2011

Purification of Fish Oils and Production of Protein Powders with EPA and DHA Enriched Fish Oils

Huaixia Yin
Louisiana State University and Agricultural and Mechanical College, hyin3@tigers.lsu.edu

PURIFICATION OF FISH OILS AND PRODUCTION OF PROTEIN POWDERS WITH EPA AND DHA ENRICHED FISH OILS

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
in

The Department of Food Science

by
Huaixia Yin
M.S., South China University of Technology, 2007
B.S., Shandong Agricultural University, 2004
December 2011

ACKNOWLEDGEMENTS

I am heartily thankful to my supervisor, Dr. Subramaniam Sathivel, whose encouragement, supervision and support from the beginning to the conclusion level enabled me to accomplish this dissertation research. There is no doubt in my mind that his talent, diligence, persistence, and positive attitude to life have profoundly shaped me and will continue to impact me in the future. My appreciation is also extended to the committee members Dr. Ioan I. Negulescu, Dr. Joan M. King, and Dr. Paul W. Wilson for their guidelines and thoughtful help during my research and study. I thank Dr. Ioan Negulescu for his guidance through my Ph.D. study and for the DSC and TG analysis for my samples. I thank Dr. Joan King for instructing my courses and all of the unconditional support and guidance for my research. I thank Dr. Paul W. Wilson for all of his support and help with my research. It has been an honor and privilege for me to have them on my committee, which I could not have asked for a better one. I also would like to thank Dr. Zhimin Xu for helping me on FAMEs analysis in his lab. Special thanks go to Dr. Peter J. Bechtel and Dr. Jesse Stine at the University of Alaska Fairbanks for their help for determination of fatty acid profile and amino acid profile.

Appreciation also goes to my laboratory fellows and friends: Luis Espinoza, Juan Li, Jie Zhang, Jianing Pu, Yuting Wan, Dr. Bob Xiang, Fathima Waheeda Mohideen, Luis Alfaro, Srijanani Sundararajan, Ahalya Kosal Ram, and Jose D. Estrada for their kind assistance and suggestions in my research. Special thanks go to Kevin E. Mis Solval for his support and help.

I am deeply indebted to my parents for their patience, encouragement, understanding, and deep love. I would also like to thank my husband Fei Deng for his consideration and encouragement that keeps pushing me forward. Without their support I could not have achieved the goal of my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Eicosapentaenoic (EPA) and docosahexaenoic (DHA) in marine oils are known to provide health benefits. However, marine oils are accompanied by saturated fatty acids. By enriching fish oils with EPA and DHA the beneficial effect of fish oils can be increased while limiting the detrimental effect of the saturated fatty acids. Fish oils need to be purified before consumption. A number of methods have been used for extracting and purifying EPA and DHA enriched fish oils; however, thermal degradation and oxidation of polyunsaturated fatty acids (PUFAs) often occurs as a result of exposure to high temperatures, solvents and adverse conditions during processing. Therefore, it is important to develop a combined purification and enrichment method for EPA and DHA that minimizes the degradation and oxidation of PUFAs. A process which produces a dry powder microencapsulated fish oil enriched with EPA and DHA enclosed in a protein and peptide coating may suffice and result in higher market value and extended shelf life. Menhaden (MO) and salmon (SO) oils were used to develop the process. MO and SO were hydrolyzed by lipase at enzyme concentrations of 250, 500, 2500 U/g oil and 50, 250, 1250 U/g oil, respectively, for up to 24h. The enzyme was subsequently inactivated and removed using centrifugal force. The fish oils enriched with EPA and DHA were purified using adsorption technology. The purified oils enriched with EPA and DHA were microencapsulated with protein and peptides. The lipase hydrolysis kinetics of MO and SO were well fitted with the *Michealis-Menten* model. Total EPA and DHA fractions increased from 21.1% to 38.9% for MO at 2500 U/g oil and from 20.1% to 32.8% for SO at 1250 U /g after 6 h of hydrolyzation. The adsorption process reduced impurities in fish oil enriched with EPA and DHA. Microencapsulated MO and SO enriched with EPA and DHA powders contained all the essential amino acids. This study demonstrated a method to enrich fish oils with EPA and DHA and to

purify the oils enriched with EPA and DHA. In addition, the study demonstrated the microencapsulation of fish oils enriched with EPA and DHA with a protein and peptide wall material.

CHAPTER 1 LITERATURE REVIEW

1.1 Oils and Long Chain ω-3 PUFA

Lipids provide a concentrated source of energy and essential fatty acids through daily dietary intake. They also serve as important constituents of cell walls and carrier of fat-soluble vitamins. In addition, lipids are essential for flavor, texture and mouthfeel of the food.

Edible oils are mainly composed of triacylglycerols (also called neutral fats or triglycerides) with phospho- and glycolipids (PL and GL, respectively) comprising a small fraction. Triacylglycerols are the combination of one unit of glycerol with three units of fatty acids. A fatty acid contains a long hydrocarbon chain and a terminal carboxylate group. Fatty acids have 3 major physiological roles: 1) fuel molecules for metabolism. Fatty acids mobilized from triacylglycerols are oxidized to provide energy for a cell or organism. Fatty acids are the main source of energy when undergoing a moderate exercise or resting. 2) Fatty acids are used to modify protein by the covalent bond to target them to membrane locations. 3) Fatty acid derivatives can serve as hormones and intracellular messengers.

Fatty acid chain may contain two to more than thirty carbon atoms with various numbers of double bonds. The fatty acids without double bonds are called saturated fatty acids; fatty acids containing one double bond are called mono-unsaturated fatty acids, while the ones that have more than one double bond are polyunsaturated fatty acids (PUFA). In systematic chemical nomenclature the position of the double bonds is given by numbering from the carboxyl group. But an alternative notation numbers the carbon atoms from the terminal methyl group, i.e., the carbon furthest from the carboxyl group, known as the ω-carbon, and the position of the first double bond from the ω-carbon is known as ω-x or n-x, where x is the carbon number on which the double bond occurs (Figure 1.1). There are two groups of essential fatty acids: ω-3 and ω-6 (or n-3 and n-6) fatty acids, because the human body system cannot introduce ω-3 and ω-6 fatty

acids, even though can create double bonds between the carboxyl group and an existing double bond. By contrast, plants can introduce a new double bond between existing double bonds and the terminal methyl group with the presence of enzymes (such as Δ^{12} and Δ^{15} – desaturases) (Ruxton and others 2005). Mammals lack these enzymes and cannot synthesize ω-3 and ω-6 fatty acids, but require a dietary source of these two essential fatty acids (Cunnane 2000). The ω-3 PUFA group mainly consists of α-linolenic acid (18:3n3) (contained mainly in vegetable oils), stearidonic acid (SA, 18:4n3), eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3). The SA, EPA, and DHA are obtained from marine sources. The ω-6 group is made up of linoleic acid (18:2n6), γ-linolenic acid (18:3n6), dihomo- γ- linolenic acid (20:3n6), and arachidonic acid (AA, 20:4n6). All of these fatty acids contain all-cis, non-conjugated double bonds, and the first double bond occurs at carbon three or carbon six counting from the terminal methyl group.

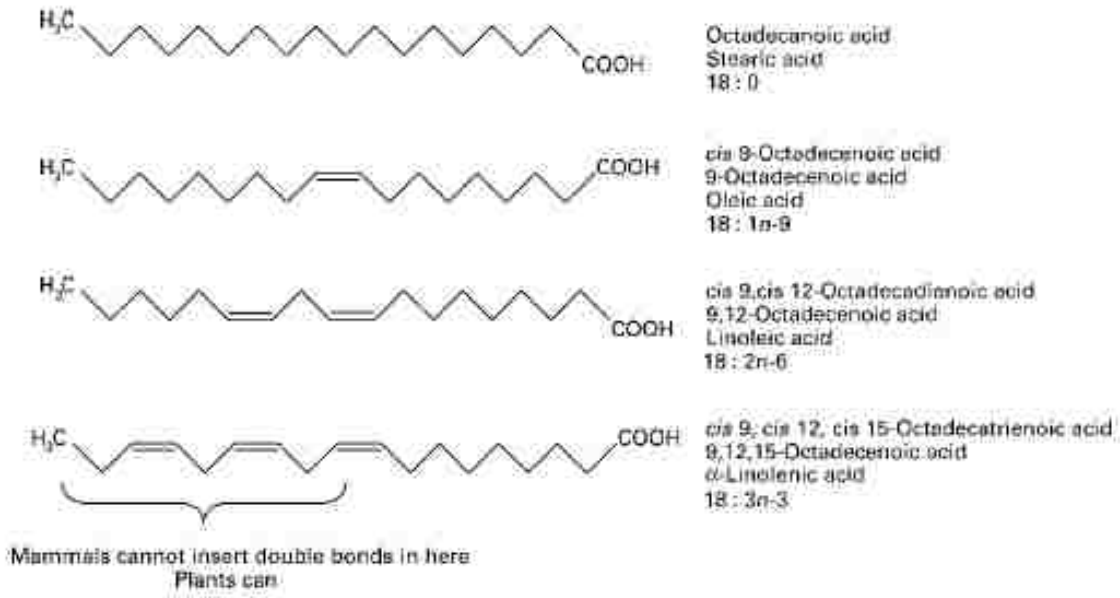


Figure 1.1 - Chemical composition of various fatty acids (Ruxton and others 2005)

Fish oils, which are rich in long chain ω-3 PUFA, have been receiving attention in the scientific and industrial areas because of its positive role in human health. The potential benefits of ω-3 PUFA include reduced risk of cardiovascular diseases, hypertension and atherosclerosis, inflammatory and autoimmune disorders. DHA is now recognized as an essential nutrient in the brain and retina for neural functioning and visual activity, respectively (Matouba and others, 2008).

1.1.1 Long Chain ω-3 PUFA and Cardiovascular Disease (CVD)

Evidence indicated that dietary long chain ω-3 PUFA has beneficial effects in the prevention and treatment of CVD and detailed systematic reviews of the evidences were provided (Bucher and others 2002; Hooper and others 2003). Based on a study of a sub-group of diabetic women, Hu and others (2003) observed a very high inverse relationship between fish intake and coronary heart disease (CHD), with a risk reduction of more than 60% for the highest fish intake and about 30% reduction for women eating one to three fish meals per month.

The GISSI-Prevenzione study (Anonymous, 1999) found that long chain omega-3 fatty PUFA supplementation (equivalent to 850 mg EPA and DHA/d) reduced the relative risk of death caused by acute myocardial infarction (MI) over 3.5 years by more than 10% and of cardiovascular death by at least 17%.

There is a lack of a definitive mechanistic explanation on the cardioprotection of long chain n-3 PUFA. It may arise from the intervention at either step leading to cardiovascular disease (i.e., atherosclerosis) or the step that ultimately causes death (e.g., MI and stroke) (Calder 2004). Ruxton and others (2005) summarized the possible mechanisms as: 1) alterations in plasma lipoprotein profiles, especially by reducing triacylglycerol concentrations and reducing the LDL: HDL cholesterol ratio; 2) reducing the endothelial activation, i.e., reduction in

expression of adhesion molecules and an increase in vascular nitric oxide (NO) production; 3) anti-arrhythmic effects on heart muscle; 4) improved plaque stability; 5) anti-thrombotic effects.

1.1.2 Long Chain ω-3 PUFA and Infant Development

It was revealed in the early study (Pullarkat and Reha 1976) that the most abundant PUFA in rat brain lipid was DHA. A lack of DHA in the last trimester of an infant may impact on the differentiation and multiplication of brain cells and hampering the function of cerebral membranes and consequently learning abilities. This could be caused by the important role of DHA in creating fluidity in neuronal membranes, which can regulate neurotransmitters, promote the enzyme activity, bind protein, and transport nutrient for the brain cells (Yehuda and others 1999; Bourre and others 1991). DHA is also very crucial for the development of visual function. Phospholipid bilayers rich in DHA are important to the normal functioning of photoreceptor cells because of their high fluidity and enhanced rates of fusion and permeability.

1.1.3 Long Chain ω-3 PUFA and Mental Health

The evidence for the role of ω-3 fatty acids in the aetiology and treatment of depression and other mental disorders were reported by many researchers (Freeman 2000, Mischoulon and Fava 2000; Haag 2003). Cenacchi and others (1993) found that elderly patients treated with DHA-rich phosphatidylserine demonstrated significant reductions in depressive symptoms compared with a placebo-controlled group. In a study with EPA, patients with unipolar depressive disorder experienced significant improvements in symptoms after 4 weeks of treatment (Nemets and others, 2002).

1.1.4 Long Chain ω-3 PUFA and Immune Function

Arachidonic acid (AA) is the most abundant fatty acid found in human immune and inflammatory cells. The balance between arachidonic acid (AA), and long chain ω-3 PUFA within immune and inflammatory cells is thought to affect the immune function and on

production of inflammatory mediators (Ruxton and others, 2005). The function of supplementation with fish oil containing long chain ω-3 PUFA was reviewed by Ruxton and others (2005). It was shown to decrease neutrophil and monocyte chemotaxis, neutrophil and monocyte respiratory burst, neutrophil binding to endothelial cells, and production of the classic inflammatory cytokines TNF, IL-1, and IL-6.

1.2. Introduction of Menhaden and Salmon Fish

In the USA, currently, menhaden and salmon oils are commercially produced and both fish oils are abundant sources of polyunsaturated fatty acids, especially EPA and DHA.

1.2.1 Menhaden Fish and Menhaden Oil

Menhaden is an Americanized name but derived from the Indian word "Munnawhatteaug", meaning "which enriches the earth". It is a silver-scaled fish, with an average length of 18 inches and weight of 5 pounds for a full-grown individual. Most of the menhaden fish consumed in the United States are caught from the Atlantic and gulf coasts.

Atlantic menhaden (*Brevoortia tyannus*) are found in estuarine and coastal waters from northern Florida to Nova Scotia. They may live up to 10-12 years. In 2000, about 30% of U.S. Atlantic coast commercial fisheries landing by weight were Atlantic menhaden. People are becoming more and more aware of the high value of menhaden oil recently, as a result, the ex-vessel value (the amount paid to fishers for their raw catch) for the Atlanta menhaden fish was increasing from $0.009/pound to $0.11/pound from year 1960 to year 2000 (National Marine Fisheries Service, Fisheries Statistics and Economics Division).

Gulf menhaden (*Brevoortia patronus*) are distributed throughout the Gulf of Mexico from the Yucatan Peninsula to Tampa Bay, FL, and are most abundant in the north central Gulf of Mexico. The primary fishing ground for gulf menhaden includes the coastal regions of Alabama, Mississippi, Louisiana, and Texas (Gulf States Marine Fisheries Commission 2002).

During the period 2000 to 2004, Gulf annual landings have averaged 575,311 metric tons. The menhaden landings in the Gulf of Mexico comprised about 11% of all U.S. landings, which is the second largest commercial fishery in the United States. Final landings of gulf menhaden in 2010 amounted to 379,727 metric tons (1,250 million standard fish), which was lower than past years because of the oil spill and weather (NOAA 2011).

Menhaden are not commonly used for human consumption because of the oily taste and bony structure. They are usually used for bait in commercial fisheries and can also be processed into fishmeal and fish oil. The following procedure describes how the fish oils are produced: Firstly, the fish are caught by a fishing technique known as "purse seining". They are pumped from the sea into refrigerated holds on the fishing vessels. Then they are transferred to temporary holding tanks until they are cooked. The fish are cooked in indirect steam cookers (165 °F, 15-20 min). After cooking, the fish are conveyed to screw press conveyors where much of the water and most of the oil are squeezed from the fish. The oil/water mix goes through several banks of centrifuges to separate the oil and water without leaving much of either with the other.

1.2.2 Salmon Fish and Salmon Oil

Seafood companies process large quantities of both farm-raised and wild salmon each year. Salmon species vary with different oceans, which primarily include Atlantic Ocean species and Pacific Ocean species. The Atlantic Ocean has only one species of salmon: Salmo salar. Pacific species belong to the genus *Oncorhynchus*, such as *Oncorhynchus masou* (cherry salmon), *Oncorhynchus tshawytscha* (Chinook salmon), *Oncorhynchus keta* (Chum salmon), *Oncorhynchus kisutch* (Coho salmon), *Oncorhynchus gorbuscha* (Pink salmon), and *Oncorhynchus nerka* (red salmon). Wright (2004) reported that several tons of fish processing waste is produced worldwide from salmon farming. The major salmon species that is raised in net-pen culture is Atlantic salmon (*Salmo salar L.*) (Sun and others 2002). Alaska's wild salmon

resource is the greatest in the world. It was reported that 99,000 metric tons of salmon byproducts were produced from 331,798 metric tons of salmon harvested in Alaska in year 2006 (ADFG 2007). The byproducts from salmon processing including heads, skins, frames, and viscera usually end up in landfills or rendering plants. These byproducts could be converted into valuable products such as salmon hydrolysate and salmon oil (Wright 2004). The hydrolysate is typically produced by maceration of byproducts containing less fat followed by enzymatic digestion of protein and removal of bones (Kristinsson and Rasco 2000). Salmon oil can be used as a feedstock for biodiesel production via a transesterification process (El-Mashad and others 2008). Salmon oils have been claimed to help maintain human health because of the high concentration of ω-3 PUFAs (Kronhout and others 1985). Much of the oil in the salmon is found in the heads (15-18% lipids). Sun and others (2002) found that Atlantic salmon viscera contained higher amounts of PUFAs than fillet portions.

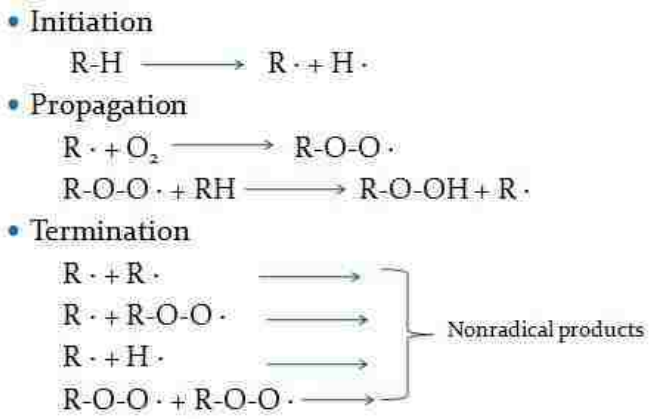
1.3 Oil Analysis and Fish Oil Deterioration

Different analyses are required at different stages of oil processing and storage. Before refining, for example, evaluating the free fatty acids (FFA) content of the oil is necessary to determine the lye amount and amount of oil loss after the neutralization process, to determine if the oil has been properly deodorized, and to serve as a quality indicator after storage. For final edible-oil products, organoleptic evaluations, peroxide value, FFA, and other analyses are utilized for assurance that the product has the required bland flavor. Active oxygen method (AOM) stability has been utilized to ensure proper shelf life. Nutritional listings of saturated, polyunsaturates, cholesterol, trans- fat, vitamins, and other product characteristics on food product labels require accurate analysis to identify the oil values. To meet the governmental regulations, trace constituents such as pesticides or trace metals also need to be analyzed (O'Brien 2003).

