Emulsions with Multiple Interfacial Layers

Figure 22 shows a comparison of oxidation rates in tuna oil-in-water emulsions stabilized by lecithin alone (primary emulsion) or lechitin plus chitosan (secondary emulsion). Lipid oxidation markers (hydroperoxide and TBARS) were measured as a function of time (0-13 day) in order to monitor differences in oxidation kinetics. The oxidative stability of the primary tuna oil emulsion was less than the secondary emulsion as determined by both lipid hydroperoxide (at day 3; Figure 22a)

and TBARS (at day 5; Figure 22b) ($P<0.05$). Transition metals, and particular iron are major prooxidatives in oil-in-water emulsions due to their ability to decompose lipid hydroperoxide into free radicals (Mancuso *et al*., 1999). However, the prooxidant activity of iron is related to its ability to interact with the droplet surface. The low amount of oxidation observed in secondary emulsions suggests that the positively charged emulsion droplets (+57 mV) inhibited iron-lipid interactions, presumably by decreasing the ability of iron to interact with the emulsion interface through electrostatic repulsive forces. Conversely, the greater oxidation rates in primary emulsion, negatively charged emulsion (-52 mV), could be due to increased interfacial iron concentrations owing to attractive forces (Mei *et al*., 1998a). Inhibition of metal-catalyzed lipid oxidation by positively charged and acceleration by negatively charge emulsion has also been observed in corn oil and salmon oil emulsion (Mei *et al*., 1998a; Mancuso *et al*., 1999; Hu *et al*., 2003). Emulsion droplets are surrounded by a "membrane" of emulsifier molecules. These membranes not only prevents the droplets from coalescing but also may protect lipids from oxidation by acting as a barrier to the penetration and diffusion of molecular species that promote lipid oxidation into the droplets (Coupland and McClements, 1996). Therefore also, lipid oxidation can be inhibited by thick interfacial membranes as was reported by Silvestre *et al*. (2000). The observed increased oxidative stability of the secondary emulsion compared to the primary emulsion (Figure 22) could therefore be due to its thicker interfacial membrane that could decrease interactions between lipid and aqueous phase prooxidants.

