CHAPTER 9

RESEARCH SUMMARY

9.1 Summary of Research Investigation

There were five studies for this dissertation. The studies were conducted in two main points: to enrich the n-3 fatty acids in tuna oil and to produce the microencapsulated tuna oil. The results were summarized as follows:

Chemical Transesterification of Tuna Oil to Enrich n-3 Polyunsaturated Fatty Acids (Chapter4); This study was aimed to enrich the amount of n-3 fatty acids, primarily EPA and DHA, in tuna oil. In this study, the fatty acid composition of tuna oil was chemically modified by using sodium methoxide ($NaOCH_3$) as catalyst. The effects of reaction time and temperature, catalyst concentration, and reactants mole ratio were studied. The lipid and fatty acid composition of the reaction products were analyzed to monitor the efficiency of transesterification. It was found that chemical transesterification could enrich n-3 fatty acids, especially EPA and DHA, in tuna oil. The increasing of EPA and DHA in tuna oil that transesterified at 80°C, 1:4 mole ratio of tuna oil to n-3 FAME and 1.5 wt% of $NaOCH_3$ were 67.5 and 47.5 %, respectively. However, loss of TG between transesterification lead to lower TG yields, which remained about 49% after tranesterification under such condition.

Emulsification of Tuna Oil in Two-Layered Interfacial Membranes (Chapter 5); This experiment was the primary experiment upon production of microencapsulated tuna oil. 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. Therefore, the tuna oil-in-water emulsions were produced using a two-stage process. 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 effect of corn syrup solids (commonly used as filling agent in the microencapsulation of oils) on the stability of emulsions stabilized by lecithin alone (primary emulsion) or by lecithin-chitosan membranes (secondary emulsion) was studied. The effect of environmental stresses, such as pH, ionic strengh, thermal treatment, freeze-thaw cycling and freeze drying were also examined. The 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.

Increasing the Oxidative Stability of Liquid and Dried Tuna Oil-in Water Emulsions (Chapter 6); 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 acids. The purpose of this experiment was to study the oxidative stability of n-3 fatty acids in 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. The lipid hydroperoxides and TBARS formation in tuna oil emulsions during storage were measured. Both liquid and dried tuna oil-in-water emulsion droplets coated by a lecithin-chitosan multilayer system had higher oxidative stability 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 antioxidant for both the liquid and freeze dried emulsions stabilized by lecithin and chitosan. These datas suggested 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. 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.

Characterization of Spray Dried Tuna Oil Emulsified in Two-Layered Interfacial Membranes (Chapter 7); To produce the microencapsulated oil, which the oil droplets surrounded by emulsifier molecules are entrapped within a wall matrix, spray drying is the most popular technique to prepare microcapsules of good quality. Therefore, the impact of spray drying on the properties and dispersibility of encapsulated tuna oil powders was examined. In this study, tuna oil-in-water emulsions containing droplets stabilized by lecithin-chitosan membranes were produced using a two-stage process. Corn syrup solids were added to the emulsions and then the emulsions were spray dried. The properties of spray dried tuna oil emulsions (e.g., water activity, moisture content, microencapsulation efficiency, color, dispersibility and microstructure) were characterized. It was shown that high quality microencapsulated tuna oil can be produced by spray drying oil-in-water emulsions containing corn syrup solids and oil droplets surrounded by multilayer interfacial membranes (lecithin-chitosan). Spray drying produced powdered emulsions consisting of smooth spheroid powder particles (diameter = 5-30 μm) containing small tuna oil droplets (diameter < 1 μm) embedded within a carbohydrate wall matrix. The powders had relatively low moisture contents (< 3%), high oil retention levels (> 85%) and rapid water dispersibility (< 2 minute).

Effect of Storage Environments on Stability of Spray Dried Tuna Oil Emulsion (Chapter 8); The chemical (e.g. oxidation) and physical properties (e.g. caking, stickiness, crystallization, dispersibility and solubility) of food powders can dramatically be changed upon storage and influence quality depending on temperature and moisture. Thus, this experiment studied the effect of storage environments on stability of spray dried tuna oil emulsion. Physicochemical properties (*e.g.*, color, dispersibility, and mean droplet diameter) and oxidative stability of the spray dried tuna oil emulsions were determined as a function of storage temperature (20 and 37°C) and relative humidity (11, 33 and 52% RH). The tristimulus color value L-, a- and b-value were used as index to monitor the color changes. Lipid hydroperoxides and TBARS formation were measured as oxidation markers. The rate and efficiency of spray dried tuna oil powders dispersion was determined by measuring the change in mean droplet diameter and droplet obscuration as a function of time. Physicochemical and oxidative stability of the spray dried emulsions were influenced depending on the function of storage temperature and relative humidity (RH). Addition of mixed tocopherol and EDTA increased in oxidative stability of spray dried multi-layer emulsion. Overall, these datas suggested 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 oxidative stable than bulk oils. This work has 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. The powdered tuna oil produced by this method has good physicochemical properties, leading to its more widespread utilization as a food additive.

9.2 Suggestion for Future Research

1. During transesterification, the remaining of triglyceride (TG) was decreased with the increasing of all reaction factors, lead to lower TG yields. Therefore it would be interesting to study further purification and separation of TG from reaction products to obtain more n-3 fatty acid enriched TG.

2. The stability of the emulsions during freeze drying may be affected the rate of freezing. It would therefore be interesting to investigate the influence of freezing rate or method on emulsion stability in future studies.

3. The effectiveness of two layer membranes consisting of lecithin-chitosan (secondary emulsions) was compared to one layer membranes consisting of lecithin (primary emulsions) to improve emulsion stability. In future studies, the performance of multilayer membranes with those of one layer membranes created from conventional surface-active food biopolymers, such as proteins or polysaccharides is suggested.

4. The effect of oil content on the properties of encapsulated tuna oil emulsified in two-layered (e.g., water activity, moisture content, microencapsulation efficiency, color, dispersibility and microstructure) should be investigated in order to know the highest oil content could be used in this system to increase the amount of n-3 fatty acids in the microencapsulated powders.

5. The retention of n-3 fatty acids in tuna oil powders during storage should be measured to evaluate the effectiveness of wall matrix in protection of n-3 fatty acids degradation.

6. Due to some constrains in using corn syrup solids as wall material resulted in pore formation on the surface. Other wall materials such as amorphous lactose should be used in combination with corn syrup solids to reduce pore and unencapsulated oil on the surface of microcapsules, because the amorphous property can limits the diffusion of nonpolar solvent into the particles leading to decrease the extractable oil and increase the encapsulation efficiency.

7. More recently, membrane-coated microcapsules have been suggested as candidates to orally deliver a wide range of compounds including drugs, nutraceuticals past the stomach to intestinal sites of absorption. In order to evaluate the suitability of coated microcapsules for oral delivery, knowledge of encapsulating agent dynamics is required under relevant physiological conditions that represent the pH conditions during the different phases of digestion.

8. The incorporation of encapsulated tuna oil as food ingredient into food products such as margarine, salad dressing, ice cream, cake or beverage should be studied and one of the most important considerations is the sensory evaluation of the product.