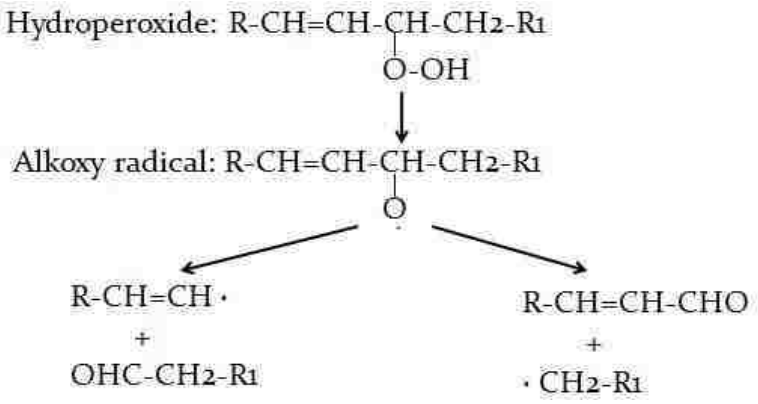
The analyses in this study include composition analysis and rancidity and stability characterization. Composition analysis includes saponification value, iodine value, fatty acid composition, and glyceride structure.

The rancidity values, such as peroxide values (PV), p-Anisidine value, thiobarbituric acid-reactive substances (TBARs), and free fatty acids (FFA), are of prime importance for quality control of edible fats and oils. The conjugated carbon double bonds of PUFAs are very easily attacked by oxygen (autoxidation) resulting in hydroperoxides (indicated by PV). This reaction rate depends on the degree of unsaturation of the fatty acid and can proceed rapidly with the presence of light, heat, oxygen, moisture and catalysts such as metals in the oil (Robles and others 1998). After a series of autocatalytic reactions, very diverse secondary oxidation products are formed including ketones, aldehydes, organic acids and other low molecular weight compounds. p-Anisdine value estimates the amount of aldehydes (mainly 2-alkenals and 2, 4-dienals) by reacting with each other to form a chromogen that is measured spectrophotometrically. The secondary lipid oxidation products can also be evaluated by the measurement of malondialdehyde (MDA) reacting with thiobarbituric acid (TBA). Because the TBA test is not highly specific for MDA, the results of this measurement are generally expressed as thiobarbituric acid-reactive substances (TBARS) (Ang and Lyon 1990).

These oxidation and decomposition reactions are summarized as follows: 1) Autoxidation:



2) Decomposition of hydroperoxides: Secondary products (e.g.,aldehydes, ketones, organic acid)



The oxygen-centered free radicals, also known as reactive oxygen species (ROS), including superoxide, hydrogen peroxide, hydroxyl ($HO^{\cdot}$), peroxyl ($ROO^{\cdot}$) and alkoxyl (RO.) play an important role in the degenerative or pathological processes of various serious diseases in humans, such as aging (Burns and others 2001), cancer, coronary heart diseases, Alzheimer's disease (Diaz and others 1997), neurodegenerative disorders, atherosclerosis, cataract, and inflammation (Aruoma 1998).

To minimize these reactions, 1) the oil processes should be done in an inert atmosphere (nitrogen or argon), if possible; 2) techniques that employ heating should be avoided; 3) EDTA can be added to inactivate the metal ions by forming chelate complex if necessary.

During the storage of purified oils, these steps are usually used to reduce the rancidity occurrence: 1) for temporary storage, diluting the sample with a solvent such as petroleum ether (low oxygen solubility); 2) sealing the oil samples under vacuum with minimum headspace (Itolman 1966); 3) storing the samples at low temperature. The deterioration rate of oil samples at -20°C is only about 1/16th that of at room temperature. Temperatures between -40°C and -80°C are required for storage of several years; 4) adding antioxidant additives to slow oxidation of PUFAs. Commonly used antioxidants are tertiary butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and octyl gallate, at concentrations of below 200ppm according to

regulations (Babovic and others 2010). But these synthetic antioxidants could be promoters of carcinogenesis (Ito and others 1986) and liver swelling (Martin and Gilbert 1968). Use of natural antioxidants has been suggested to avoid the potential toxicity of these synthetic antioxidants with an increased demand from consumers (Ahn and others 2002). Many researchers have reported antioxidant effects of extracts from plants, such as rosemary, sage, thyme, grape seed, and pine bark (Barbut and others 1985; Buckley and others 1995; Babovic and others 2010; Demiral and Ayan 2011). Most of the natural antioxidants are phenolic compounds or polyphenols. Their antioxidant activity is based on their structure, hydrogen-donating potential and ability to chelate metal ions. They may show higher efficiency than endogenous or synthetic antioxidants (Soobratte and others 2005).

1.4 Oil Purification

Sterol, waxes, lipid soluble vitamins, phenolics and other minor constituents comprise the unsaponifiable matter of oils. Also, during the processing and storage, food lipids undergo chemical and physical changes, and these changes should not exceed a desirable limit. The impurities produced from processing and storage may decrease product quality or processing efficiency. These impurities include moisture, dust, protein degradation products, free fatty acids, phosphatides, oxidation products, pigments and trace elements (e.g. copper, iron, sulfur, and halogens), polysaccharides and chlorinated pesticide residues (Young and others 1994). The objective of purifying fish oil was to remove the impurities which cause the unattractive color or taste or are harmful components. In the meantime, some of the natural components such as omega-3 fatty acids and tocopherols should be preserved.

The conventional oil purification methods include degumming, neutralization, bleaching, and deodorization. The following figure summarizes the major refining stages of oil processing and the type of impurities removed during each step.

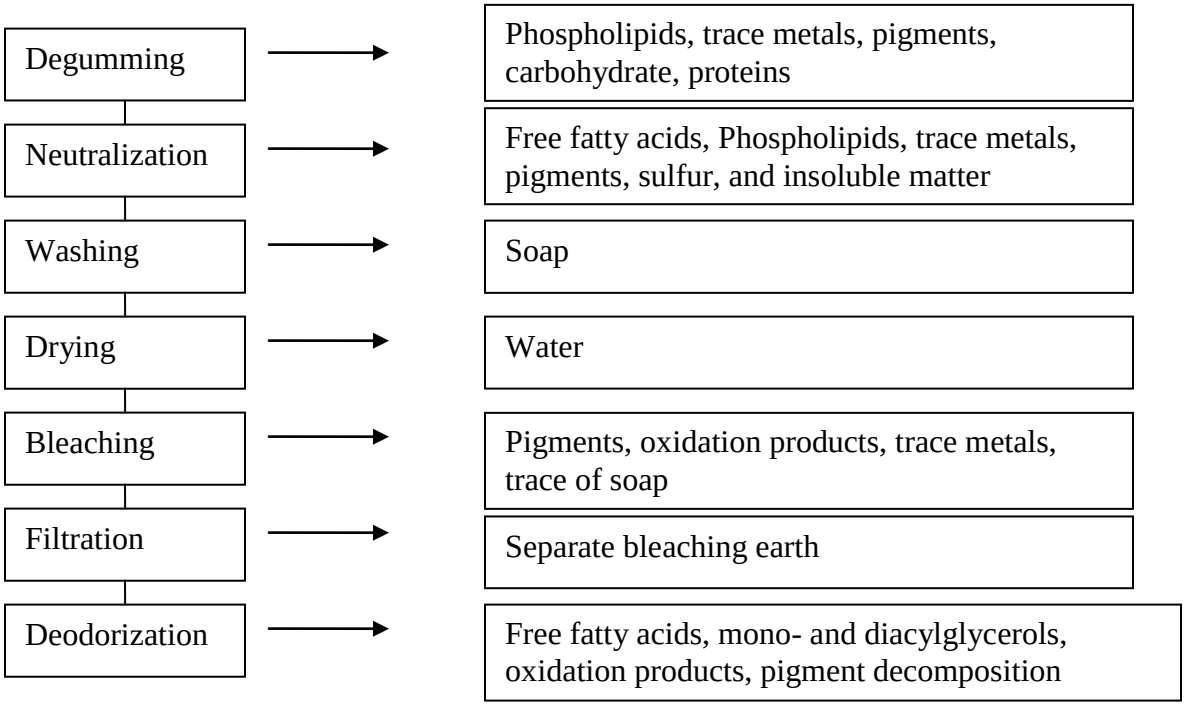


Figure1.2 - Refining stages of edible oils and the major impurities removed.

1.4.1 Conventional Oil Purification Methods

- **Degumming**

Degumming is usually carried out after separating the oil from the micella. The gums are phospholipids and they are classified as hydratable and nonhydratable phospholipids (Subramanian and others 1999). With the presence of water or acids the phospholipids become hydrated and can be removed (Hui 1996). In the water-degumming process, the hydratable phospholipids are easily removed from the oil by treatment with water or steam. For example, soybean oil contains large amounts of phospholipids, to remove the gum the oils are usually treated by adding 2-3% water, agitating the mixture at about 50°C, and separating the hydrated phospholipids by settling or centrifugation (Fennema 1996). During acid degumming, the hydratability of the nonhydratable is increased by the acid (phosphoric or citric acid) and then

they can be removed from the crude oil. The lipids removed by degumming are α-lipids because they are easily reacted with water and readily precipitate as oil-insoluble hydrates (Wiedermann 1981).

Degumming has shown to effectively reduce lead, copper, arsenic, and zinc in menhaden oil (Elson and Ackman 1978). The degumming methods include water degumming, superdegumming, total degumming, ultrafiltration process, acid treatment, and enzymatic degumming. Acid degumming is a simple and widely used method, by which phosphorus or citric acids are used and the phosphorus content after degumming is about 15-80 mg/kg depending on the different oil resources and quality. The problem with acid degumming is that it is not suitable for all types of oils. Superdegumming and total degumming were designed as modified processes of acid degumming, but the problem is that they are not cost-effective. The ultrafiltration process is an effective way to preserve the thermal sensitive and bioactive components in the oil, but it also has poor economic performance problem. The enzymatic degumming is to use enzyme to hydrolyze nonhydratable phospholipids into their hydratable form. This enzymatic degumming process produces high quality oil with low oil loss but it is not mature so far (Yang and others 2008).

Marine oils are not usually degummed before being refined. The removal of phospholipids and mucilaginous materials in such oils is carried on together with the removal of free fatty acids by alkali-refining (Shahidi and others 1997).

- **Neutralization**

Neutralization is also called alkali refining. It is performed on edible oils to remove free fatty acids and other gross impurities such as phosphatides, proteinaceous, and mucilaginous substances. Alkali refining may cause both physical and chemical changes in the oil. The alkali (normally diluted sodium hydroxide) added to the oil reacts with free fatty acids present to form

soap. Gums absorb alkali and are coagulated by hydration, much of the pigments are degraded, adsorbed on the gums or made water-soluble by the alkali and the insoluble matter is entrained with other coagulated materials (Bimbo and Crowther 1991). After adding alkali to crude oils, the mixture is slightly heated to break the emulsion and then the soapstock is removed by centrifugation (Cowan 1976; Carr 1978). The refined oil is washed with warm water to remove the last traces of soap (Kwon and others 1984). These impurities, if not removed, could affect the color, foam and smoke characteristics and/or cloudiness of the oil in later stages of processing that involve high temperatures.

The soap which is removed in the refining process is diluted with water and acidified to form fatty acids. The fatty acids are centrifuged to remove the aqueous phase, dried and used for production of fatty acids, soap or feed manufacturing. This product is called acid oil or acidulated soapstock by the oil industry (Bimbo and Crowther 1991). Alkali refining removes the β-lipids since they are nonwater precipitable and require addition of alkali or acid to be removed from the oil (Wiedermann 1981).

- **Bleaching**

Normally, after alkali-refining, the oil would be bleached. Bleaching refers to the process for removing color compounds, natural pigments (e.g. carotenoids, chlorophyll, xanthophyll and polyphenols) and oxidation products; it also removes some suspended mucilaginous and colloid-like matter (Chang 1967). The process will improve the color, flavor, appearance, and oxidative stability of the oil. The usual method of bleaching is by adsorption of the color producing substance and other compounds on an adsorbent material. Commonly used bleaching materials are natural clay (or earth), activated clay (or earth) and carbon (Cowan 1976). If the oil is easily bleachable, natural clay is usually used; if the oil is not readily bleachable, acid-activated clay is

used. Bleaching power of the acid-activated clay is greater than that of the natural clay (Boki and others 1989).

- **Deodorization**

After several purification steps, the oil may contain volatile odor and flavor-active components originally present in the crude oil, the "soapy" odor created by alkali-refining, and the "earthy" odor generated by bleaching (Chang 1967). Deodorization is the last major step in the refining of edible oils. It is done to remove undesirable odors and flavors from the oil and ensure their shelf-life stability (Gavin 1978). The deodorization is a steam distillation process to strip the volatile compounds from the nonvolatile oil (Bimbo and Crowther 1991). The conventional procedure is to steam strip the oil at 200-240°C. For most edible oils, deodorization does not have any significant effect on the fatty acid composition and sufficient tocopherols remain in the finished oil after the process. But for marine oils, the temperature may cause high oxidation of polyunsaturated fatty acids (PUFA). Dimamarca and others (1990) developed a process for deodorizing fish oil by high vacuum distillation at lower temperatures (< 150°C). They produced purified fish oil without destroying the long chain PUFA.

The main problems with the conventional purification methods are that they are not only labor-intensive but also expensive. An easy-operating and economical method is necessary in the edible oil purification industry. In this study an adsorption method with combined adsorbents was applied.

1.4.2 Adsorption

An adsorption process is an alternative method to refine fish oils. This technique involves mass transfer of adsorbate from the fluid phase to the adsorbent surface until the thermodynamic equilibrium of the adsorbate concentration is reached. Adsorption is a cost effective method with less oil loss and less lipid oxidation because of the mild conditions applied. Different kinds of

adsorbents were used in purification of edible oils, such as activated earth, activated carbon, kiselguhr or diatomaceous earth (Bera and others 2004), metal oxide and metal phosphate adsorbents (Chapman 1994). Activated earth is by far the most common adsorbent for purification and color improvements of fats and oils (Du and others 2006; Lara and Park 2004). Activated earth is produced by the activation of bentonite using mineral acids under heating for a few hours. Activation is a process that involves a series of chemical reactions, resulting in strongly protonated clay mineral surface and increased specific surface area from an original 40-60 to about 200 m^2 per gram of dry clay (Hymore 1996). Proctor (1996) reported a novel adsorbent rice hulls for edible oil processing, which are the co-products for rice processing. The reason was that rice hulls are rich in amorphous silica, and are very effective in binding phospholipids in adsorption process. Bera and others (2004) used charred sawdust as adsorbent to refine several kinds of edible oils, and indicated it was an effective, economical, and cost-effective method for the refinery industries. Activated carbons have been widely used as adsorbents in technologies related to pollution abatement, pharmaceutical, and food industries due to their highly porous structure, big internal surface area and large adsorption capacity (Song and others 2005).

1.5 Enrichment of ω-3 Polyunsaturated Fatty Acid

Consumption of EPA and DHA in a concentrated form may be more beneficial than general fish oil itself because the concentrates contain less saturated fatty acid. It has been suggested that PUFA concentrates, devoid of more saturated fatty acids, are much better than marine oils themselves because they allow daily intake of total lipids to remain as low as possible (Wanasundara and Shahidi 1998). With the consumer awareness of benefits from PUFAs, there's a great demand for pharmaceutical industries to prepare highly pure PUFA concentrations. Therefore, rapid and reliable methods to enrich PUFAs are required.

The most developed techniques used to concentrate PUFAs include urea fractionation, low temperature fractional crystallization, salt solubility methods, gas chromatography, and thin-layer chromatography. These techniques usually fractionate the fatty acids based on the number of double bonds or the chain length. However, not all those methods are applicable to preparative or production scale (Robles and others 1998). There are some newly developed processes to concentrate PUFAs, such as supercritical fluid technology and lipase-catalyzed hydrolysis reaction.

1.5.1 Urea Fractionation

The urea fractionation method is one of the common methods being used to concentrate PUFAs. The theory of fractionation of organic compounds with urea is: When urea crystallizes from a solution of fatty acids of varying degrees of unsaturation, the saturated and monounsaturated fatty acids (long and straight-chain molecules), are first included in crystals and precipitates as hexagonal crystals, while the PUFAs remain in solution.

Molina and others (1996) developed a process for purifying PUFAs from various sources, the marine *microalga I galbana* and the *diatom P. tricornutum,* using urea fractionation and reverse phase chromatography techniques (Figure 1.3). This process produced a fraction containing a highly pure form of eicosapentaenoic acid with a high yield from *P. tricornutum* biomass. But a large quantity of organic solvent was required and the cost was high ($188/g).