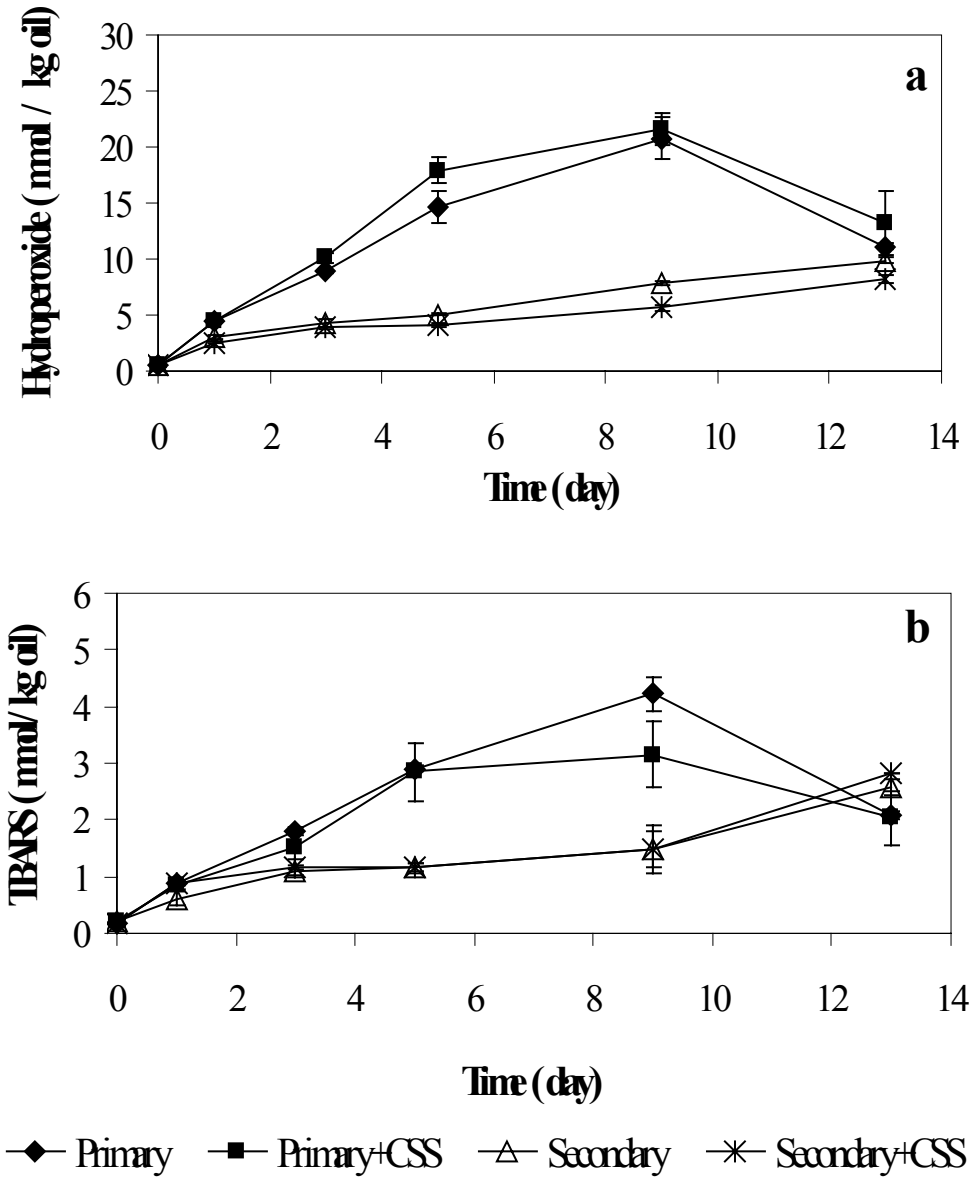

Figure 22 Formation of lipid hydroperoxide (a) and TBARS (b) in primary (lecithin alone) and secondary (lecithin and chitosan) emulsions in the absence and presence of 20 wt% corn syrup solids (CSS) during storage at 37°C.

Drying lipid emulsion requires the presence of a bulking agent. These bulking agents are often carbohydrates such as corn syrup solids. Sugars and polysaccharides have been reported to alter lipid oxidation rates in oil-in-water emulsion (Ponginebbi *et a*l., 1999). Therefore, the impact of

bulking agents on the rate of lipid oxidation in liquid primary and secondary emulsions lipid hydroperoxide and TBARS were determined in the presence and absence of corn syrup solids. In both the primary and secondary emulsions, corn syrup solids had no major impact on lipid oxidation rates ($P \geq 0.05$, Figure 22).

6.4.2 Influence of Mixed Tocopherol Isomers on the Oxidation of the Secondary Emulsions

While lipid oxidation was slower in the secondary emulsions lecithin-chitosan stabilized tuna oil-in-water emulsion compared to the primary emulsions (Figure 22), oxidation markers were still observed to increase over time. Therefore the ability of varying concentrations of the lipid-soluble chain breaking antioxidant, mixed tocopherol (0-1000 ppm), to further decrease lipid oxidation was determined. Mixed tocopherols were tested as the chain breaking antioxidant because lipophilic antioxidants have been reported to be more effective than hydrophilic antioxidants in oil-in-water emulsions (Frankel *et al*., 1994) and moreover, mixed tocopherol have been found to be more effective than α-tocopherol (Chen *et al*., 2002, Djordjevic *et al*., 2004; Hu *et al*., 2004). Because mixed tocopherol contains δ- and γ-isoforms that are higher stable, so mixed tocopherol is less susceptible to participate in side reactions (prooxidative acitivity of tocopherol) than α-isoform (Wagner *et al*., 2004). Due to corn syrup solids have no impact on lipid oxidation (Figure 22), so they were included in the emulsion since they will be used in later experiments on dried emulsions. None of the mixed tocopherol concentrations tested was able to inhibit the oxidation of the secondary tuna oil-in-water emulsion until 13 days of storage (Figure 23a and b). At day 13, all three tocopherol concentrations inhibited lipid hydroperoxide formation in a similar manner (Figure 23a) with

inhibition ranging from 17 to 27% compared to the control at day 13. At day 13, differences were also seen in the effectiveness of mixed tocopherols when lipid oxidation was measured with TBARS with 500 and 1000 ppm mixed tocopherols inhibiting lipid oxidation more effectively ($P<0.05$) than 100 ppm (Figure 2b). No difference in effectiveness in the ability of 500 and 1000 ppm mixed tocopherol to inhibit TBARS formation was observed with TBARS being 43 to 45% lower than the control at day 13 ($P<0.05$).

