CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Thailand has become the world's largest exporter of canned tuna and largest importer of fresh and frozen tuna. Canned tuna exports accounted for 77% of total canned seafood exports of Thailand in 2001, which was about 300,000 metric tons of tuna and valued about $670 million (Nidhiprabha *et al*., 2003). Tuna processing produces 30-35% product yield, 20-35% of solid waste and 20-35% of liquid waste (Prasertsan *et al*., 1988). Normally, utilization of tuna waste or by-product is in the form of low value applications, such as fishmeal or pet food (Yamprayoon and Virulhakul, 1999). One possible way to use the tuna wastes is extraction of oil for human consumption.

There are a number of processes that can be used to convert raw tuna or tuna wastes into oil. These consist of wet rendering, dry rendering, hydrolysis, and solvent extraction. The wet rendering process is worldwide used in the factories producing fish oil. The major steps of this process are cooking, pressing and separating (Bimbo, 1990). Crude oil from precooked and non-precooked tuna waste (Skipjack's heads) could be separated by a wet rendering method, yielding 2.8 and 4.8%, respectively (Chantachum *et al*., 2000). Another processing technique is solvent extraction. Oil from tuna wastes could be obtained using solvent extraction. The results showed that eyes and heads of tuna contained 14-18% oil while that from the gut contained only 2.8-3.9% oil (Kungsuwan *et al*., 1996).

Tuna oil and other marine oils such as squid oil are rich in n-3 polyunsaturated fatty acids (n-3 PUFAs), especially eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, 22:6) that have been shown to be important for prevention a range of human diseases and disorders (Uauy and

Valenzuela, 2000; Harris, 2001; 2004). Due to their beneficial health properties, n-3 PUFAs have great potential as functional food ingredients. Generally, tuna oil has complex mixtures of fatty acids with varying chain lengths and degree of unsaturation. Overconsumption of tuna oil to obtain n-3 PUFAs may increase the intake of cholesterol and other saturated fatty acids by consumers (Haagsma *et al*., 1982; Shahidi and Wanasundara, 1998). Therefore, concentration or enrichment of tuna oil in n-3 PUFAs could help avoid the consumption of saturated fatty acids.

The triglyceride (TG) form of n-3 PUFAs is considered to be nutritionally more favorable than methyl or ethyl esters of fatty acids as seen from experimental results indicating impaired intestinal absorption of methyl or ethyl esters of n-3 PUFAs in animals (Shahidi and Wanasundara, 1998). Thus, TG of n-3 PUFAs is often promoted as being more natural than other fatty acid derivatives. Transesterification is a reaction that exchanges carbonyl groups of fatty acids within and between TG molecules and it is used to modify the structure and composition of oils to improve the physical and nutritional properties of TG (Basheer *et al*., 1995).

Utilization of oils high in n-3 fatty acids in food is limited due to their high susceptibility 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). Spray drying is one common method of encapsulating oils such as fish oils. An emulsion of oils is prepared with water soluble components such as carbohydrates, gums and proteins. This emulsion is then spray dried to produce an encapsulated powder. The physicochemical properties of liquid emulsion before spray drying are critical to their ability to encapsulate oils. Obtaining a stable liquid emulsion is a pre-requisite for proper encapsulation in spray dried powders (Faldt and Bergenstahl, 1995).

One of the most important and widely used methods of improving the stability of emulsions is to utilize emulsifiers (Dickinson, 1992). Each type of emulsifier has its own particular advantages and disadvantages. For examples, some emulsifiers are highly effective at generating small emulsion droplets during homogenization because of their rapid adsorption rates, but are poor at providing long-term stability against droplet-droplet repulsive interactions, e.g., some small molecule surfactants (McClements, 1999). On the other hand, some emulsifiers are highly effective at imparting long-term stability to emulsions, but are inefficient to create emulsions with small droplet sizes during homogenization, e.g., some polysaccharides and proteins (McClements, 1999).