1.5.2 Low Temperature Fractional Crystallization

Low temperature fractional crystallization is also known as winterization. This technique is based on melting points of the fatty acids. The more the double bonds the lower the melting points. Therefore, the PUFAs can be separated from saturated fatty acids by controlling the temperature. Low temperature fractional crystallization is usually carried out in organic solvents,

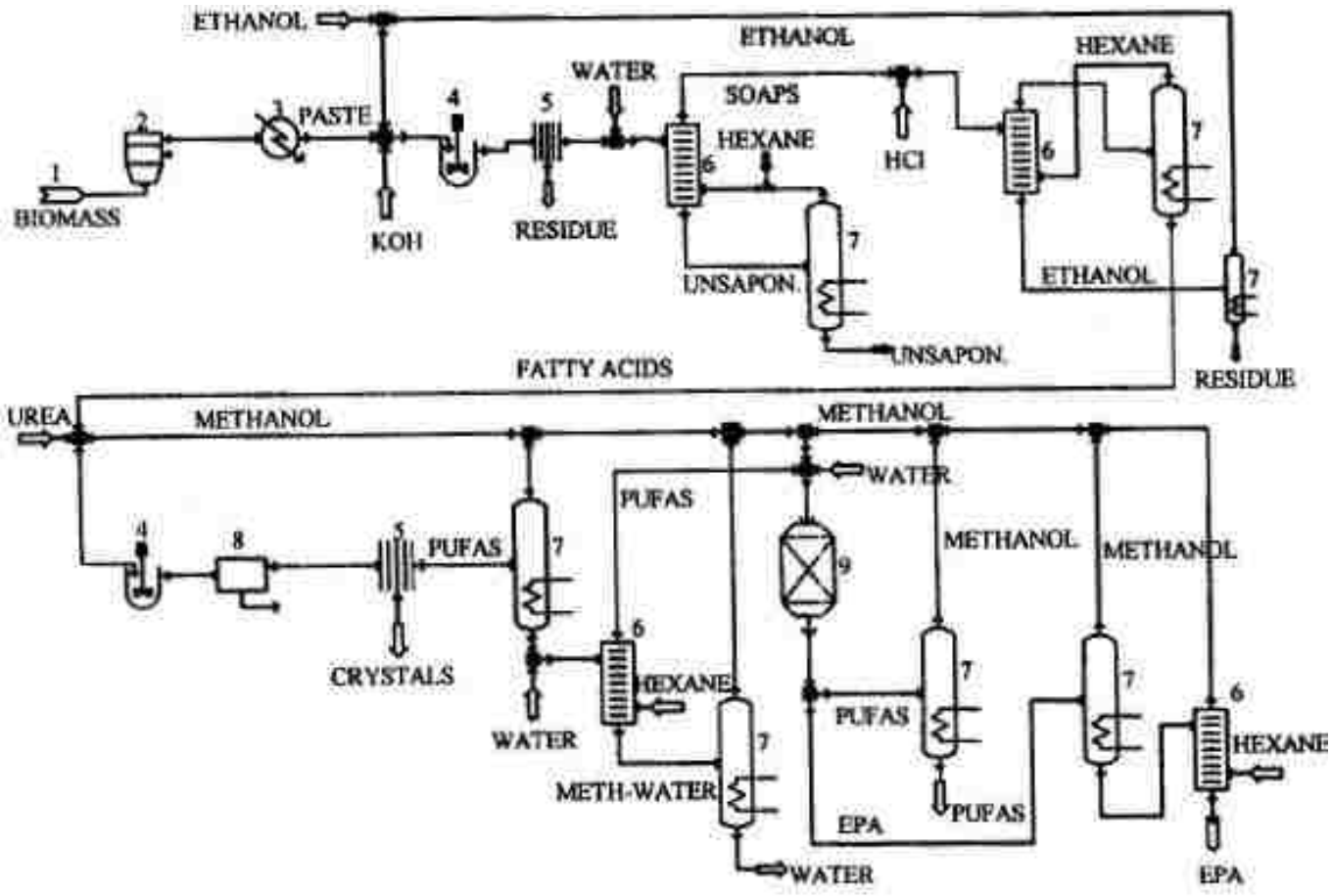


Figure 1.3 - Indicative flowchart of EPA purification from wet microalga biomass.
(1) culture from photobioreactor; (2) centrifugation; (3) freezer; (4) stirred tank under N2 atmosphere; (5) cake filtration; (6) solvent extraction; (7) vacuum distillation; (8) crystallizer; (9) HPLC preparative column.

such as acetone and different hydrocarbons. The solubility of fatty acids in solvents increases with greater unsaturation and decreased with chain length, which is opposite for melting points (Scholfield 1979). Arachidonic acid and its esters are liquid below -80ºC, so they always appear in the final filtrates of any solvent fractionation process. Arachidonic methyl acid ester with concentrations of up to 95% was obtained by a series of temperature fractionations (Shinowara and Brown 1940).

1.5.3 Salt Solubility Methods

As with low temperature fractional crystallization, the salt solubility method is also one of the oldest methods concentrating PUFAs. The solubility of fatty acid metal salts in organic solvents varies depending on the nature of the metal ion, the chain length, double bond number, and temperature. Lithium soaps in acetone and alcohol is mostly used because the lithium salts of polyenoic fatty acids are soluble in 95% acetone while less unsaturated acids are relatively insoluble. Thus PUFAs may be separated from saturated and mono-unsaturated fatty acids (Marldey 1964). The method is not considered a good one because of the safety concern of the lithium and utilization of acetone.

1.5.4 Silica Gel Thin-layer Chromatography

The thin-layer chromatographic method is a simple, reliable and inexpensive way to concentrate PUFAs (Shantha and Ackrnan 1991). Thin-layer chromatography (TLC) is performed on a glass, plastic, or metal plate coated with the stationary phase and requires only small quantities of samples. The separation is achieved because different compounds ascend the TLC plate at different rates in the mobile phase. The silica gel is commonly used for lipid separations. Shantha and Ackman (1991) concentrated tetraene, pentaene and hexaene methyl esters from several natural sources to more than 85% with TLC plates. However, TLC is more an analytical method than a preparative method.

1.5.5 Preparative-scale Gas Chromatography

Analytical gas-liquid chromatography provides excellent separation of polyunsaturated fatty acid esters but is difficult to scale-up. Hardy and Keay (1967) developed a procedure for the continuous preparation of highly purified methyl esters of octadecatetraenoic acid (C18:4), eicosapentaenoic acid (C 20: 5) and docosahexaenoic acid (C 22:6) from cod liver oil using a 1.52 m × 6.35 mm column. Four steps were involved: cod liver oil saponification, PUFA

concentration with the urea method, preparative gas chromatography, and purification of PUFA fractions by thin layer chromatography on silicic acid plates.

1.5.6 Supercritical Fluid Technology

Supercritical fluids are unique because they have liquid-like densities and at the same time values of transport properties (e.g., viscosity and diffusion coefficient) intermediate between liquids and gases (McHugh 1986). Therefore they can be attractive media for extractions. The solubility of a solute in a supercritical fluid has been shown to be directly related to fluid density (concentration) (Chrastil 1982). For example, the solubility of a substance in a fluid can increase dramatically with pressure. This is more obvious near the critical point where the fluid is highly compressible and even a small increase in pressure can induce a large increase in fluid density. Supercritical fluids exhibited retrograde and nonretrograde behaviors depending on the pressures used (Brule and Corbett 1984). At pressures near the critical point, a moderate temperature increase can cause a large decrease in fluid density resulting in a decrease in solute solubility. This is known as retrograde behavior. At much higher pressures, the fluid becomes less compressible and an increase in temperature induces a much less dramatic decrease in density. Thus, at higher pressures, an increase in temperature can cause an increase in solubility, which is called nonretrograde behavior. Concentration of PUFAs from fish oil is not as effective as from its methyl or ethyl esters. It was indicated that at the pressures below 250atm the esters exhibit retrograde behavior (Nilsson and others 1988).

For food applications CO_2 is the solvent of choice because it is nontoxic, non-flammable, readily available and relatively inexpensive. It's more suitable for the fish oil process because moderate temperatures (critical temperature and pressure are 31ºC and 7.3 MPa) are required and in a non-oxygen environment, which limits autoxidation, decomposition and/or polymerization of the PUFAs in fish oil. Eisenbach (1984) reported a process of CO_2 supercritical fluid

fractionation of fatty acid ethyl esters in the retrograde region at 150 atm and found that esters were separated primarily by carbon number and very concentrated (90-98%) fractions were obtained. At higher temperatures, supercritical CO_2 becomes less dense and solubility of the esters is decreased because of its retrograde property; the shorter chain components become more soluble than the longer chain ones, therefore the separation between these two are achieved.

1.5.7 Lipase-catalyzed Hydrolysis Enrichment

Most of the methods described above produce PUFA concentrate in the form of free fatty acids or their corresponding alkyl esters. There's a strict limit for the content of FFA in fish oils for human consumption. It has been reported that methyl and ethyl esters of unsaturated fatty acids hydrolyze at a slower rate than their corresponding acylglycerols (Yang and others 1989). The acylglycerol form of PUFA is considered to be nutritionally more favorable than methyl or ethyl esters of fatty acids. Several studies revealed the fact that alkyl esters of n-3 fatty acids can impair intestinal absorption in laboratory animals (El-Boustani and others 1982; Hamazaki and others 1987; Lawson L.D. and Hughes 1988). From a marketing point of view, acylglycerols (mono-, di-, and triacylglycerols) are often promoted as being more "natural" than free fatty acids and their methyl or ethyl esters (Haraldsson and Hoskuldsson 1989).

Much attention has been given to microbial lipases to produce n-3 PUFA concentrates in the form of acylglycerols by hydrolysis of marine oils (Tanaka and others 1992; Hoshino and others 1990; Shimada and others 1994; Yadwad and others 1991; Maehr and others 1994).). Lipases are one of the most important hydrolytic enzymes that can specifically hydrolyze carboxyl esters of triglycerides into free fatty acids and partial acylglycerols. The advantages of lipase hydrolysis compared with chemical methods are saving energy and avoiding the formation of undesirable oxidation products, polymers, and isomeric conversion of natural cis-PUFAs to deleterious trans-PUFAs. Another unique characteristic that lipases offer is selectively (i.e.,

substrate, positional, and sterospecificity) concentrating on targeted fatty acids in triglycerides (Jaeger and Reetz 1998). Breivik and others (1997) have reported that lipase-catalyzed enzymatic production of EPA and DHA concentrate from fish oil had shown potential in producing a high quality product because of the mild conditions of the process. The reason that EPA and DHA can be concentrated by the lipase-assisted hydrolysis is because the 5 or 6 double bonds in EPA and DHA cause the molecules to be bent, not linear, so that they lie close to the ester bond and the lipase is less likely to hydrolyze the EPA and DHA ester bond (Bottino and others 1967).

The rate of enzymatic reactions could be affected by several factors, including: (i) the nature of enzyme (activity, position- and substrate-specificity, etc.); (ii) the concentrations and ratios of reactants; (iii) the composition of oils or fatty acid mixtures; (iv) whether an organic solvent is used and its nature; (v) the water content and the water elimination system; (vi) temperature and pH; (vii) method of agitation; and (viii) the type of reactor (Robles and Molina 1998).

Wanasundara and Shahidi (1998) systematically described the hydrolysis and separation methods to produce PUFAs from marine oils using eight different microbial lipases. The flow diagram is shown as in Figure 1.4. It consists of five steps: hydrolysis of triglycerides, inactivation of lipase, neutralization of free fatty acids, separation of n-3 PUFAs with hexane, and evaporation of hexane.

Many researchers adopted the same or similar processes to evaluate the effects of different lipases to enrich PUFAs from various sources of marine oils (Sun and others 2002; Okada and Morrisey 2007). Table 1.1 summarized the properties of some of the lipases that have been used and their effectiveness on different oils.

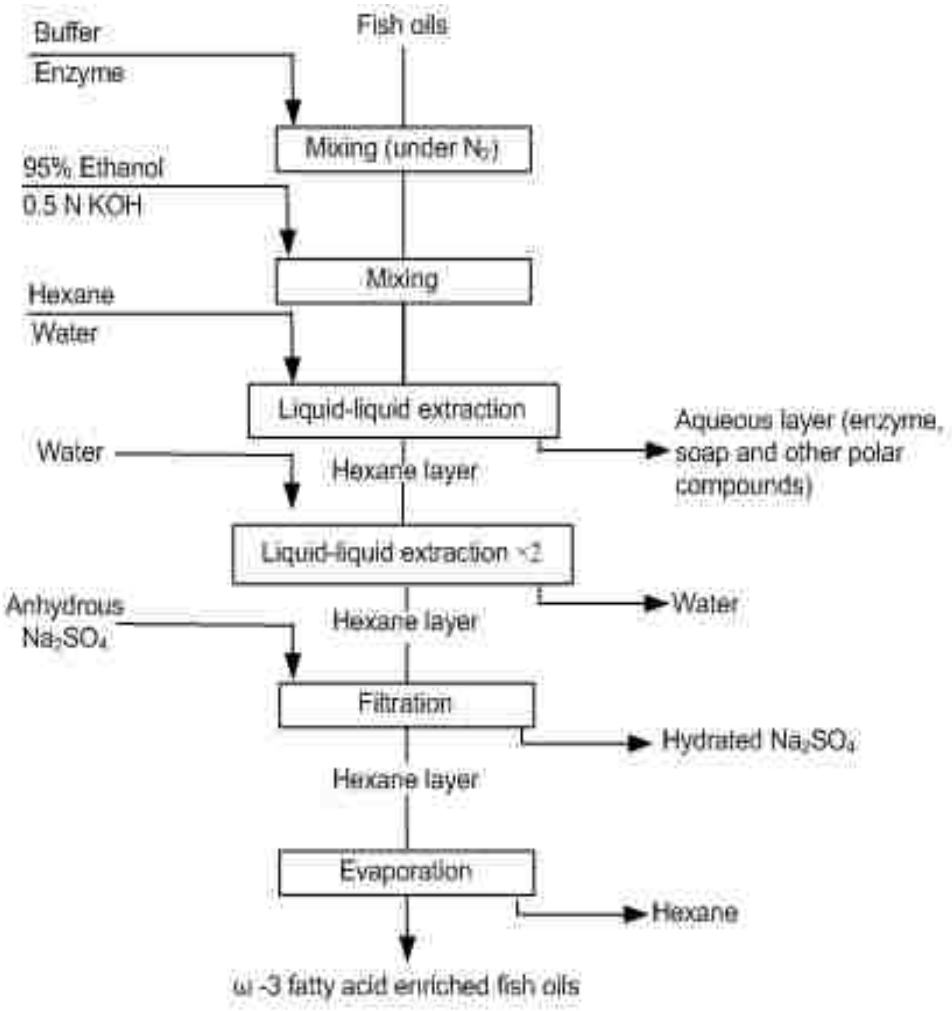


Figure 1.4 – Enzymatic hydrolysis of marine oils and separation of acylglycerols and free fatty acids (Wanasundara and Shahidi 1998).

Table 1.1 Effectiveness of lipase on EPA and DHA enrichment of marine oils

Lipase sources	Literature author (year)	Oil	Effectiveness	Lipase properties
Aspegillus niger	Sun and others (2002)	Atlanta salmon oil	Ineffective in increasing EPA and DHA	Origin: Fungal Optimal temperature and pH: 30-40°C, 6.5 Positional specificity: 1, 3-specific
	Wanasundara and Shahidi (1998)	Seal blubber oil and menhaden oil	Ineffective in hydrolyzing saturated fatty acids	
	Okada and Morrissey (2007)	Sardine oil	No increase in EPA and minor increase in DHA	
Pseudomonas fluorescens	Sun and others (2002)	Atlanta salmon oil	Ineffective in concentrating EPA and DHA	Origin: Bacterial Optimal temperature and pH 45-55°C; 8.0 Positional specificity: none-specific
Candida rugosa	Sun and others (2002)	Atlanta salmon oil	Increased 42% EPA at 12h, increased 72% DHA at 12h	Origin: Yeast Optimal temperature and pH: 30-50°C; 7.0 Positional specificity: none-specific
	Okada and Morrissey (2007)	Sardine oil	Increased EPA from 26.87% to 33.74%, DHA from 13.62% to 29.94%.	
Rhizopus oryzae	Sun and others (2002)	Atlanta salmon oil	Ineffective in concentrating EPA and DHA	Origin: Fungal Optimal temperature and pH: 30-45°C, 7.0 Positional specificity: 1, 3-specific
	Wanasundara and Shahidi (1998)	Seal blubber oil and menhaden oil	Increased DHA, decreased EPA	
Mucor javanicus	Sun and others (2002)	Atlanta salmon oil	Ineffective in concentrating EPA and DHA	Origin: Fungal Optimal temperature and pH: 30-45°C, 7.0 Positional specificity: 1, 3-specific
	Okada and Morrissey (2007)	Sardine oil	No increase in EPA and minor increase in DHA	
Pseudomonas cepacia	Sun and others (2002)	Atlanta salmon oil	Increased 60% EPA at 12h, increased 58% DHA at 12h	Origin: Bacterial Optimal temperature and pH: 30-65°C, 7.0 Positional specificity: none-specific

(table 1.1 continued)

Candida cylindracea	Okada and Morrissey (2007)	Sardine oil	Increased EPA and DHA contents	Optimal temperature and pH: 30-50°C, 6.5 Positional specificity: none-specific
Mucor miehei	Wanasundara and Shahidi (1998)	Seal blubber oil and menhaden oil	Ineffective in hydrolyzing saturated fatty acids in both oils	Optimal temperature and pH: 30-45°C, 6.5-7.5 Positional specificity: 1, 3-specific
Rhizopus niveus	Wanasundara and Shahidi (1998)	Seal blubber oil and menhaden oil	Increased 25.3% n-3 fatty acids in seal blubber oil, increased 23% n-3 fatty acids in menhaden oil	Optimal temperature and pH: 30-45°C, 5.0-8.0 Positional specificity: 1, 3-specific
Candida cylindracea	Wanasundara and Shahidi (1998)	Seal blubber oil and menhaden oil	Increased 50% EPA and 5 fold of DHA in seal blubber oil; increased 60% EPA and 70% DHA in menhaden oil	Optimal temperature and pH: 30-50°C, 5.0-8.0 Positional specificity: none-specific
Chromobacterium viscosum	Wanasundara and Shahidi (1998)	Seal blubber oil and menhaden oil	Increased 54% EPA and ineffective to DHA in seal blubber oil; increased 50% EPA and ineffective to DHA in menhaden oil	Positional specificity: none-specific
Geotrichum candidum	Wanasundara and Shahidi (1998)	Seal blubber oil and menhaden oil	Increased 46% EPA and 2.5 fold of DHA in seal blubber oil; increased 38% EPA and 50% DHA in menhaden oil	Origin: Fungal Optimal temperature and pH: 30-45°C, 6.0-8.0 Positional specificity: none-specific
Pseudomonas sp.	Wanasundara and Shahidi (1998)	Seal blubber oil and menhaden oil	Increased 64% EPA and ineffective to DHA in seal blubber oil; increased 50% EPA and ineffective to DHA in menhaden oil	Origin: Fungal Optimal temperature and pH: 40-60°C, 5.0-9.0 Positional specificity: none-specific

(table 1.1 continued)

Aspergillus oryzae	Matouba and others (2008)	Salmon Oil	Not very effective (increased 16.8% of EPA+DHA)	Optimal temperature: 37°C Positional pH: 7 Specificity: 1, 3- specific

Defects with this process are that a lot of organic solvents mostly hexane are utilized and many chemicals were added to the oils. All these researches focused on the effectiveness of lipase on the PUFAs enrichment but little information on scale up of the process is available.

1.6 Microencapsulation

1.6.1 Microencapsulation of Fish Oil

Microencapsulation (ME) has been defined as 'the technology of packaging solid, liquid and gaseous materials in small capsules that release their contents at controlled rates over prolonged periods of time'. Such technologies are of significant interest to the pharmaceutical sector (e.g. for drug and vaccine delivery), but also have relevance for the food industry. The industrial production of foods often requires not only the addition of functional ingredients to control flavor, color, texture or preservation properties but also ingredients with potential health benefits are included. Shahidi and Han (1993) summarized the six reasons for applying microencapsulation in the food industry: to reduce the core reactivity with environmental factors; to decrease the transfer rate of the core material to the outside environment; to promote easier handling; to control the release of the core material; to mask the core taste; and finally to dilute the core material when it should be used in only very small amounts.