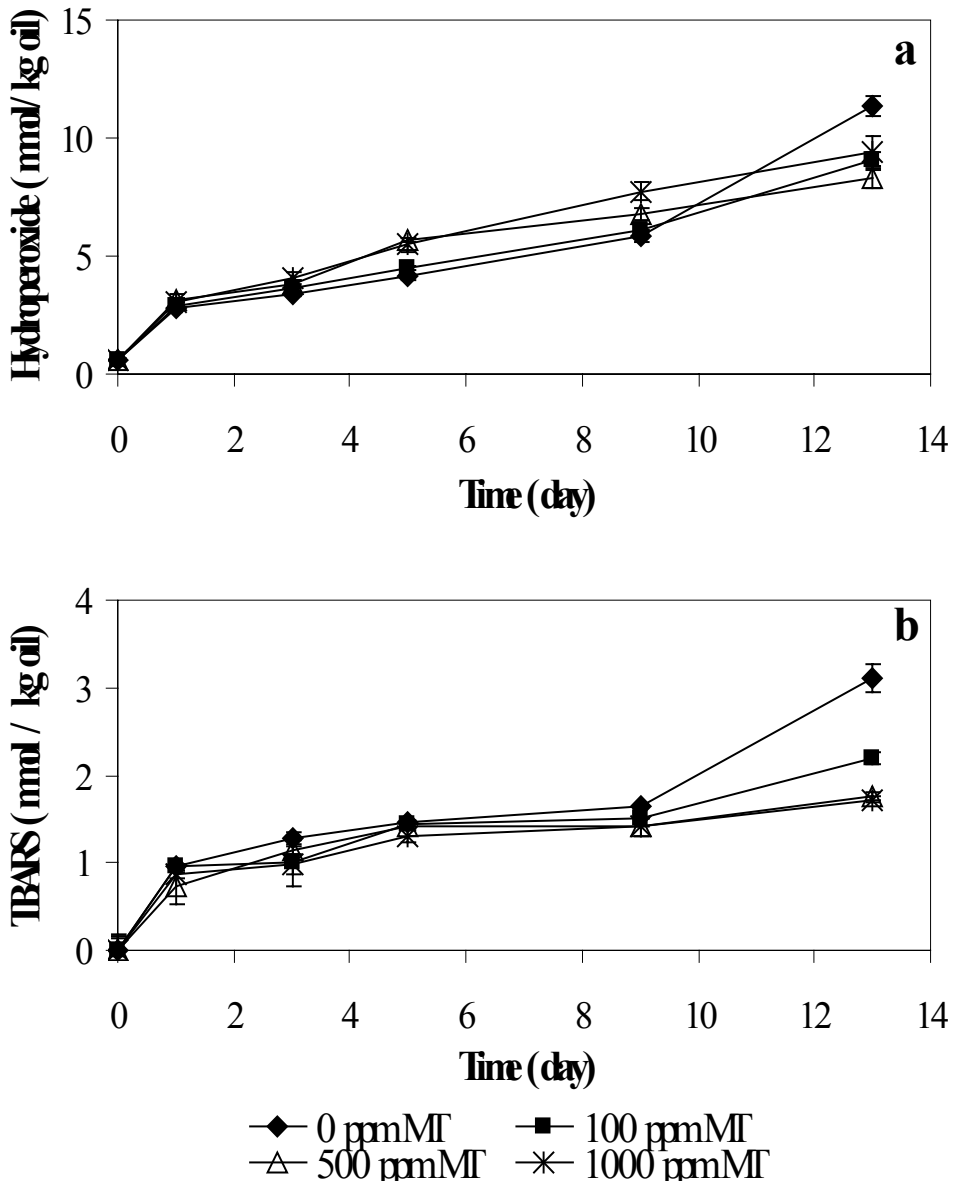

Figure 23 Formation of lipid hydroperoxide (a) and TBARS (b) in secondary emulsions secondary (lecithin and chitosan) emulsions containing 0, 100, 500 and 1000 ppm mixed tocopherol (MT) during storage at 37°C.