In this study, the beneficial attributes of different kinds of emulsifiers are combined to create emulsions with improved stability. An anionic emulsifier (lecithin) that rapidly adsorbs to the surface of oil droplets during homogenization will be used to produce a primary emulsion with small droplet sizes, and then a cationic biopolymer (chitosan) will be added to the system to produce secondary emulsions containing droplets coated with an emulsifier-biopolymer membrane. Chitosan was selected as a potential stabilizer of food emulsions because of its unique functional attributes, natural abundance, and under utilization (Ogawa *et al*., 2003a). Chitosan recently is pending for "generally recognized as safe" (GRAS) status within the United States for general application in foods and beverages. So chitosan can now be legally incorporated into food products means that novel chitosan-based technologies developed in other industries can be applied to foods.

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 which partial release of encapsulated oils and the released oil then may be more exposed and undergo rapid oxidation (Rosenberg *et al*., 1990; Shimada *et al*., 1991). 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 decreased below or increased above the monolayer. 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). Hence the evaluation of the effects of the physical properties on the quality of food powders is become more complicated. A better understanding of the relationship between physical and chemical properties is essential for future designing of stable encapsulated products.

1.2 Objectives of the Research

The objectives of this research were:

1. To determine the effects of reaction conditions for the production of tuna oil enriched with n-3 fatty acids by chemical transesterification.

2. To study the emulsification of tuna oil in two layered interfacial membrane and study the effects of environmental stresses on the physicochemical properties of

emulsion. The effectiveness of antioxidants for improving the oxidative stability of tuna oil emulsion was also studied.

3. To study the spray drying condition of tuna oil emulsion on the properties of microencapsulated powders and to investigate the effect of storage environments on stability of microencapsulated tuna oil.

1.3 Hypothesis

Tuna oil transesterified by chemical should enrich in n-3 fatty acids, especially EPA and DHA when compared to the original tuna oil. The physicochemical properties of liquid tuna oil-in-water emulsion and storage conditions may influence the quality of encapsulated tuna oil. It is hypothesized that the composition, microstructure and physical state are the key parameters in lipid oxidative stability. In this work, the impacts of processing and storage conditions on the properties of tuna oil emulsion before and after spray drying were studied. Data obtained from this work may lead to a novel method of improving the properties of encapsulated oils in the food industry.

1.4 Scope and Limitation of the Research

1. Crude tuna oil from canning industry (Chotiwat Manufacturing Co., Ltd., Songkhla) was refined and preparation of FAME of n-3 fatty acids.

2. The fatty acid composition of tuna oil was chemically modified to enrich n-3 PUFAs, primarily EPA and DHA, by using sodium methoxide ($NaOCH_3$) as catalyst. The effects of reaction time and temperature, catalyst concentration, and reactants mole ratio were studied. Lipid and fatty acid composition of tuna oil were analyzed before and after transesterification.

3. The effect of filling agent (corn syrup solids) on the stability of tuna oil emulsions stabilized by lecithin alone or by lecithin-chitosan membranes was studied. The effect of

environmental stresses, such as pH, ionic strengh, thermal treatment, freeze-thaw cycling and freeze-drying were also examined.

4. The oxidative stability of tuna oil emulsions coated by lecithin alone or by lecithin-chitosan was examined. The ability of the antioxidants mixed-tocopherol and EDTA on the stability of the emulsions was also studied.

5. The impact of spray drying on the properties and dispersibility of encapsulated tuna oil powders was investigated.

6. The effects of relative humidity (RH) and temperature on dispersibility, color, and oxidative stability of spray-dried microencapsulated tuna oil were examined.

1.5 Expecting Outcomes

In this project, tuna oil from waste products of canned-tuna will be enriched with n-3 PUFAs such as EPA and DHA in order to increase the value of by- products from tuna processing. Furthermore, microencapsulated tuna oil will be produced to improve oxidative stability so that their shelf life would be prolonged and the data from this research may lead to a novel method of improving the properties of encapsulated oils in the food industry and may be used to produce oxidatively and physically stable n-3 fatty acids in functional foods.