A microcapsule is a small sphere with a wall around the core materials. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called shell, coating, wall material, or membrane. The microcapsules may range from several millimeters in size (0.2-5000 μm) and have multitudes of shapes, depending on the materials and methods used to prepare them (Balassa and Fanger 1971).

Fish oil provides the main dietary source of long-chain ω-3 polyunsaturated fatty acids such as eicosopentaenoic acid (EPA) and decosohexaenoic acid (DHA), which are prone to oxidative degradation. This disadvantage has presented obstacles to the widespread use of fish oils in the food industry. When we attempt to add fish oils to foodstuffs the drawbacks limit their use to low-level addition and require special processing and storage methods to introduce fortified foods. Microencapsulation is an effective method for oxidative stabilization of edible oils. In these systems, stabilization occurs because the wall materials act as a physical and a permeability barrier for molecular oxygen diffusion (Matsuno and Adachi 1993). Recently many studies on microencapsulation of fish oils have been reported, most of which used polysaccharide or starch as wall materials (Gharsallaoui and others 2007).

1.6.2 Protein/Peptides as Wall Materials

Proteins have been widely used for excellent functional properties, which allow them to be a good coating material for the microencapsulation by spray-drying. In addition, proteins have high binding properties for flavor compounds (Gharsallaoui and others 2007). Not only are the functional properties, proteins were also reported for nutritional value. Proteins are indispensable nutrients for the structure and function of all living organisms. Inadequate intake of protein results in a reduction or cessation of growth and a loss of weight (Wilson 2002). Appropriate intake of protein can be used to synthesize new tissues and generate energy (Shiau 2002). The mechanism by which proteins fuel the muscle is that the branched chain amino acids (BCAAs, including leucine, isoleucine, and valine) in proteins are oxidized by muscle during exercise to provide energy once short-term glycogen stores are expended (Kasperek and Snider 1987; Wagenmakers 1998).

Proteins are also known for their benefits in preventing some diseases, mainly obesity and cardiovascular disease, which are two of today's health concerns. Obesity is a global public health problem that reached alarming proportions. In the U.S., the percent of adults over 20 years of age with BMI values of 25 kg/m^2 or higher (overweight and obese) increased from 64.5 to 66.3 according to data obtained in 1999-2000 and 2003-2004 (Ford and Mokdad 2008). It is estimated that 3.8 million men and 3.4 million women die every year from cardiovascular diseases (CVD), which have become the main cause of death worldwide (WHO, 2004).

The most commonly used proteins in food industry are egg protein, whey protein, soy protein, and collagen.

- **Egg protein**

According to the information from the American Egg Board, eggs have been traditionally used as the standard of comparison for measuring protein quality because of their essential amino acid profile and high digestibility. The most abundant amino acid in egg protein is one of the essential amino acids leucine. Leucine is reported to be an inhibitor of protein degradation in skeletal muscle (Nakashima and others 2007; Tischler and others 1982) and liver (Meijer and Dubbelhuis 2004). In addition, in vitro and in vivo experiments both indicated that leucine can stimulate muscle protein synthesis (Suryawan and others 2008; Tischler and others 1982). Layman and Rodriguez (2009) observed that leucine is a critical element in regulating muscle protein synthesis and may be the key amino acid for optimizing skeletal muscle mass and muscle recovery from both resistance and endurance exercise.

- **Whey protein**

Whey protein is a milk protein ingredient that's been widely used for emulsification and coating properties (Kurt and Kilincceker 2011). In addition, whey protein is one of the highest quality proteins available for commercial use because of the higher concentrations of branched

chain amino acids (BCAA) and essential amino acids compared with other sources of protein (Boza and others 2000). Consumption of whey protein in combination with resistance training exercise can help adults build and maintain valuable muscle mass and manage weight through increasing satiety and maintaining lean body mass (U.S. Dairy Export Council). Calcium, magnesium, zinc, B-vitamins, and certain lipid fractions in whey may help reduce the risk of cardiovascular disease. Whey peptides may be involved in inhibiting platelet aggregation and lowering cholesterol levels (U.S. Dairy Export Council).

- **Soy protein**

Studies on both animals (Lin and others 2004) and humans (Anderson and others 1995) have shown that consumption of soy protein has beneficial impacts on risk factors for cardiovascular disease including lowering liver or blood triglyceride, total and LDL cholesterol levels, increasing HDL cholesterol and the ratio of HDL/LDL cholesterol. In 1999 the U.S. FDA (1999) claimed that soy protein could prevent the risk of coronary heart disease by lowering the cholesterol level, which promoted the production and consumption of soy protein in Western countries. The intake of soy protein can suppress appetite because the peptides released from soy protein can stimulate cholecystokinin (CCK) release (Nishi and others 2001), which in turn contributes to food intake suppression (Reidelberger 1994).

- **Collagen**

Collagen is a protein that's been widely used in food industries to improve the elasticity, consistency, and stability of foods in an empirical way (Santana and others 2011). As a natural polymer, collagen was used as drug delivery system materials (Berthold and others 1998) because of its excellent biocompatibility, compared to that of synthetic polymers. Collagen exhibits superior biocompatibility compared with other natural polymers because collagen is one

of the major components of extracellular matrix and plays important roles in various biological events.

- **Gelatin**

Gelatin is derived from the partial hydrolysis of collagen and contains high levels of hydroxyproline, proline and glycine. It is the most commonly used functional biopolymer in the food industry for food design, texture control, and emulsion stabilization. Another remarkable function of gelatin is its ability to serve as a barrier for sensitive food ingredients against unfavorable chemical and mechanical stresses (Kaushik and Roos 2007).

- **Dairy peptides**

Peptides can be rapidly absorbed by the bloodstream and fuel the muscles. It's widely accepted and produced because of the rapid digestion speed and the health benefits with cardiovascular systems. Ballard and others (2009) conducted a study on the effects of NOP – 47 (Nitric oxide peptide) on vascular function in 20 healthy men and women. The results revealed that ingestion of NOP-47 resulted in a 28% increase in artery dilation and a significant peak forearm blood flow.

- **Consumption of a blend of proteins**

Paul (2009) blended isolated soy protein (ISP), whey protein, and casein and investigated the digestion rates of the blended proteins. He found that these three proteins has a digestion rates in the order of whey protein>ISP>casein, which created a "time-release" effect that could prolong the delivery time of the absorbed amino acids to muscle thus resulting in a faster muscle recovery. In addition, consumption of a mix of proteins may have more benefits for sports performance and provides nutritional advantages over consuming only one type of protein.

The current dietary reference intake for the general population is 0.8 g/kg body weight per d (Otten and Meyers 2006). Individuals experiencing regular exercise training require more

dietary protein than sedentary individuals (Campbell and others 2007). Protein intake necessary to support N balance in strength athletes ranges from 1.2 to 1.7 g/kg body weight per day (Campbell and others 2007; Tipton and Witard 2007; Rodriguez and others 2009).

1.7 Objectives

1) To characterize the melting point, enthalpy, and rheological properties of unpurified menhaden oil and red salmon oil, and to determine the effects of temperature on the viscosity and oxidation rates of the unpurified menhaden oil and red salmon oil

2) To determine the performance of chitosan, activated earth or activated alumina as an adsorbent and the combination of the 3 adsorbents to remove FFA and peroxides from the unpurified menhaden and salmon oils. To characterize the fish oils during the purification process on minerals, color, moisture content, insoluble impurities, water activity, and thermal properties.

3) To purify the menhaden and salmon oil using adsorption columns packed with chitosan, activated alumina and activated earth and to determine the adsorption kinetics for removal of FFA and peroxides from the unpurified menhaden oil and red salmon oil by the adsorption columns.

4) To develop fish oils containing enriched EPA and DHA by an enzymatic process with no organic solvent and chemicals involved

5) To develop a protein powder containing fish oil with enriched EPA and DHA targeting on people seeking products having benefits on the cardiovascular system and/or managing weight by exercise.

CHAPTER 2 PHYSICAL PROPERTIES AND OXIDATION RATES OF UNREFINED FISH OILS

2.1 Introduction

Knowledge of physical and chemical properties of fish oil is essential for the design of a proper refining process, the analysis of production cost, and final quality evaluation. Impurities including moisture, dust, protein degradation products, free fatty acids, phosphatides, oxidation products, pigments and trace elements (e.g. copper, iron, sulfur, and halogens), polysaccharides and chlorinated pesticide residues, either inherent in the raw material or resulting from processing and from storage of the crude oil may decrease product quality (Young and others 1994). Refining is required to remove these impurities from the crude fish oil (Wiedermann 1981). However, removing impurities may cause oxidation and oil loss and alter the thermal and rheological properties of the fish oils (Sathivel and others 2003). Lipid oxidation is one of the limiting factors of fish oil acceptability, especially fish oil that contains high amounts of polyunsaturated fatty acids. Understanding rheological properties is essential in controlling fluid transfer, velocity, and energy requirements for pumping and heating fish oil and it can provide a basis for better control over fluid behavior during the process of fish oil refining (Huang and Sathivel 2008).

Menhaden oil is rich in eicosapentaenoic (12.8 - 15.4%) and docosahexaenoic (6.9 - 9.1%) acids; the primary market for the oil is in aquaculture feeds (IFFO.net). In 2005, the estimated Gulf of Mexico menhaden oil production was 46,528 metric tons. Currently, menhaden oils are commercially produced by a wet rendering method. The oil extracted by this process may be suitable for human consumption if the oil is purified of impurities such as proteins, free fatty acids, and oxidized components. In 2006, 99,000 metric tons of salmon byproducts including heads, skins, frames, and viscera were generated from 331,798 metric tons of salmon harvested

in Alaska (Fiorillo 2005). The byproducts which could be converted into valuable products such as salmon hydrolysate and salmon oil usually end up in landfills or rendering plants (Wright 2004). Hydrolysate is typically produced by maceration of byproducts containing less fat fish byproducts followed by enzymatic digestion of protein and removal of bones (Kristinsson and Rasco 2000). Huang and Sathivel (2008) separated the byproducts of red salmon processing into solid and liquid portions by mechanically pressing, thereby producing fish meal and fish. Salmon oil can be used as a feedstock for biodiesel production via a transesterification process (El-Mashad and others 2008).

A large number of reports are available on the mechanism of lipid oxidation (Labuza 1971; Karel 1992; Hamilton and others 1997) and the influence of temperature during storage on the quality of the stored oil (Lopez-Duarte and Vidal-Quintanar 2009; Romen-Nadal and others 2007). However, information on the effects of temperature on oxidation rate and changes in viscosity of menhaden and salmon oils is needed to obtain reliable predictive models for assessing the quality and predicting the purification process of fish oils.

The objectives of the present study were: 1) to evaluate the thermal stability, melting point, enthalpy, and rheological properties of menhaden and salmon oils, and 2) to determine the effects of temperature on the viscosities and oxidation rates of menhaden and salmon oils.

2.2 Materials and Methods

2.2.1 Peroxide Value and Free Fatty Acids in Unrefined Fish Oils

Unrefined menhaden fish oil extracted using a rendering process was obtained from a commercial source (Aylesworth's Fish&Bait, Inc, St. Petersburg, FL). Unrefined salmon oil was produced from salmon processing byproducts including viscera, heads, skins, frame, and discarded fish obtained from a large commercial plant in Alaska. They were characterized for peroxide value (PV) and free fatty acids (FFA). PV of the unrefined fish oils was determined by

a titration method according to AOAC 965.33 (1999). The results were expressed in terms of milliequivalent of peroxides per Kg of oil (meq/Kg). FFA content of the unrefined oil was also determined using a titration method (AOCS Ca 5a-40, 1998) and the percentage of FFA was expressed as oleic acid equivalents.

2.2.2 Density, Specific Gravity, Water Activity, and Moisture Content of Unrefined Fish Oils

Bulk density of the unrefined oils was determined in triplicate using a 25 mL glass-measuring cylinder at 25°C. The sample was filled to 25 mL, the weight to volume ratio determined, and bulk density values reported as g per mL. Specific gravity of the unrefined fish oils was determined in triplicate using a 25 mL glass-measuring cylinder. The net weight of the oil (g) was divided by the net weight of water (g) at 25°C to obtain the specific gravity. A calibrated Rotronic water activity meter (AwQUICK, Rotronic Instrument Corp., Huntington, New York, USA) was used to measure the water activity of the unrefined oils at 25^{o}C. The moisture content was measured according to the Karl Fischer titration method using a Mitsubishi Karl Fischer Moisturemeter (Mitsubishi Chemical Analytech Co., Ltd., Japan).

2.2.3 Thermal Properties of Unrefined Fish Oils

The differential scanning calorimetric experiments were conducted using a Differential Scanning Calorimeter (Model DSC Q20/ RCS 90, TA Instruments, New Castle, DE). Approximately 0.5 mg of the unrefined oil sample was placed in the aluminum sample vessel. The sample vessel was then placed on the sample platform while an empty aluminum vessel was placed on the reference platform. To determine the phase transition of the unrefined oil sample, a linear heating rate of 5°C/min over a temperature range of -90 to 100°C was used. The data collected was plotted and the thermogram peak points were used to determine the melting points.

A baseline was drawn through the beginning to the end of the endotherm peak. The area encircled by the peak and the baseline was integrated for estimating the enthalpy (ΔH).

2.2.4 Rheological Properties of Unrefined Fish Oils

Rheological properties of the unrefined oil samples were measured in triplicate using an AR 2000 Rheometer (TA Instruments, New Castle, DE) fitted with a plate geometry (acrylic plates with a 40-mm diameter, having a 400 μm gap between the two plates). Each sample was placed in the temperature-controlled parallel plate and allowed to equilibrate to 5, 10, 15, 20, 25, and 30°C. The shear stress was measured at 5, 10, 15, 20, 25, and 30°C at varying shear rates from 10 to 200 s^{-1}. The mean values of triplicate samples were reported.

The power law (Eq. 2.1) was used to analyze the flow behavior index of the unrefined oil sample.

$$\sigma = K\gamma^n \qquad (2.1)$$

Where σ = shear stress (Pa.s), γ = shear rate (s^{-1}), K = consistency index ($Pa.s^n$), and n = flow behavior index. The logarithms were taken on both sides of Eq. 1, and a plot of log σ versus log γ was constructed. The resulting straight line yielded the magnitude of log K (i.e., intercept) and n (i.e., slope).

The effect of temperature on apparent viscosity was described through the Arrhenius relationship as described in Eq. 2.2 (Rao 1999).

$$k = Ae^{(-E_a / RT)} \qquad (2.2)$$

where k is the reaction rate constant, A is the frequency factor, Ea is the activation energy (kJ/ mol), R is the gas constant (8.314 J $mol^{-1}K^{-1}$), and T is the temperature (K).

Apparent viscosity of the unrefined menhaden oil sample was measured at 5, 10, 15, 20, 25, and 30°C at a shear rate of 200 s^{-1} using the AR 2000 Rheometer. A plot of Ln (apparent viscosity) versus 1/T (i.e., 1/absolute temperature) was constructed for each menhaden oil sample. The slope of the straight line, the intercept and the regression coefficient were calculated using the trend line of the plot. The magnitude of Ea was calculated as the slope of the plot multiplied by the gas constant, and A was an exponential of the intercept.

2.2.5 Thermal Effect on the Lipid Oxidation and Free Fatty Acids of Unrefined Fish Oils

For lipid oxidation rate study, 50 mL of oil samples were placed in amber bottles and were separately heated in a water bath at 35, 45, 55, 65, 75, and 85°C as described by Huang and Sathivel (2008). Another amber bottle containing oil was held at room temperature, ca. 25°C ± 1°C. Samples were drawn every hour for PV (primary lipid oxidation) and FFA analysis for up to 6 h. The effects of temperature on oxidation rates were described through the Arrhenius relationship as shown in Eq. 2.

For lipid oxidation at different storage temperatures study, 500 ml of oil samples placed in glass containers were stored at 4^{o}C and 50^{o}C in Hotpack environmental chambers (Hotpack Inc., Philadelphia, PA). Another glass container containing fish oil was held at room temperature. The PV and FFA analyses were conducted for the stored samples from week 0 to week 6. The oil samples were analyzed at week 0 after they reached the storage temperature of 4°C, room temperature, or 50 ^{o}C.

2.2.6 Statistical Analysis

Analysis of Variance (ANOVA) was conducted to evaluate the significance of observed differences among treatment means (SAS version 8.2, SAS Institute Inc., Cary, NC), followed by the post-hoc Tukey's studentized range test (SAS 2002).

2.3 Results and Discussion

2.3.1 Peroxide Value, Free Fatty Acids, Density, Specific Gravity, Water Activity, and Moisture Content of Unrefined Fish Oils

The initial peroxide value (PV) and FFA content of the unrefined menhaden oil was 5.70 meq/Kg and 3.80%, respectively (Table 2.1), while salmon oil had a peroxide value at 5.66 meq/Kg and FFA value at 0.67%, respectively (Table 2.2) . PV is a good indicator of lipid oxidation. Gracey and others (1999) have reported that the oil with PV less than 5 meq/Kg can be considered as fresh oil, while an edible oil with a PV of 7.5 meq/Kg is unacceptable for human consumption (Robards and others 1988; Schnepf and others 1991). The initial FFA of the oil was higher than the acceptable level of FFA in purified fish oil that has been reported to be 0.15% (Young 1986). Higher FFA indicates that the oil will have a higher refining loss when the oil is neutralized.

The menhaden oil bulk density (g/mL) was 0.93 ± 0.02, which was a little higher than values reported by Sathivel (2005) for red salmon oil (0.9 g/mL) and pink salmon oil (0.81 g/mL). The unrefined salmon oil has similar bulk density as reported in red salmon oil. The water activity of unrefined menhaden oil (0.52 ± 0.0) was slightly lower than those values reported by Sathivel (2005) for red salmon oil (0.57) and pink salmon oil (0.53). The salmon oil exhibited much lower water activity (0.37±0.02) than reported by Sathivel (2005) and it could be caused by the oil extraction process and the batch difference. Specific gravities of the unrefined menhaden and salmon oils at room temperature were 0.93 ± 0.00 and 0.91±0.01, respectively. The moisture content in the unrefined menhaden oil was 0.15% ± 0.00% and 988.32 ± 25.66ppm. Moisture in the oil increases the FFA content of the oil by lipid hydrolysis reaction (Bhattacharya and others 2008).