6.4.3 Influence of the Combination of Mixed Tocopherol and EDTA on the Oxidation of the Secondary Emulsions

The combination of a metal chelator and lipid-soluble chain breaking antioxidant can be very effective in controlling lipid oxidation in oil-in-water emulsions (Djordjevic *et al*., 2004). To determine if the combination of chelator and lipid-soluble antioxidant was effective in the lecithin-chitosan stabilized tuna oil-in-water emulsions, mixed tocopherol (500 ppm) and varying concentration of EDTA (0, 12, 60 and 120 μ M) were added in combination and the emulsions were incubated at 37°C for 13 days in the dark. As seen previously (Figure 23), mixed tocopherol were only able to decrease lipid hydroperoxides at the end of the incubation period. In the presence of EDTA, all concentrations of EDTA were able to further decrease in lipid hydroperoxide concentrations after 1 day of storage ($P<0.05$; Figure 24a). There was no significant difference ($P\geq0.05$) in hydroperoxide concentrations in emulsions containing 12, 60 and 120 μM EDTA. At day 13, lipid hydroperoxide concentration (Figure 24a) was 25-34% lower in EDTA treated emulsions. EDTA at 60 and 120 μM were more slightly effective in retarding TBARS formation than 12 μM EDTA after 13 days of storage (Figure 24b). No differences in TBARS concentrations were observed between the samples containing 60 and 120 μM EDTA ($P\geq0.05$). At day 13, TBARS concentration (Figure 24b) was 8-34% lower in EDTA treated emulsions. The combination of EDTA and mixed tocopherol improved inhibition of lipid oxidation compared with mixed tocopherol isomers alone as indicated by both hydroperoxide and TBARS concentrations (Figure 24a and b). These results are consistent with the report that the prooxidant effect of tocopherol in emulsion can be decreased by metal chelators such as EDTA, phosphoric, malonic and citric acids (Cillard and Cillard, 1986; Mancuso *et al*, 1999b)

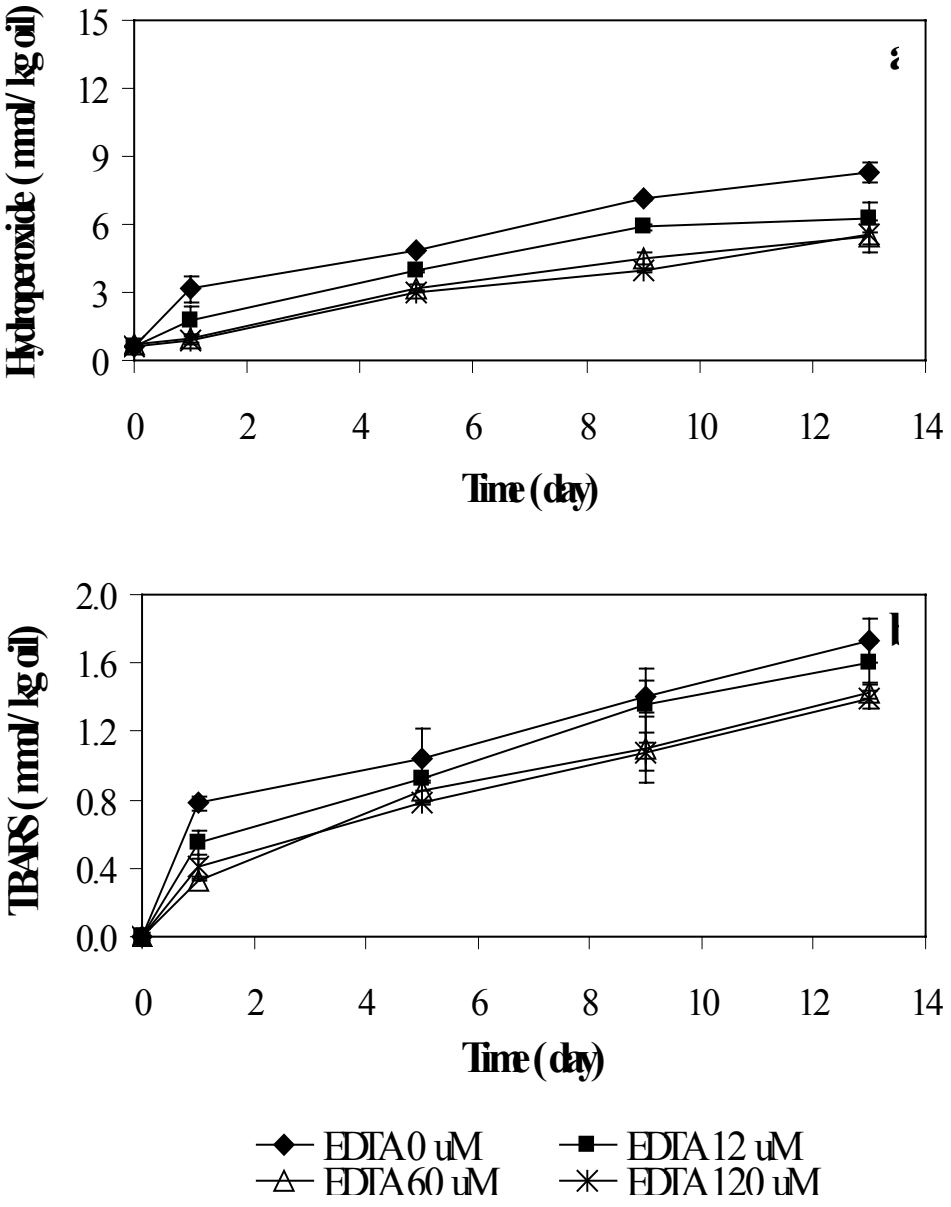

Figure 24 Formation of lipid hydroperoxide (a) and TBARS (b) in secondary emulsions (lecithin and chitosan) containing

500 ppm mixed tocopherol isomers and 0, 12, 60 and 120 μM ethylenediaminetetraacetic acid (EDTA) during storage at 37°C.

6.4.4 Influence of Freeze Drying EDTA on the Oxidation of the Secondary Emulsions

To determine if the properties of the emulsion droplet interfacial membrane also influence lipid oxidation in dried emulsions, both primary (lecithin) and secondary (lecithin-chitosan) tuna oil-in-water emulsions were freeze dried and lipid hydroperoxides and TBARS were measured for 14 days during storage in the dark. As seen with the liquid emulsion, both lipid hydroperoxides and TBARS were much lower in the secondary emulsion (Figure 25a and b). The ability of mixed tocopherol (500 ppm) and/or EDTA (12 μM) to further increase the oxidative stability of the freeze dried secondary tuna oil-in-water emulsion was also evaluated (Figure 26). Since the previous study showed that lipid hydroperoxides and TBARS concentrations were very low in the freeze dried secondary emulsions without antioxidant stored at 37°C (Figure 25), incubation of freeze dried emulsions containing antioxidants was performed at 55°C. Compared to control freeze-dried emulsions (no antioxidant), addition of mixed tocopherol or EDTA, alone, caused significant ($P<0.05$) decrease in lipid hydroperoxide concentrations ranging from 29.8 to 49.6% and 63.7 to 76.1% for mixed tocopherols or EDTA, respectively, during the first 5 days of storage (Figure 26a). EDTA was able to decrease lipid hydroperoxide better than mixed tocopherols throughout storage ($P<0.5$). Mixed tocopherols or EDTA also decreased TBARS concentrations in the freeze-dried emulsions from 1-5 days of storage ($P<0.05$, Figure 26b). No statistical difference was observed in TBARS concentrations in samples

containing mixed tocopherols or EDTA during first 5 days of storage ($P \geq 0.05$). The combination of EDTA and mixed tocopherol hydroperoxide was more effective than mixed tocopherol alone in inhibiting lipid hydroperoxide formation during the entire incubation period ($P < 0.05$; Figure 5a). No differences in lipid hydroperoxide concentrations were observed between EDTA alone and the EDTA, mixed tocopherol combination until 14 days of storage ($P \geq 0.05$). The combination of mixed tocopherol and EDTA tended to have lower TBARS concentrations than the individual antioxidants however these differences were not significant (Figure 26b).

From the results (Figure 23 and 26), not only mixed tocopherol seem to be more effective in the dried system than in the non-dried system but also the control samples. These results could be indicating that lipid oxidation in dried emulsions was lower than in liquid emulsions which can not be explained by the results from this study.