Table 2.1 - Initial peroxide value, free fatty acids, density, specific gravity, and water activity of unrefined menhaden oil

Properties	
PV (meq/Kg)	5.70 ± 0.34
FFA (%)	3.80 ± 0.23
Bulk density (g/mL)	0.93 ± 0.02
Specific gravity[a]	0.93 ± 0.00
Water activity (a_W)	0.52 ± 0.00
Moisture content (%)	0.15 ± 0.00

Values are means ± SD of 3 determinations.
[a]Specific gravity was measured at room temperature.

Table 2.2 - Initial peroxide value, free fatty acids, density, specific gravity, and water activity of unrefined salmon oil

Properties	
PV (meq/Kg)	5.66±0.12
FFA (%)	0.67±0.01
Bulk density (g/mL)	0.91±0.01
Specific gravity[a]	0.91±0.01
Water activity (a_W)	0.37±0.02
Moisture content (ppm)	988.32 ± 25.66

Values are means ± SD of 3 determinations.
[a]Specific gravity was measured at room temperature.

2.3.2 Thermal Properties of Unrefined Fish Oils

The DSC thermograms (Figure 2.1 and 2.2) show the endothermic peaks for the unrefined menhaden and salmon oils. The melting points of the unrefined menhaden and salmon oils ranged from -69.5 to 27.2°C and -78.94 to 12.23°C, which were slightly different to the reported melting point range of -69.6 to -0.36°C, -64.7 to 20.8°C, and -64.5 to 14.9°C for red salmon, pink salmon oils (Sathivel 2005), and pollock oil (Sathivel and others 2008a), respectively. The low melting point of the menhaden and salmon oils was attributed to triacylglycerols which contain unsaturated fatty acids (Tan and Che Man 2002). The first melting peak of menhaden and salmon oils around -36.4°C and -44.71°C may be attributed to the presence of polyunsaturated fatty acids (Sathivel and others 2008a). Oil samples with a higher

degree of unsaturated fatty acids melt at lower temperatures, whereas those with a higher degree of saturated fatty acids melt at higher temperatures. The second and the third peaks of menhaden oil appear around the temperatures of -17.2 and -6.3°C, respectively. This melting point at -6.3°C may be attributed to the presence of linoleic acid (C18:2) and linolenic (C18:3) fatty acids, whose melting points range from -5.0 to -13.0°C (Sathivel and others 2008b). Salmon oil has an endothermic peak at -11.05°C, which could also be caused by linoleic acid (C18:2) and linolenic (C18:3) fatty acids. The peaks melted close to 0^{o}C which might be attributed to the moisture in the sample (Berjak and others 1992; Conner and Bonner 2001). Physical properties of triglycerides are more complex than those of the individual fatty acids because they contain three fatty acid groups. In this study, we did not analyze the triglyceride composition of the oil samples. All three peaks for the unrefined menhaden oil were not sharp; this might be attributed to the presence of impurities, such as phospholipids, ketones, and other materials in the unrefined fish oil (Sathivel 2005). A similar DSC thermogram was reported for unrefined pollock oil by Sathivel and others (2008a) and for unrefined red salmon oil by Huang and Sathivel (2008). Crude fish oil contains impurities such as phospholpids, free fatty acids, aldehydes, ketones, water, and pigments. Those impurities melt uncharacteristically compared to pure fatty acids. Fatty acids have their own melting points; therefore, sharp peaks were not observed for the two oils. Sathivel and others (2008b) reported that the DSC thermograms of purified catfish oil showed sharper and narrower peaks than unrefined catfish oil. The melting points of fish oil were sharper after each purification step that removed impurities from the oil.

Enthalpy of the oil explains whether oil changes from one physical state to another either by absorbing (endothermic) or releasing (exothermic) heat (Zhao and Yalkowsky 1999). It is the amount of thermal energy which must be absorbed or evolved for 1 gram of a substance to

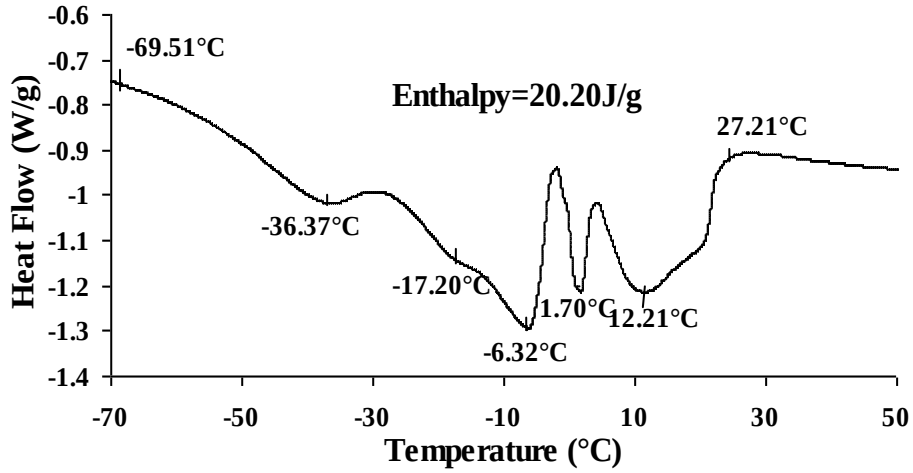


Figure 2.1 - DSC thermogram of unrefined menhaden oil with heat flow.

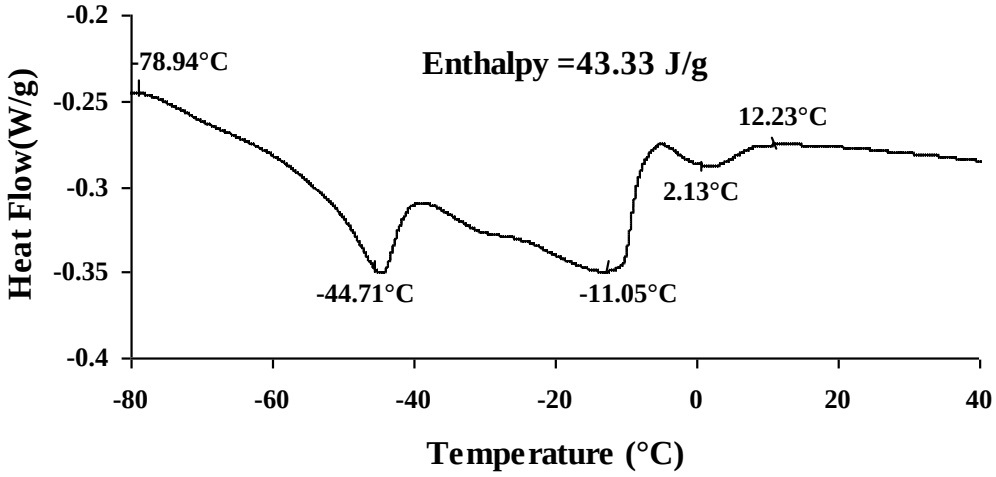


Figure 2.2 - DSC thermogram of unrefined salmon oil with heat flow.

change states from a solid to a liquid, and the temperature at which it occurs is called the melting point. The enthalpy of the menhaden and salmon oil was 20.2 and 43.33 kJ/kg, which are smaller than the reported enthalpy values for catfish oil (73.9 to 84.7 kJ/Kg) by Sathivel and others (2008b). Tan and Che Man (2002) have reported that the oils with higher degrees of saturation required higher enthalpies during melting. The study demonstrated that menhaden and

salmon oil required less enthalpy for melting due to high amount of long chain polyunsaturated fatty acids in the oil.

2.3.3 Rheological Properties of Unrefined Fish Oils

The power law parameters for the unrefined menhaden and salmon oil are given in Table 2.3 and 2.4. The flow behavior index (n) of the unrefined menhaden oil sample ranged from 0.56 to 0.93, which indicated that non-Newtonian fluid behavior was observed at the lower temperatures (from 5°C to 25°C), while menhaden oil had a Newtonian behavior at 30°C. The flow behavior index (n) of the unrefined salmon oil sample ranged from 0.99 to 0.96, which indicated that the unrefined salmon oil had a Newtonian behavior at 5 to 30°C. The consistency index (K) values for the unrefined menhaden and salmon oil were higher at lower temperatures (Table 2.3 and 2.4). More than 40 fold increases in the magnitude of the K values between 5°C and 30°C was observed, which was much higher than that of unrefined salmon oil and pollock oil reported by Sathivel and others (2008a). The presence of impurities in the fish oil might be a reason that the K values of menhaden oil were higher than those reported for unrefined pollock oil. Table 2.2 and Figure 2.2 showed changes in apparent viscosity of the unrefined menhaden oil as a function of temperature. The activated energy (E_a) indicated the energy barrier that must be overcome before the elementary flow process can occur (Rao 1999). The magnitudes of Ea for unrefined menhaden and salmon oil were calculated according to Arrhenius equation (shown in Figure 2.3 and 2.5), which are 50.37 and 31.80 kJ/mol, respectively. The predicted viscosity obtained by the Arrhenius equation agreed with the experimental viscosity (Figure 2.4 and 2.6). The degree of fit, as shown by the R^2 value of 0.9837 (Figure 2.4) and 0.9977, indicated that changes in apparent viscosity with temperature could be modeled by the Arrhenius equation for the unrefined menhaden and salmon oils. This study also showed that the magnitude of the

apparent viscosity of the unrefined menhaden and salmon oil was greatly influenced by temperature.

Table 2.3 - Apparent viscosity at different temperatures of the unrefined menhaden oil

Temperature (°C)	*n*	*K* (Pa.s^n)	Apparent viscosity (Pa.s)
5	0.56 ± 0.02^f	2.17 ± 0.02^a	0.22 ± 0.01^a
10	0.68 ± 0.03^e	0.69 ± 0.02^b	0.13 ± 0.01^b
15	0.79 ± 0.02^d	0.26 ± 0.01^c	0.085 ± 0.00^c
20	0.83 ± 0.01^c	0.17 ± 0.02^d	0.067 ± 0.00^{cd}
25	0.85 ± 0.02^b	0.11 ± 0.02^e	0.050 ± 0.00^{de}
30	0.93 ± 0.01^a	0.047 ± 0.00^f	0.033 ± 0.00^f
Ea (KJ/mol)			50.37 ± 2.10
μ_∞			7.02E-11 ± 5.62E-12

Values are means ± SD of 3 determinations.
[a-g]Means with the same superscript letter in each row are not significantly different (P>0.05).
n = flow behavior index; *K* = consistency index; Ea = activation energy; μ_∞ = the frequency factor.

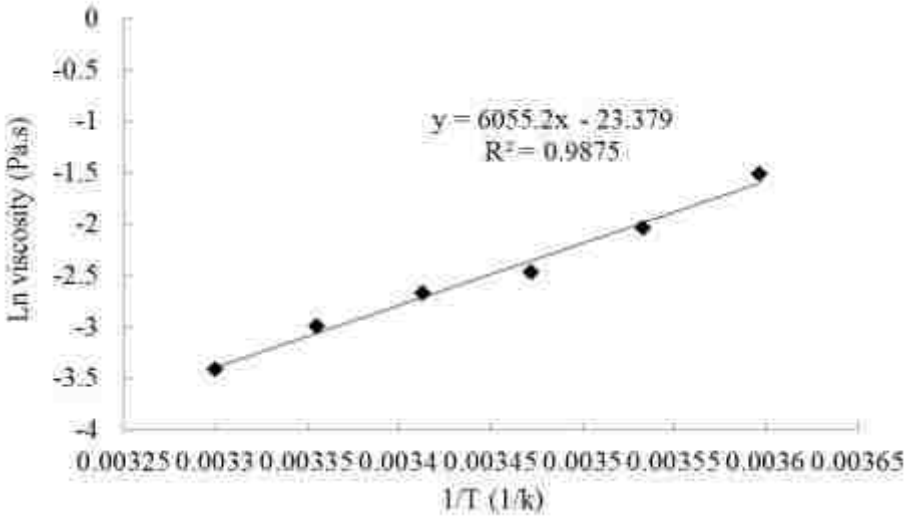


Figure 2.3 - The Arrhenius plot for apparent viscosity of the unrefined menhaden oil.

2.3.4 Lipid Oxidation Kinetics of Unrefined Fish Oils

Peroxide value is a useful indicator of lipid oxidation, especially at the beginning of lipid oxidation (Choe and Min 2005). The lipid oxidation, as indicated by the peroxide values, increased with increasing time and temperature. The unrefined menhaden oil stored at 25^{o}C and

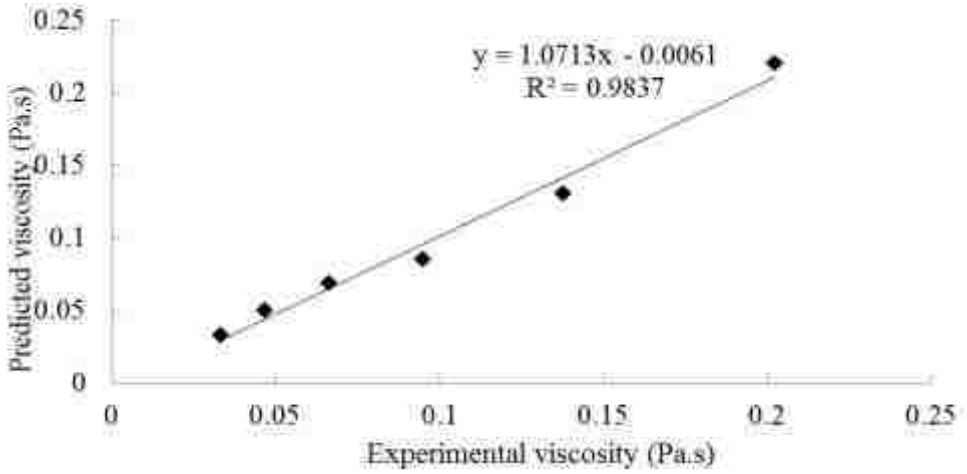


Figure 2.4 - Predicted apparent viscosity from the Arrhenius plot vs the experimental apparent viscosity of the unrefined menhaden oil.

Table 2.4 - Apparent viscosity at different temperatures of the unrefined salmon oil

Temperature (°C)	*n*	*K* (Pa.s^n)	Apparent viscosity (Pa.s)
5	0.99±0.01^a	0.147±0.00^a	0.137±0.00^a
10	0.99±0.01^a	0.107±0.00^b	0.102±0.00^b
15	0.99±0.00^a	0.084±0.00^c	0.079±0.00^c
20	0.98±0.01^a	0.071±0.00^d	0.063±0.00^d
25	0.96±0.01^b	0.061±0.00^e	0.050±0.00^e
30	0.96±0.01^b	0.050±0.00^f	0.041±0.00^f
Ea (KJ/mol)			31.80±1.55
μ_∞			1.09E-07±6.43E-09

Values are means ± SD of 3 determinations.
$^{a-g}$Means with the same superscript letter in each row are not significantly different (P>0.05).
n = flow behavior index; *K* = consistency index; Ea = activation energy; μ_∞ = the frequency factor.

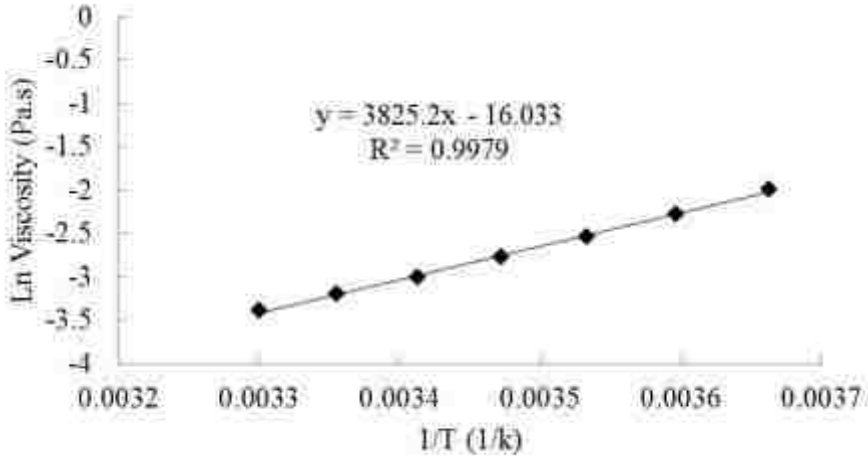


Figure 2.5 - The Arrhenius plot for apparent viscosity of the unrefined salmon oil.

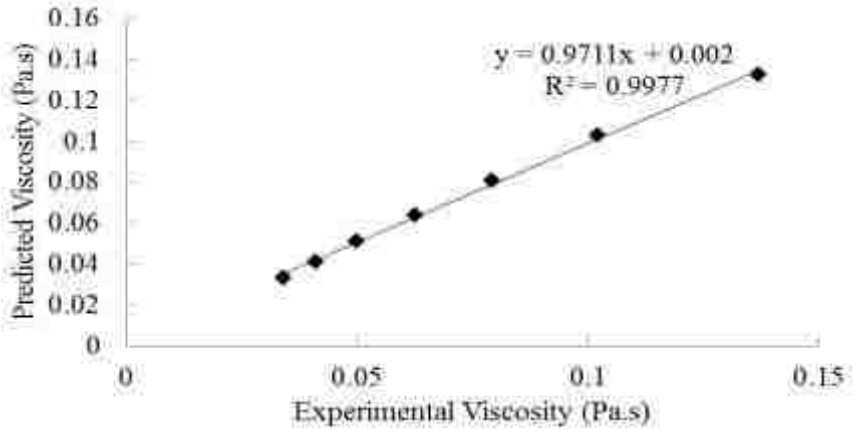


Figure 2.6 - Predicted apparent viscosity from the Arrhenius plot vs the experimental apparent viscosity of the unrefined salmon oil.

35°C for 6 hours exhibited minimal lipid oxidation, whereas those oils at 45-85°C showed greater lipid oxidation after 6 hours (Figure 2.7). Similar results were observed for unrefined salmon oil (Figure 2.10). The oxidation rate of oil sample at 85°C was the highest compared with the oil stored at 25°C, 35°C, 45°C, 55°C, 65°C, and 75°C. The lipid oxidation rates of menhaden and salmon oil can be predicted by the Arrhenius model (Figure 2.8 and 2.11). The activation energy (Ea) for lipid oxidation of the unrefined menhaden and salmon oil was 30.9 and 27.73 kJ/mol, which was lower than the reported E_a value by Huang and Sathivel (2008) for lipid oxidation of the unrefined salmon oil (51.3 kJ/mol). The E_a value for unrefined menhaden and salmon oil was in accordance with the previously reported Ea values for lipid oxidation of edible oils (24 to 240 kJ/mol) (Frankel 1993; Tan and others 2001). This study showed that the magnitude of lipid oxidation for unrefined menhaden and salmon oil was greatly influenced by temperatures. The degree of fit, as shown by the R^2 value of 0.9563 and 0.9957 (Figure 2.9 and 2.12), indicated that the lipid oxidation rate in relation to temperature could be well described by the Arrhenius equation for the unrefined menhaden and salmon oil. In general, the hydrolysis of oil triacylglycerols occurs during heating due to the presence of moisture and lipase enzymes in the oil. The FFA content of both the unrefined menhaden and salmon oil had not significantly

changed with increased temperature in our study, which suggested that the presence of the lipase enzymes in the oil samples might have been inactivated due to the heating.