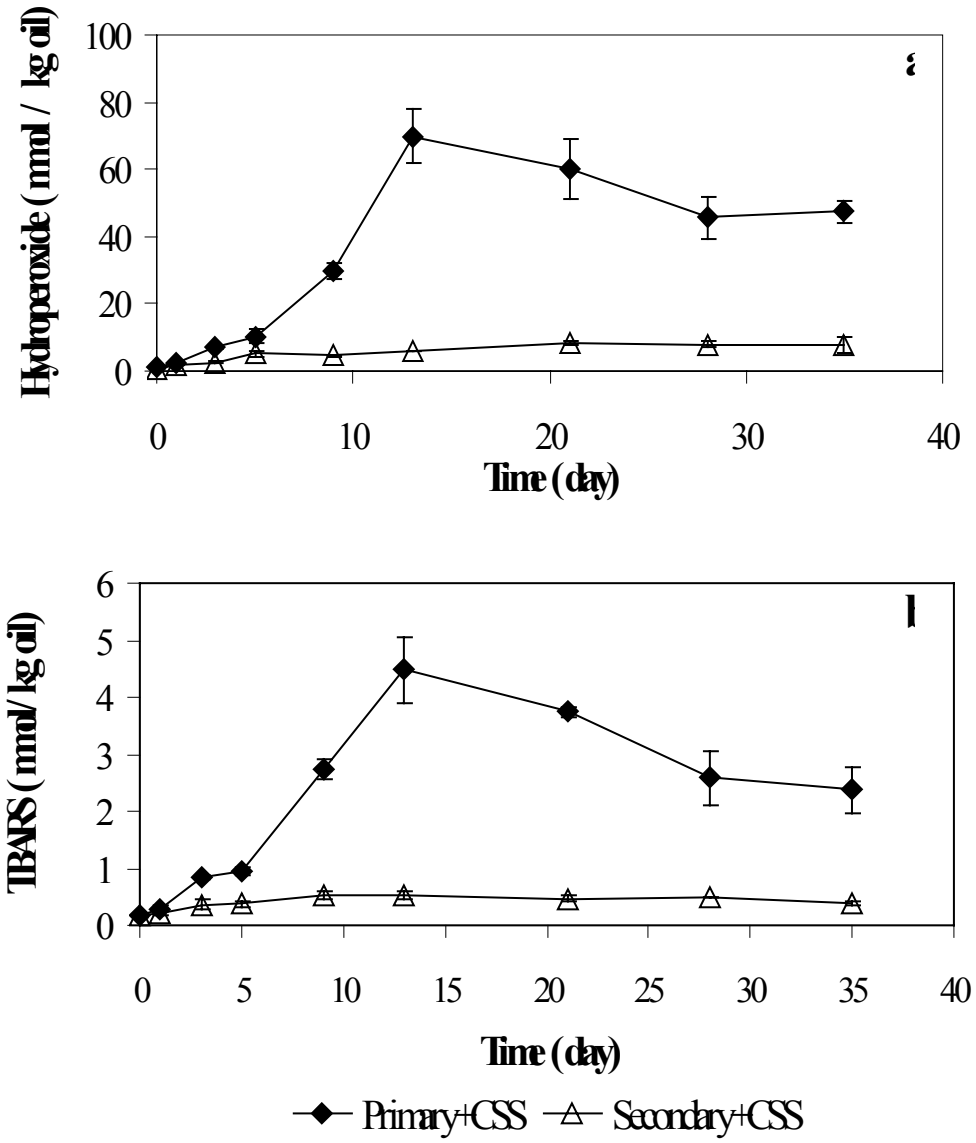

Figure 25 Formation of lipid hydroperoxide (a) and TBARS (b) in freeze dried primary (lecithin alone) and secondary (lecithin and chitosan) emulsions during storage at 37° C.

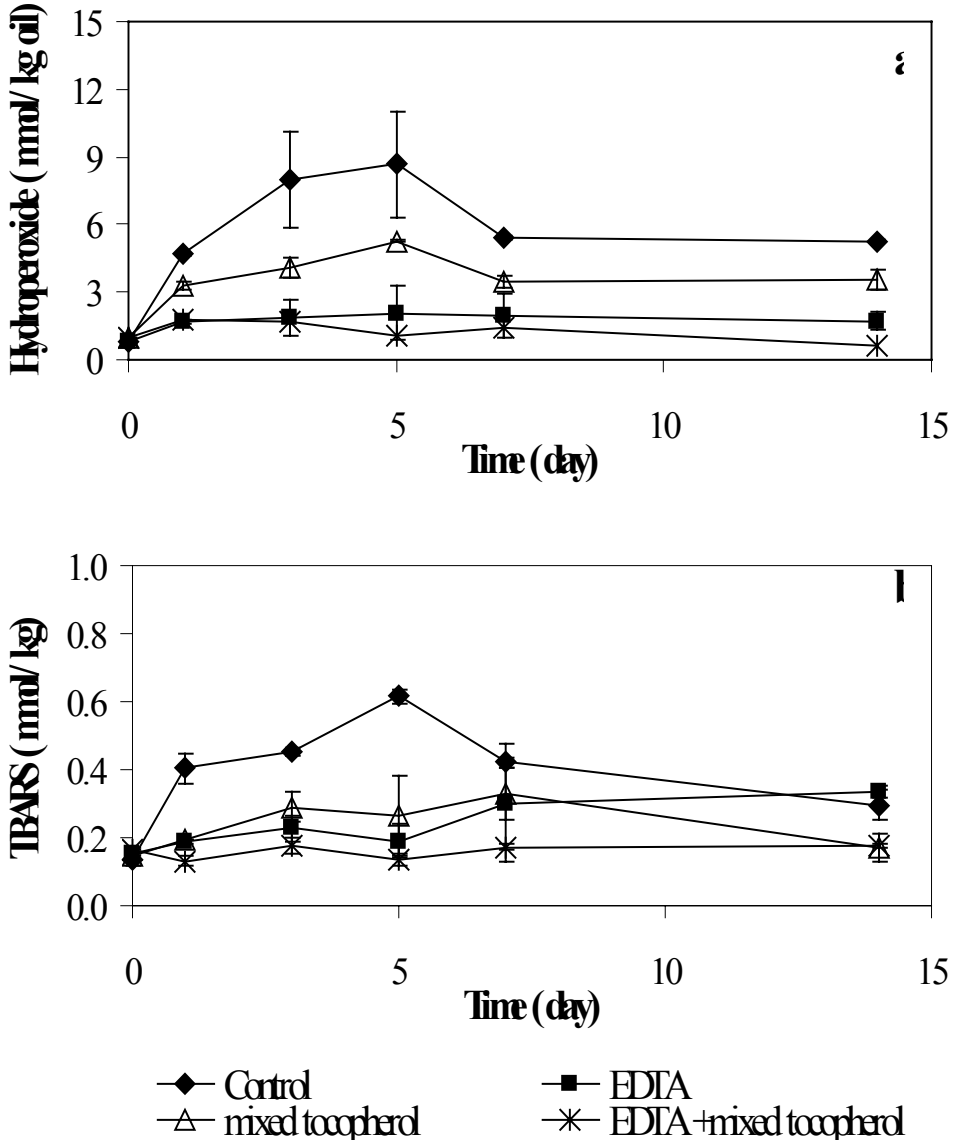

Figure 26 Formation of lipid hydroperoxide (a) and TBARS (b) in freeze dried secondary emulsions (lecithin and chitosan) containing 500 ppm mixed tocopherol and/or 12 μM ethylenediaminetetraacetic acid (EDTA) storage at 55°C.

6.5 Conclusion

This study has shown that the oxidative stability of both liquid and dried tuna oil-in-water emulsion droplets coated by a lecithin-chitosan multi-layer system is higher than emulsion droplets coated with only lecithin. The improved oxidative stability of the emulsion droplets is likely due to its cationic nature that can repel prooxidative metals and possibly formation a thicker interfacial region that could decrease interactions between lipids and water-soluble prooxidants. Combination of mixed tocopherol and EDTA was the most effective of the antioxidant tested at increasing the oxidative stability of both the liquid and freeze dried emulsions stabilized with lecithin and chitosan suggesting that control of prooxidant metals was the effective method to prevent lipid oxidation. These data suggest that tuna oil-in-water emulsions stabilized by lecithin-chitosan membranes may be used to produce oxidatively and physically stable n-3 fatty acids in functional foods.

6.6 Concision of Further Study

The utilization of the multilayer interfacial membrane emulsion system in combination with corn syrup solids may prove to be an effective means to improve the stability of microencapsulated oils. Because spray drying is the most popular technique to prepare microcapsules of good quality, therefore, the impact of spray drying on the properties of microencapsulated tuna oil powders was further studied.