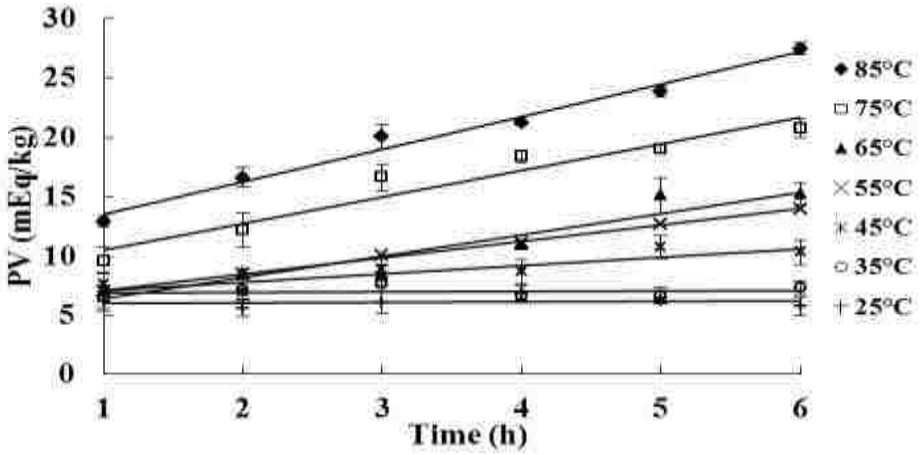


Figure 2.7 - Effect of time on peroxide formation in the unrefined menhaden oil at different temperatures.

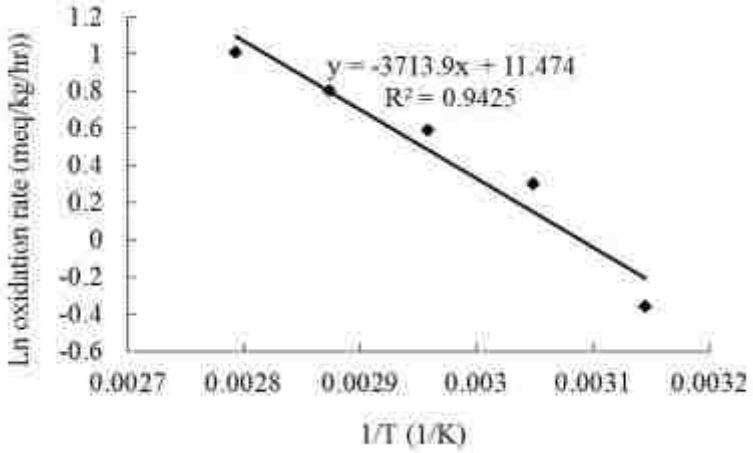


Figure 2.8 - The Arrhenius plot for the peroxide values of unrefined menhaden oil.

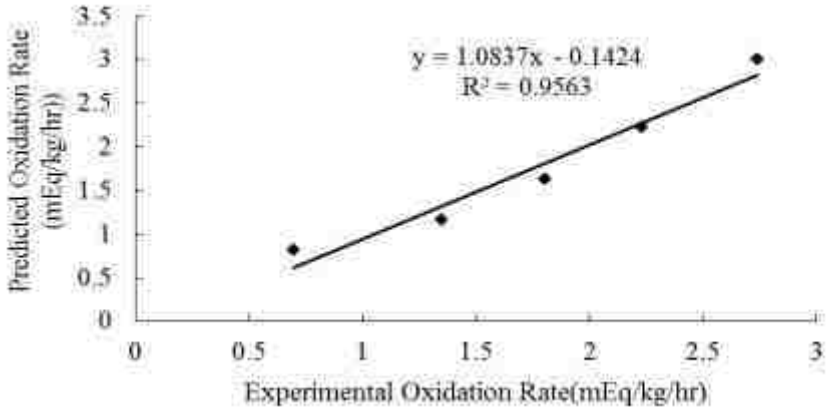


Figure 2.9 - Predicted lipid oxidation rate from the Arrhenius plot vs the experimental oxidation rate of the unrefined menhaden oil.

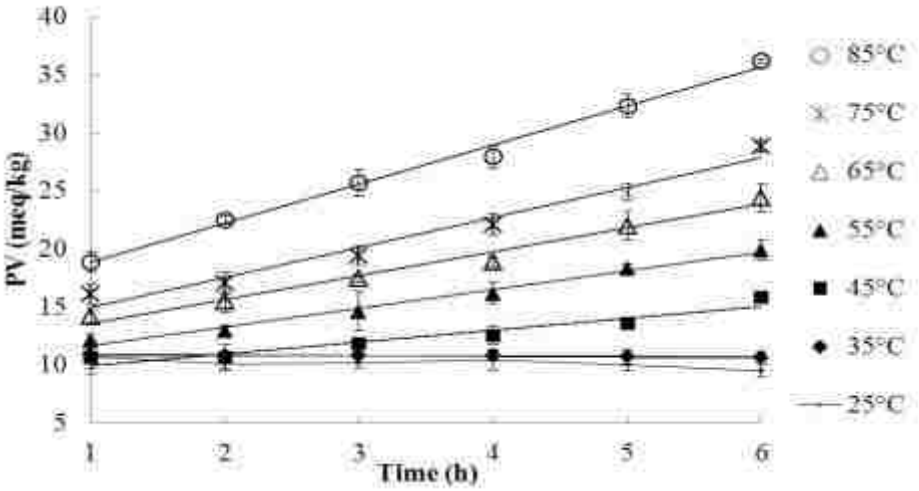


Figure 2.10 - Effect of time on peroxide formation in the unrefined salmon oil at different temperatures.

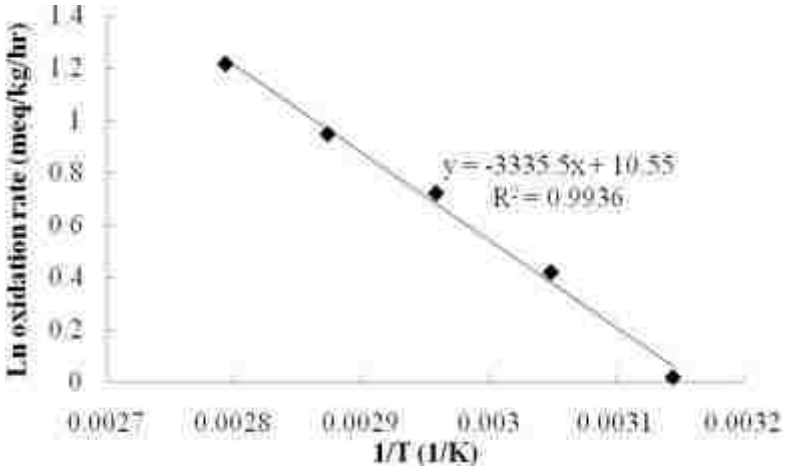


Figure 2.11 - The Arrhenius plot for the peroxide values of unrefined salmon oil.

The menhaden and salmon oil storage study showed an increase in the formation of primary oxidation products in the unrefined menhaden oil with increased storage time at 4°C, room temperature, and 50°C (Figure 2.13 and 2.15). The amount of accumulated hydroperoxide for the oil stored at room temperature was the highest since the first week of storage for the two oil samples. Both of the oils stored at 50°C had a lower rate of hydroperoxide formation than

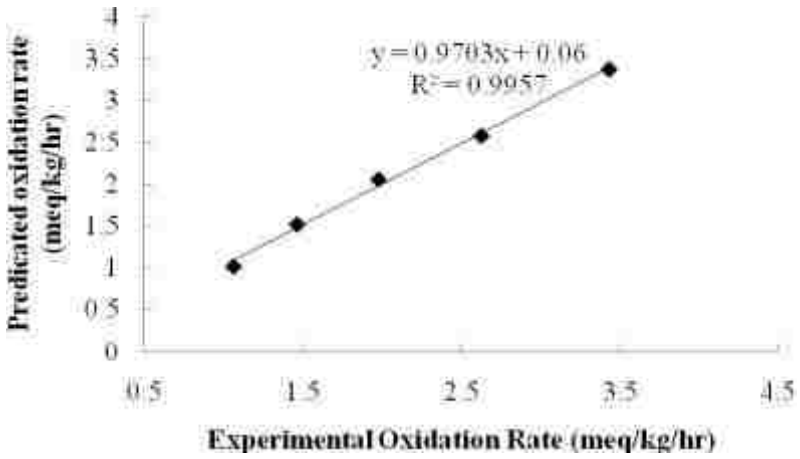


Figure 2.12 - Predicted lipid oxidation rate from the Arrhenius plot vs the experimental oxidation rate of the unrefined salmon oil.

that of the oil stored at room temperature, which might be attributed to decomposition of primary hydroperoxides at an elevated temperature. The rate of decomposition of primary hydroperoxides is higher at the elevated temperature and/or longer storage period; therefore, hydroperoxides might not have accumulated in the oil stored at 50°C compared to the oil at room temperature (Aidos and others 2002). The accumulation of the primary oxidation products in the oil depends on the nature of the oil and the conditions under which the oxidation occurs (Guillen and Goicoechea 2009). This study demonstrated that the rate of oxidation of the unrefined menhaden and salmon oils was influenced by both temperature and storage time. Similar patterns of formation and decomposition of hydroperoxides were reported for herring oil stored at room temperature and elevated temperatures (Aidos and others 2002).

The unrefined menhaden oil samples stored at 4^{o}C, room temperature, and 50^{o}C had similar FFA values at first 3 weeks of storage, while the FFA value of the oil stored at 50^{o}C was higher than that of the oil samples stored at 4^{o}C and room temperature after 6 week of storage (Figure 2.14). The FFA increased from 3.72%±0.04 to 4.82%±0.06 at 50^{o}C, while it increased from 3.61±0.1% to 3.92%±0.07 and from 4.05%±0.9 to 4.27%±0.04 at 4°C and at room

temperature, respectively during 6 weeks of storage. The unrefined salmon oil samples stored at 50°C had higher FFA values from the first week of storage (Figure 2.16). After 6 weeks of storage, the FFA increased from 0.66%±0.00 to 1.51%±0.03 and from 0.66%±0.01 to 0.98%±0.05 at 50°C and at room temperature, respectively, while it decreased from 0.67±0.01% to 0.52%±0.02 at 4°C. The increased FFA content in oil during storage was probably due to the oil hydrolysis that was influenced by the initial moisture content and presence of lipase in the oils.

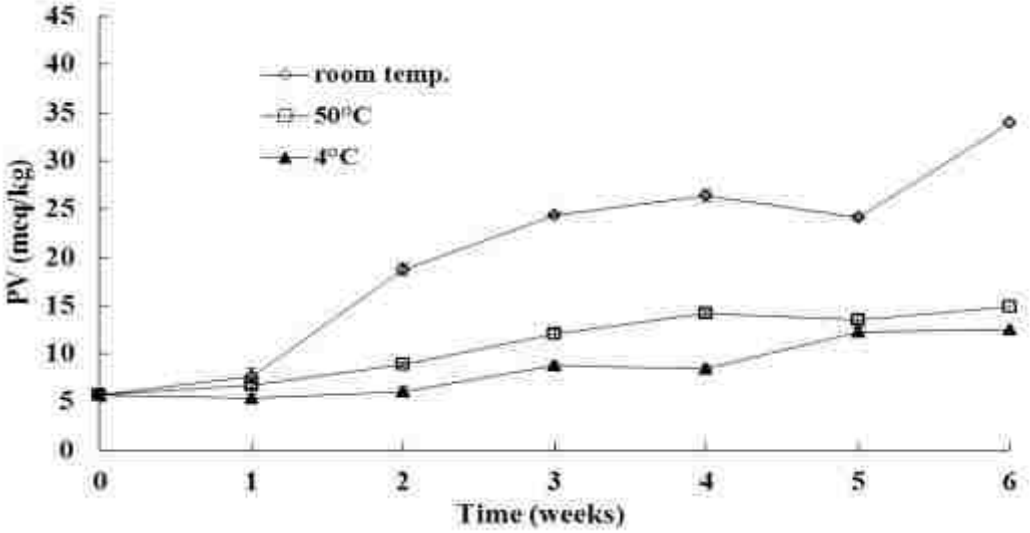


Figure 2.13 - Peroxide values of the unrefined menhaden oil during 6 weeks of storage.

2.4 Conclusions

Non-Newtonian fluid behavior was observed for unrefined menhaden oil at temperatures ranging from 5 to 25°C, while Newtonian behavior was evident at 30°C. Unrefined salmon oil exhibited Newtonian fluid behavior at temperatures ranging from 5 to 30°C. The peroxide values of the unrefined menhaden and salmon oils increased with increasing time and temperature. The 6 week storage study showed that the rate of hydroperoxide formation of the oil stored at 4 °C

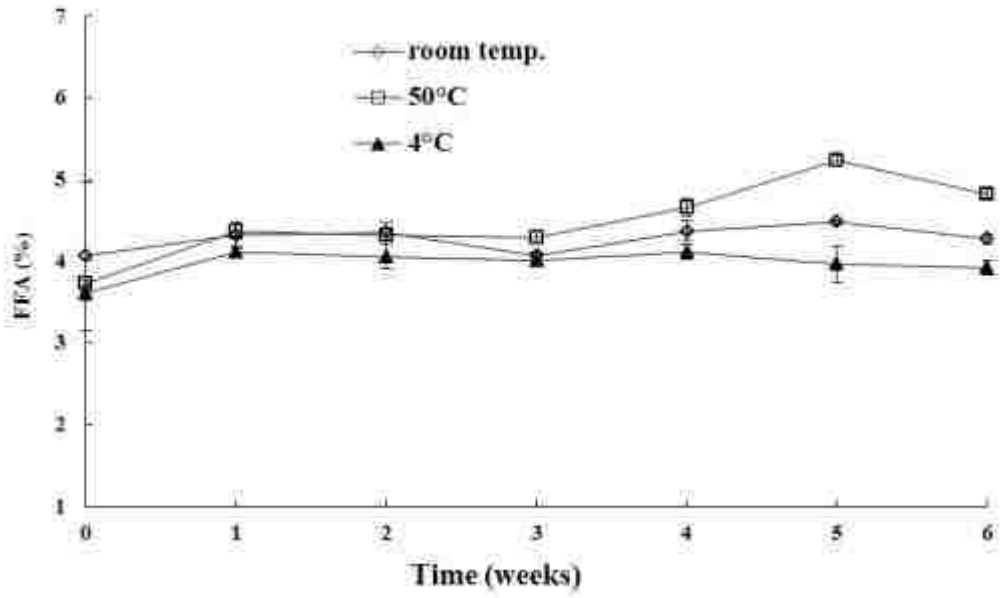


Figure 2.14 - FFA contents of unrefined menhaden oil during 6 weeks of storage.

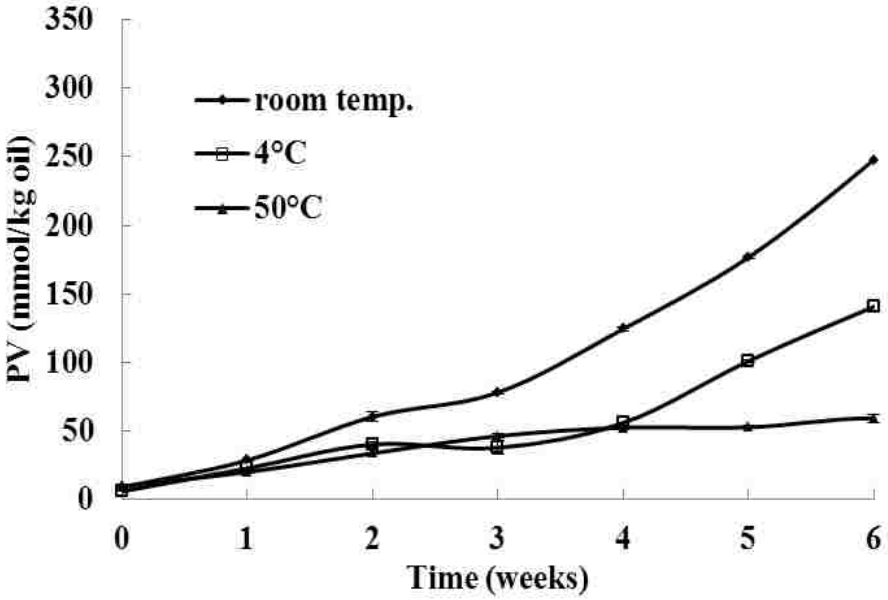


Figure 2.15 - Peroxide values of the unrefined salmon oil during 6 weeks of storage.

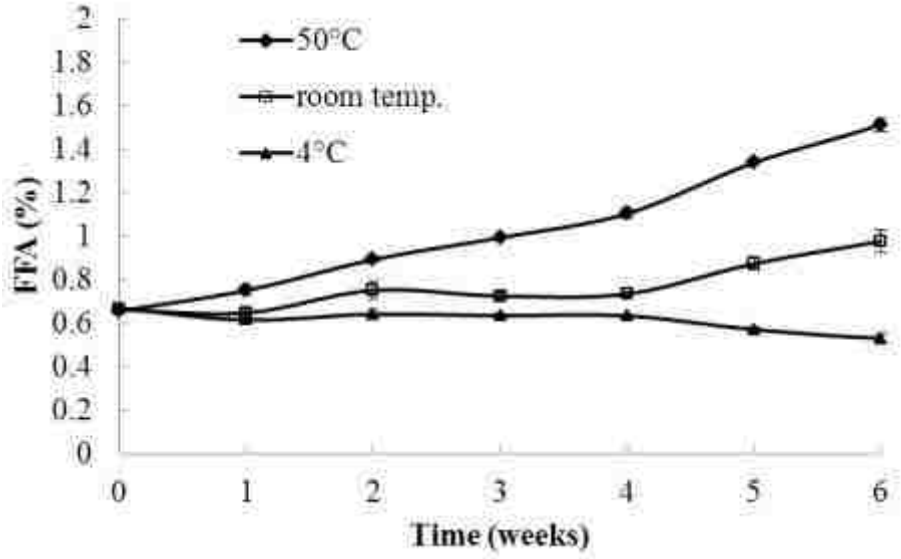


Figure 2.16 - FFA contents of unrefined salmon oil during 6 weeks of storage.

was lower than that of the oil stored at room temperature and at 50°C. The FFA content of the oil increased during storage due to hydrolysis that was influenced by the initial moisture content of the oil. The study demonstrated that changes in the magnitude of apparent viscosity and lipid oxidation of the unrefined menhaden and salmon oil with temperature could be well described by the Arrhenius equation. This study further provides information on the temperature dependent viscosity and lipid oxidation rates of unrefined menhaden and salmon oils, parameters that are useful for optimizing unit operations for oil purification processes and final quality evaluation. As each oil purification step involves different temperature conditions, it is important to predict the apparent viscosity values and lipid oxidation rates of the oils at different temperatures.

CHAPTER 3 PURIFYING FISH OILS WITH BATCH ADSORPTION PROCESSES

3.1 Introduction

The objective of purifying fish oil is to remove the impurities which cause the unattractive appearance or constitute harmful components to human health. Concurrently, some natural components such as health beneficial pigments, omega-3 fatty acids, and tocopherols should be preserved. Conventional oil purification methods include degumming, neutralization, bleaching, and deodorization. Degumming is a process to remove phospholipids from oil with the presence of water or acids (Hui 1996). Neutralization is performed on edible oils to remove free fatty acids and other impurities by adding alkali (usually diluted sodium hydroxide). Alkali refining may cause both physical and chemical changes in the oil. Bleaching refers to the process for removing color compounds, natural pigments (e.g. carotenoids, chlorophyll, xanthophyll and polyphenols) and oxidation products as well as some suspended mucilaginous and colloid-like matter (Chang 1967). Deodorization is a steam distillation process to strip the volatile compounds from the nonvolatile oil (Bimbo and Crowther 1991). The problems with the conventional purification methods are that they are not only labor-intensive, they are also expensive. An easily operated and economical purification method is needed by the edible oil purification industry. An adsorption method could be that method. This technique involves mass transfer of adsorbate from the fluid phase to the adsorbent surface until the thermodynamic equilibrium of the adsorbate concentration is reached. Different kinds of adsorbents have been used in purification of edible oils, such as activated earth, activated carbon, kiselguhr or diatomaceous earth (Bera and others 2004), and metal oxide and metal phosphate adsorbents (Chapman 1994). Activated earth is produced by the activation of bentonite using mineral acids under heating for a few hours and is by far the most common adsorbent for purification and color improvements of fats and oils (Du and others 2006; Lara and Park 2004). Activated alumina is

a porous dry powder made by thermal treatment of aluminum hydroxide comprising a series of non-equilibrium forms of partially hydroxylated aluminum oxide (Al_2O_3). The surface of activated alumina is a complex mixture of aluminum, oxygen, and hydroxyl ions which combine in specific ways to produce both acid and base sites, which are the cause of surface activity and ~~so,~~ are important in adsorption applications (Fleming 1998). Chitosan, the product of deactylation of chitin and composed of N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) residues, is the only cationic polysaccharide in nature. Chitin is widely found in the exoskeleton of crustaceans, the cuticles of insects and the cell walls of fungi. Chitosan has very good adsorption capacity for dyes and metal ions due to the presence of a large number of free amino ($-NH_2$) groups that can serve as the coordination and reaction sites (Huang and others, 2011). Mucha and Balcerzak (2005) reported chitosan as a water adsorbing agent.

In this chapter, activated earth, activated alumina, and chitosan were used to adsorb the impurities from menhaden and salmon fish oils. However, using a single adsorbent was not effective in removing all the impurities because of the diversity of these impurities and the limitation of each adsorbent. Huang and Sathivel (2010) used a combined adsorption (activated earth) and neutralization process to purify salmon oil and it was more effective in reducing FFA, peroxides, and moisture contents than either the adsorption or neutralization process alone. However, the neutralization process could cause a higher oil loss (Appendix 1), therefore, a combined adsorption purification process using three adsorbents (chitosan, activated earth and activated alumina) was developed. The objectives of this study were to purify menhaden and salmon oil using a combined chitosan, activated alumina and activated earth adsorption process and to evaluate the quality of the purified fish oils.

3.2 Material and Methods

3.2.1 Fish Oil Production and Purifying Methods

Unrefined menhaden fish oil (MO) extracted using a rendering process was obtained from a commercial source (Omega Protein Inc., Houston.TX). Unpurified salmon oil (SO) was produced from salmon processing byproducts including viscera, heads, skins, frame, and discarded fish obtained from a large commercial plant in Alaska. The unpurified salmon oil was extracted and stored at -40ºC until used. Shrimp chitosan obtained from Green Pastures Products Inc. (O'Neill, NE) was used in this study. Activated alumina was obtained from Zapp's Potato Chips Inc. (Gramercy, LA) and activated earth was obtained from the BASF Chemicals Division, Geismar, LA.

The batch adsorption study was conducted in glass containers. Thirty grams of MO/SO was placed into each glass container and 1.5 g of an adsorbent (chitosan, activated earth or activated alumina) was added separately. The adsorption reaction was carried out with constant agitation using a magnetic stirrer at 22 ± 1ºC. Experiments were repeated three times. Five different batch adsorption processes were used to purify MO/SO: process #1 involved purification of MO/SO by 5% (wt/wt of oil) chitosan (MCH/ SCH), process #2 involved purification of MO/SO by 5% (wt/wt of oil) activated earth (MAE/SAE), process #3 involved purification of MO/SO by 5% (wt/wt of oil) activated alumina (MAA/SAA), process #4 involved combined MO/SO purification processes of 5% (wt/wt of oil) chitosan, 1.5% chitosan plus 3.5% activated earth, and 5% activated alumina (M4/S4) in 3 steps, and process #5 involved combined MO/SO purification processes of 5% (wt/wt of oil) chitosan, 4% chitosan plus 1% activated earth, and 5% activated alumina (M5/S5) in 3 steps.

3.2.2 Peroxide Value (PV), Free Fatty Acids (FFA), Moisture, and Iodine Value (IV) in Unrefined and Refined Fish Oils

The PV and FFA of the oil samples were measured by the methods described in 2.2.1. The moisture content was measured according to the method in 2.2.2. Iodine value which is a measure of the unsaturation of the oils was measured following the AOCS official method Cd 1-25 (1998). It was expressed in terms of number of centigrams of iodine absorbed per gram of sample (% iodine absorbed). All of the analyses were repeated three times.

3.2.3 Color of the Unrefined and Refined Fish Oils

Color of the unrefined fish oils was determined using the chroma meter LABSCAN XE (Hunterlab, VA, USA). The data of colors were reported in CIELAB color scales (L* value is degree of lightness to darkness, a* value is degree of redness to greenness, and b* value is degree of yellowness to blueness). Chroma and hue angle value were calculated as equation 3.1 and equation 3.2, respectively.

$$\text{Chroma} = [a^{*2} + b^{*2}]^{\frac{1}{2}} \qquad (3.1)$$

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \qquad (3.2)$$

Total color difference (TCD) was calculated from the L*, a* and b* values according to equation 3.3 as described by Topuz and others (2008).

$$\text{TCD} = [(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2]^{\frac{1}{2}} \qquad (3.3)$$

where L is degree of lightness to darkness, L_0 is initial value of L, a is degree of redness to greenness, a_0 is initial value of a, b is degree of yellowness to blueness, and b_0 is initial value of b.

3.2.4 Fatty Acid Profile and Mineral Concentrations in Unrefined and Refined Fish Oils

Fatty acid composition of oil samples was determined at the USDA-ARS Laboratory, University of Alaska Fairbanks, AK. FAMEs were prepared using a modified method of Maxwell and Marmer (1983). A 20 mg sample of fish oil was dissolved in 4.5 mL isooctane and 500 µL of internal standard (10 mg methyl tricosanoate (23:0)/mL isooctane) and 500 µL 2 N

KOH (1.12 g/10mL MeOH) was added to the mixture. The mixture was vortexed for 1 min and centrifuged to the separate upper layer. The separated upper layer was mixed with 1 mL of saturated ammonium acetate solution and the aqueous layer was removed and discarded. The mixture was centrifuged and the upper layer of the mixture was separated. Then 1 mL of distilled water was added to the separated upper layer and centrifuged, then 2-3 g anhydrous sodium sulfate was added, vortexed, and kept for 20-30 min. The mixture was centrifuged and the liquid containing methyl ester was separated. A 0.5 mL aliquot of isooctane containing methyl ester and 0.5 mL of isooctane were added to the amber GC vial. The fatty acid analysis was done with a GC model 7890A (Agilent) fitted with a FAMEWAX™ (30m, 0.32mm x 0.25µm, Restek, Bellefonte, PA) column. Data was collected and analyzed using the GC ChemStation program (ver E.02.00.493 Agilent Technologies, Inc.). Helium was used as the carrier gas at an average velocity of 64cm/sec. Injector and detector temperature were held at 250°C and 280°C, respectively. A split injection (50:1 split ratio) was used and the oven programming was 195°C to 240°C at a rate of 5°C/min and held 2min for a total run time of 11 min. An autosampler performed the GC injection of standards and samples. The injection volume was 1 µL. Samples were identified by comparing retention times to standards. The standards used were: Supelco 37, PUFA #1, PUFA #3, and cod liver oil from Supelco (Bellefonte, PA). Data were expressed as percent of total integrated area.

Mineral content of salmon oil samples was determined according to AOCS Ca17-01 and AOCS Ca 20-99 (1998) and reported as ppm. The mineral profile analysis of the oil samples was carried out in triplicate by the acid digestion method involving microwave technology (CEM microwave, MDS-2000, CEM 3 5 Corp., Matthews, N.C., U.S.A.). A 0.5 g sample was placed in a vessel and 6 mL HNO3 was added. The sealed vessel was heated until digestion was completed. The samples were cooled for 5 min. The inductively coupled argon plasma system (Model

CIROS, SPECTRO Analytical Instruments, Kleve, Germany) was utilized to determine the mineral profile.

3.2.5 Rheological Properties of the Unrefined and Refined Fish Oils

Rheological properties of the oil samples were measured in triplicate using an AR 2000 Rheometer (TA Instruments, New Castle, DE) fitted with a plate geometry (plastic plates with a 40-mm diameter, having a 200 μm gap between the two plates). Each sample was placed in the temperature-controlled parallel plate and allowed to equilibrate to 25^{o}C. The shear stress was measured at varying shear rates from 10 to 200s^{-1}. Apparent viscosities of the oil samples were measured at 25^{o}C at a shear rate of 200 s^{-1} using the AR 2000 Rheometer. The mean values of triplicate samples were reported.

The power law (Eq. 3.4) was used to analyze the flow behavior index of the oil samples.

$$\sigma = K\gamma^{n} \qquad (3.4)$$

where σ = shear stress (Pa.s), γ = shear rate (s^{-1}), K = consistency index (Pa.s^{n}), and n = flow behavior index. The logarithms were taken on both sides of Eq. 1, and a plot of log σ versus log γ was constructed. The resulting straight line yielded the magnitude of log K (i.e., intercept) and n (i.e., slope).

3.2.6 Thermal Properties of Unrefined and Refined Fish Oils

Thermal stability of the oil samples were analyzed using the Thermogravimetric Analyzer (Model Q50, TA Instruments, New Castle, DE). Approximately 1-1.2 mg of the oil sample was added to an aluminum pan, the pan was placed in the furnace, and the exact sample weight was determined. The sample was heated to 700^{o}C under air atmosphere at an increasing rate of 5^{o}C/min. Sample weight differences were automatically recorded every 0.5 sec.

Collected data were analyzed and plotted using the TA Universal Analyzer Software. The graph was normalized based on the sample weight basis.

The differential scanning calorimetric experiments were conducted using a Differential Scanning Calorimeter (Model DSC 2920, TA Instruments, New Castle, DE). Approximately 0.5-1 mg of the unpurified oil samples were placed in aluminum sample vessels. An empty aluminum vessel was placed on the reference platform. To determine the phase transition of the unpurified oil samples, a linear heating rate of 5^{o}C/min over a temperature range of -75 to 100^{o}C was used. The thermogram peak was used to provide an estimate of enthalpy (ΔH).

3.2.7 Statistical Analysis

Means and standard deviations of the data collected were reported. Multiple comparison was performed at the significant level of $P < 0.05$ using SAS version 8.2 (SAS Institute Inc., 2002). Tukey's studentized range tests were performed to detect significant differences among the different treatments.

3.3. Results and Discussion

3.3.1 Peroxide Value (PV), Free Fatty Acids (FFA), Moisture, and Iodine Value (IV) of Unrefined and Refined Fish Oils

PV is a good indicator of initial lipid oxidation. The initial PV of unpurified menhaden and salmon oil were 24.22±1.24 and 38.59±0.42 meq/kg of fish oil (Tables 3.1 and 3.2). The results show that the MAE and SAE which are the menhaden and salmon oils purified by the activated earth have the lowest peroxide values (13.36 ±1.82 meq/Kg and 20.63±0.45 meq/Kg) among these oils. It indicated that activated earth can effectively adsorb primary oxidation compounds compared with chitosan and activated alumina from both menhaden and salmon oils. Huang and Sathivel (2010) reported that the adsorption principles of activated earth on the

oxidization products could be related to hydrogen bonding, competition for adsorption sites; electrostatic field strength and intraparticles diffusion of molecules.

Free fatty acid content (FFA) is one of the most harmful impurities in fish oils. Reducing FFA is a very important goal in oil purification process. Higher FFA indicates that the oil will have a higher refining loss when purified by neutralization. Activated alumina decreased the free fatty acids of the menhaden and salmon oils from 2.76±0.29% and 2.40±0.05% to 2.14±0.06% and 1.75±0.03% after 1h of adsorption. Activated alumina is an amorphous aluminum oxide from aluminum trihydrate. The reason that it can decrease the free fatty acid concentration may be caused by the combination between aluminum oxides and the free fatty acids. Neither chitosan nor activated earth was effective in reducing FFA from the SO, which is in agreement with Huang and Sathivel (2010). The M4 and M5 had similar FFA contents compared with MAA, while S4 and S5 had similar FFA contents compared with SAA.

Chitosan was the most effective adsorbent in reducing the moisture content, which decreased the moisture content of the menhaden and salmon oils from 3560±0.42 ppm and 631.60±37.9 ppm to 591.45±0.5 ppm to 162.6±6.4ppm, respectively. Chitosan is recognized as a hydrophilic compound and when added to the fish oils, it can adsorb water rapidly. Activated earth and activated alumina can also remove the moisture because of their adsorption capacities and reduced the moisture to 495.55±10.25 ppm and 399.95±2.05 ppm, respectively. M4 and M5 had the lowest moisture contents (256.55±6.72ppm and 271.37±16.38ppm) among all the menhaden oil samples. Similarly, S4 and S5 had the lowest moisture contents (143.3±3.0ppm and 141.3±3.3ppm) among all the salmon oil samples.

Iodine value (IV), also called iodine adsorption value or iodine number or iodine index, measures the degree of unsaturation of the oil and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample. Iodine value has been one of the standard

parameters related to chemical composition and quality of oils and is a way to tell the change in the fatty acid profile during processing or storage of the oil. In this study, the IV of the unrefined and refined menhaden oils were around 179.71 to 181.18 cg I_2/g oil, while the IV of salmon oils ranged from 170 to 178 cg I_2/g oil which is a little higher than reported values (147.8-170 cg I_2/g oil) for Atlantic salmon (*S. salar*) by Afseth and others (2006). Statistically there were no differences among the fish oil samples for IV. It means that the adsorption process may not change the degree of unsaturation and composition of fatty acid profile on both oils.

Table 3.1 – PV, FFA, moisture, and IV of the unrefined and refined menhaden oils

Sample	PV (meq/kg)	FFA (%)	Moisture (ppm)	IV (cg I_2/g oil)
MO	24.22±1.24^{a}	2.76±0.29^{a}	3560±0.42^{a}	180.40±3.21^{a}
MCH	17.29±0.06^{b}	2.82±0.10^{a}	591.45±0.49^{d}	179.98±2.14^{a}
MAE	13.36±1.82^{c}	2.74±0.05^{a}	897.9±16.97^{c}	180.37±2.45^{a}
MAA	22.58±1.06^{a}	2.14±0.06^{b}	986.25±11.67^{b}	181.18±0.56^{a}
M4	15.94±0.41^{bc}	2.00±0.09^{b}	256.55±6.72^{e}	179.71±4.01^{a}
M5	15.88±0.50^{bc}	2.04±0.08^{b}	271.37±16.38^{e}	180.70±2.89^{a}

Values are means ± SD of triplicate determinations. ^{abcd}Means with the same letters in each column are not significantly different (P > 0.05). MO = unrefined menhaden oil; MCH = process involved purification of MO by 5% (wt/wt of oil) chitosan for 1h, MAE = process involved purification of MO by 5% (wt/wt of oil) activated earth for 1h; MAA = process involved purification of MO by 5% (wt/wt of oil) activated alumina for 1h; M4 = process involved combined MO purification processes of 5% (wt/wt of oil) chitosan, 1.5% chitosan plus 3.5% activated earth, and 5% activated alumina for 1h, respectively; M5 = process involved combined UPO purification processes of 5% (wt/wt of oil) chitosan, 4% chitosan plus 1% activated earth, and 5% activated alumina for 1h, respectively. PV = peroxide value; FFA = free fatty acid content; IV = iodine value.

Table 3.2 – PV, FFA, moisture, and IV of the unrefined and refined salmon oils

	PV (meq/kg)	FFA (%)	Moisture (ppm)	IV (cg I_2/g oil)
SO	38.59±0.42^{a}	2.40±0.05^{a}	631.6±37.90^{a}	176.02±4.21^{a}
SCH	35.20±0.20^{b}	2.32±0.07^{a}	162.6±6.36^{d}	177.68±1.63^{a}
SAE	20.63±0.45^{d}	2.45±0.05^{a}	495.55±10.25^{b}	177.19±5.32^{a}
SAA	34.19±1.02^{b}	1.75±0.03^{b}	399.95±2.05^{c}	170.57±4.09^{a}
S4	24.34±0.17^{c}	1.76±0.02^{b}	143.3±2.97^{d}	175.95±0.57^{a}
S5	26.03±1.48^{c}	1.80±0.14^{b}	141.3±3.25^{d}	174.22±10.43^{a}

Values are means ± SD of triplicate determinations. ^{abcd}Means with the same letters in each column are not significantly different (P > 0.05). SO = unrefined salmon oil; SCH = process involved purification of SO by 5% (wt/wt of oil) chitosan for 1h, SAE = process involved purification of SO by 5% (wt/wt of oil) activated earth for 1h; SAE = process involved

(table 3.2 continued)
purification of SO by 5% (wt/wt of oil) activated alumina for 1h; S4 = process involved combined SO purification processes of 5% (wt/wt of oil) chitosan, 1.5% chitosan plus 3.5% activated earth, and 5% activated alumina, for 1h, respectively; S5 = process involved combined UPO purification processes of 5% (wt/wt of oil) chitosan, 4% chitosan plus 1% activated earth, and 5% activated alumina, for 1h, respectively. PV = peroxide value; FFA = free fatty acid content; IV = iodine value.

3.3.2 Color of Unrefined and Refined Fish Oils

The main pigments in fish oils are chlorophyll and carotenoids, notably astaxanthin and canthaxanthin, which are known for benefits for human health. Carotenoids pigments have been shown to be adsorbed from oil by adsorbents (Brekke 1980). Besides removing impurities from the fish oils, another purpose of this study is to retain these health beneficial pigments. Traditionally activated earth is a bleaching agent and can remove a lot of the pigments from oils. Tables 3.3 and 3.4 show the lightness (L*), redness (a*), yellowish (b*) values of the unrefined and refined fish oils. MAE and SAE having much lower or even negative a* values indicate that a reduction in red pigments occurred during the adsorption process by activated earth. The activated earth purified oil (MAE and SAE) had a large change in the lightness (L*), redness (a*), yellowish (b*) values and thus had total color difference (TCD) of 23.39 and 52.50 compared with the raw oil indicating that the color changed dramatically compared with the unrefined menhaden and salmon oils. To retain more pigments, processes 4 and 5, the amount of the activated earth was reduced from 5% to 1.5% and 1%, respectively, and as a result, the TCD of M4 and M5 dropped to 22.83 and 19.38, and S4 and S5 dropped to 26.03 and 17.95, respectively. Of the three adsorbents, chitosan changed color the least and had a TCD of 0.75 for salmon oil. So the chitosan is considered to have little effect on color change of the oil. All of these results were consistent with the results by visual evaluation.

Table 3.3- Color of the unrefined and refined menhaden oils

Sample	L*	a*	b*	Chroma	Hue angle	TCD

(table 3.3 continued)

MO	32.72±0.29^{d}	14.42±0.07^{a}	52.79±0.43^{d}	55.55±0.44^{d}	80.61±0.04^{d}	
MCH	38.56±0.16^{c}	13.85±0.03^{a}	65.05±0.31^{c}	67.55±0.30^{bc}	83.59±0.08^{c}	13.59±0.14^{d}
MAE	48.69±0.07^{a}	2.72±0.05^{c}	65.25±0.17^{c}	65.75±0.17^{c}	93.12±0.04^{a}	23.39±0.20^{a}
MAA	40.11±0.26^{c}	13.82±0.01^{a}	67.55±0.43^{bc}	69.98±0.43^{b}	83.94±0.05^{c}	16.51±0.08^{c}
M4	43.85±0.12^{b}	9.76±0.32^{b}	72.18±0.18^{a}	73.80±0.20^{a}	87.59±0.24^{b}	22.83±0.19^{a}
M5	41.75±0.38^{c}	12.80±0.04^{a}	69.87±0.49^{b}	72.07±0.46^{ab}	85.06±0.08^{c}	19.38±0.06^{b}

Values are means ± SD of triplicate determinations. ^{abcd}Means with the same letters in each column are not significantly different (P > 0.05). See Table 3.1 for brief description of MO, MCH, MAE, MAA, M4, and M5. TCD = Total Color Difference.

Table 3.4 - Color of the unrefined and refined salmon oils

	L*	a*	b*	Chroma	Hue angle	TCD
SO	37.27±0.05^{e}	21.23±0.05^{b}	61.00±0.08^{a}	65.17±0.09^{a}	76.24±0.02^{e}	
SCH	37.38±0.20^{e}	21.88±0.09^{a}	61.23±0.32^{a}	65.60±0.34^{a}	75.76±0.02^{f}	0.75±0.25^{e}
SAE	52.94±0.03^{a}	-3.75±0.03^{f}	17.55±0.04^{d}	18.37±0.03^{d}	108.55±0.12^{a}	52.50±0.05^{a}
SAA	38.25±0.09^{d}	20.18±0.03^{c}	61.38±0.11^{a}	65.21±0.11^{a}	77.29±0.02^{d}	1.49±0.08^{d}
S4	47.17±0.19^{b}	5.40±0.03^{e}	42.85±0.23^{c}	43.95±0.24^{c}	89.70±0.04^{c}	26.03±0.10^{b}
S5	44.53±0.07^{c}	9.42±0.02^{d}	49.59±0.07^{b}	51.12±0.07^{b}	85.75±0.01^{c}	17.95±0.03^{c}

Values are means ± SD of triplicate determinations. ^{abcd}Means with the same letters in each column are not significantly different (P > 0.05). See Table 3.2 for brief description of SO, SCH, SAE, SAA, S4, and S5. TCD = Total Color Difference.

3.3.3 Fatty Acid Methyl Ester (FAME) Composition of Unrefined and Refined Fish Oils

Tables 3.5 and 3.6 show the compositions of the main fatty acids in the unrefined and refined menhaden and salmon oils. No differences were found among these fatty acids in the unrefined and refined menhaden and salmon oils. The reason is that these processes were conducted at room temperature without heating and the air in the amber bottles was also replaced by nitrogen so there were no factors causing the oxidation of the unsaturated fatty acids. The EPA and DHA concentrations ranged between 16.86-17.07% and 6.38-6.86% for menhaden oils and 11.49-11.77% and 10.34-10.89% for salmon oils, respectively.

Table 3.5 – Fatty acid methyl ester (%) of the unrefined and refined menhaden oils

Sample	MO	MCH	MAE	MAA	M4	M5
C14	10.63±0.13[a]	10.98±0.06[a]	10.54±0.05 [a]	10.67±0.10 [a]	10.63±0.14[a]	10.21±0.10[a]
C16	19.19±0.05[a]	19.51±0.06[a]	18.69±0.15 [a]	19.00±0.11 [a]	18.88±0.12[a]	18.08±0.06[a]
C16:1n7	11.21±0.15[a]	11.79±0.17[a]	11.31±0.06 [a]	11.52±0.04 [a]	11.42±0.03[a]	11.24±0.06[a]
C18:1n9	8.00±0.07[a]	8.42±0.08[a]	8.07±0.03 [a]	8.10±0.06 [a]	8.15±0.05[a]	7.73±0.02[a]
C20:5n3	16.86±0.18[a]	16.76±0.14[a]	17.02±0.17 [a]	17.07±0.15 [a]	16.89±0.07[a]	16.87±0.18[a]
C22:6n3	6.53±0.03[a]	6.86±0.09[a]	6.61±0.09 [a]	6.66±0.06 [a]	6.64±0.13[a]	6.38±0.02[a]

Values are means ± SD of triplicate determinations. Fatty acids less than 5% are not reported. [a]Means with the same letters in each column are not significantly different (P > 0.05). See Table 3.1 for brief description of MO, MCH, MAE, MAA, M4, and M5.

Table 3.6 – Fatty acid methyl ester (%) of the unrefined and refined salmon oils

	SO	SCH	SAE	SAA	S4	S5
C16	12.32±0.03[a]	12.30±0.31 [a]	12.43±0.19[a]	12.67±0.06 [a]	12.55±0.23[a]	12.51±0.19[a]
C16:1n7	5.14±0.05[a]	5.20±0.12[a]	5.21±0.07[a]	5.34±0.06[a]	5.27±0.14[a]	5.25±0.06[a]
C18:1n9	11.89±0.03[a]	12.01±0.23[a]	12.15±0.16[a]	12.33±0.04[a]	12.22±0.15[a]	12.11±0.18[a]
C20:1n11	7.03±0.05[a]	7.12±0.09[a]	7.20±0.10[a]	7.23±0.08[a]	7.24±0.13[a]	7.16±0.12[a]
C20:5n3	11.49±0.07[a]	11.64±0.19[a]	11.76±0.22 [a]	11.76±0.09[a]	11.77±0.15[a]	11.71±0.15[a]
C22:1n11	11.40±0.14[a]	11.58±0.16[a]	11.65±0.20[a]	11.68±0.13[a]	11.67±0.23[a]	11.59±0.13[a]
C22:6n3	10.89±0.15[a]	11.08±0.12[a]	11.16±0.28[a]	11.34±0.39[a]	11.13±0.52[a]	11.11±0.12[a]

Values are means ± SD of triplicate determinations. Fatty acids less than 5% are not reported. [a]Means with the same letters in each column are not significantly different (P > 0.05). See Table 3.2 for brief description of SO, SCH, SAE, SAA, S4, and S5.

3.3.4 Mineral Concentrations of the Unrefined and Refined Fish Oils

Tables 3.7 and 3.8 listed the mineral and heavy metal contents of unrefined and refined oil samples. B, Fe, Zn, Al, Ca, Mg, Na, and Ar were the most abundant minerals in unrefined menhaden and salmon oils. After the five adsorption processes, most of these mineral contents decreased. Compared with chitosan and activated alumina, activated earth was most effective in reducing B, Fe, and Zn from the menhaden oil. AE decreased the iron contents from 19.15ppm to 7.08ppm, which is below the acceptable level (8 ppm) for iron (Bimbo, 1998). For the salmon oil, activated earth effectively removed the Fe, Zn, Ca, S, and Na. Activated alumina removed significant amounts of Ca and Mg from the menhaden fish oil. Chitosan had highest capacity for

adsorbing potassium which decreased from 7.83 to 4.95ppm. Chitosan effectively decreased the Al levels in both menhaden and salmon oils; however, activated alumina and activated earth increased the Al levels in the oil samples. This could be caused by the alumina in the adsorbents. Activated earth has a porous aluminium/silicate composition with a pore diameter of 50,000 Angstroms. The difference in absorption capacity of these minerals is related to the physical structure of these adsorbents, because the adsorbate interaction potential largely depends on the pore size and geometry of the adsorbents (Yang 2003). MO and SO contained high contents of P (45.36 ppm and 38.60ppm) and they were reduced to 15.65 ppm and 17.88 ppm by activated earth. The phosphorus present in fish oils may be attributed to the phospholipids in the oil which is the main components of the gum presents in oils. Another complex that phosphorus can form is calcium-phosphate complexes (Young 1986). Even though all the three adsorbents were effective in reducing minerals or heavy metals from menhaden and salmon oils, there were still considerable amounts of minerals left in the oils after the adsorption processes. Huang and Sathivel (2010) reported that the neutralization process could reduce most of the minerals (Ca, Fe, Mg, P, Na, Ar) in salmon oils to trace amounts. This could be caused by the washing step during the neutralization process which removes the water soluble impurities, especially phospholipids from the raw oil and most of minerals and heavy metals precipitated with the soap as saponification occurred during neutralization. The problem with the neutralization method is the increase of Na because of the addition of NaOH solution (Huang and Sathivel 2010).

Table 3.7 - Minerals and heavy metal concentration (ppm) of unrefined and refined menhaden oils

	MO	MCH	MAE	MAA	M4	M5
Boron	30.6 ± 1.13^{a}	25.75 ± 1.90^{c}	24.8 ± 0.42^{c}	28.31 ± 1.38^{b}	19.75 ± 1.21^{d}	22.55 ± 1.34^{cd}
Copper	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
Iron	19.15 ± 0.78^{a}	10.43 ± 1.24^{c}	7.08 ± 0.59^{d}	15.4 ± 0.42^{b}	8.07 ± 0.64^{d}	10.3 ± 0.28^{c}
Manganese	<1	<1	<1	<1	<1	<1
Zinc	4.50 ± 0.32^{a}	4.06 ± 0.65^{b}	1.08 ± 0.52^{d}	3.48 ± 0.36^{c}	0.99 ± 0.19^{d}	1.06 ± 0.07^{d}
Aluminum	5.34 ± 1.27^{b}	3.83 ± 0.78^{c}	6.06 ± 0.78^{a}	6.94 ± 0.72^{a}	4.59 ± 1.82^{b}	4.73 ± 0.23^{bc}

(table 3.7 continued)

Barium	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Cadmium	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Chromium	1.80±0.08[a]	1.24±0.17[b]	0.89±0.13[c]	1.31±0.56[b]	1.19±0.37[b]	1.26±0.19[b]
Calcium	37.75±1.48[a]	33.05±1.34[a]	23.56±1.36[b]	19.7±2.40[c]	17.65±1.03[c]	17.6±1.27[c]
Cobalt	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Magnesium	8.86±0.21[a]	7.67±0.71[a]	5.11±0.06[b]	4.58±0.23[b]	3.16±0.25[c]	3.88±0.03[c]
Lead	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Molybdenum	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
Phosphorus	45.36±1.36[a]	20.34±1.22[b]	15.65±0.38[c]	18.34±2.65[bc]	17.56±1.78[bc]	17.41±0.89[bc]
Potassium	7.83±0.46[a]	4.95±0.92[c]	6.06±0.48[b]	6.05±1.06[b]	4.78±0.28[c]	4.81±0.02[c]
Nickel	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
Selenium	<14	<14	<14	<14	<14	<14
Arsenic	18.55±1.62[a]	16.25±0.92[a]	<4	<4	<4	<4
Sodium	36.15±1.20[a]	21.9±0.57[b]	22.2±1.75[b]	20.6±1.53[b]	17.1±1.41[c]	20.6±0.28[b]

Values are means ± SD of duplicate determinations. [abcd]Means with the same letters in each column are not significantly different (P > 0.05). See Table 3.1 for brief description of MO, MCH, MAE, MAA, M4, and M5.

Table 3.8 - Minerals and heavy metal concentration (ppm) of unrefined and refined salmon oils

	SO	SCH	SAE	SAA	S4	S5
Boron	36.20±1.70[a]	32.50±1.98[b]	34.80±1.84[a]	29.15±1.16[c]	33.00±2.21[b]	29.15±1.31[c]
Copper	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Iron	5.90±0.24[a]	5.39±1.56[a]	1.25±0.04[c]	3.03±0.30[b]	1.86±0.15[c]	1.52±0.02[c]
Manganese	<1	<1	<1	<1	<1	<1
Zinc	4.85±0.64[a]	4.06±2.65[ab]	1.73±0.62[b]	3.48±1.36[ab]	3.93±0.38[ab]	3.69±0.87[ab]
Aluminum	12.60±1.27[b]	7.83±3.78[c]	16.00±0.94[a]	16.25±1.48[a]	11.23±0.82[b]	10.60±0.71[b]
Barium	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Cadmium	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Chromium	1.47±0.08[a]	1.24±0.17[a]	1.20±0.23[a]	1.28±0.24[a]	1.19±0.37[a]	1.15±0.26[a]
Calcium	42.87±0.58[a]	29.86±2.25[b]	17.90±2.26[c]	25.50±3.11[b]	27.65±4.03[b]	26.10±6.93[b]
Cobalt	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Magnesium	8.53±0.49[a]	7.67±0.71[ab]	7.30±0.26[ab]	7.47±0.36[ab]	5.16±0.29[b]	4.42±0.18[c]
Lead	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
Molybdenum	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
Phosphorus	38.60±2.26[a]	25.75±0.35[b]	17.88±0.21[d]	25.65±2.33[b]	21.50±0.37[c]	22.70±1.13[bc]
Potassium	26.18±2.23[a]	8.37±2.17[bc]	6.06±0.48[c]	8.21±1.70[bc]	4.70±0.68[c]	5.25±0.29[c]
Nickel	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
Sulphur	71.9±0.70[a]	71.15±3.04[a]	50.75±0.64[c]	59.85±0.07[b]	67.55±10.82[ab]	50.65±1.48[c]
Selenium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Arsenic	6.07±1.10[a]	4.84±0.35[b]	<4	<4	<4	<4
Sodium	59.60±5.66[a]	45.30±4.10[b]	19.40±1.70[d]	25.30±3.82[cd]	28.00±3.11[c]	30.05±1.63[c]

(table 3.8 continued)
Values are means ± SD of duplicate determinations. [abcd]Means with the same letters in each column are not significantly different (P > 0.05). See Table 3.2 for brief description of SO, SCH, SAE, SAA, S4, and S5.

3.3.5 Rheological Properties of the Unrefined and Refined Fish Oils

The flow behavior index (*n*), consistency index (K) values, and apparent viscosity of unrefined and refined fish oil samples were tested at 25 °C. The results are listed in tables 3.9 and 3.10. The flow behavior index of MO (0.78) was much less than 1, which indicates that it exhibited shear thinning properties (Paredes and others 1989). The purified menhaden oils and all of the salmon oil samples were considered as Newtonian fluids at room temperature with *n* value very close to 1. The consistency index is an indicator of the viscous nature of the samples, and the higher K values indicate a more viscous consistency (Paredes and others 1989). This result is in agreement with the apparent viscosity of the oils. All the oil samples became less viscous after the purification processes. The viscosity of the oil could be affected by two factors, one of which is the impurities, such as minerals, insoluble impurities, free fatty acids, and moisture; the other factor is the fatty acid composition and the degree of unsaturation of the fatty acids. The interaction between oil and impurities may form an aggregated colloidal dispersion system and the oil exhibits higher viscosity (Teeter and Cowan 1956). More unsaturated fatty acids will cause low viscosity of oils.

Table 3.9 – Rheological properties of the unrefined and refined menhaden oils

	n	K (Pa.s^n)	Viscosity (Pa.s)
MO	0.78±0.02^b	0.45±0.02^a	0.05±0.00^a
MCH	0.98±0.03^a	0.24±0.01^b	0.03±0.00^b
MAE	0.97±0.01^a	0.23±0.01^b	0.03±0.00^b
MAA	0.98±0.01^a	0.23±0.01^b	0.03±0.00^b
M4	0.99±0.00^a	0.22±0.00^b	0.03±0.00^b
M5	0.99±0.01^a	0.22±0.00^b	0.03±0.00^b

(table 3.9 continued)
Values are means ± SD of triplicate determinations. [ab]Means with the same letters in each column are not significantly different (P > 0.05). See Table 1 for brief description of MO, MCH, MAE, MAA, M4, and M5. *n* = flow behavior index, K = consistency index.

Table 3.10 - Rheological properties of unrefined and refined salmon oils

	n	K (Pa.s^n)	Viscosity (Pa.s)
SO	0.98±0.02^a	0.051±0.001^a	0.046±0.001^a
SCH	0.99±0.01^a	0.046±0.001^b	0.044±0.000^{ab}
SAE	0.99±0.00^a	0.045±0.001^b	0.042±0.000^{bc}
SAA	0.99±0.00^a	0.044±0.001^b	0.043±0.000^{bc}
S4	0.99±0.00^a	0.044±0.001^b	0.042±0.000^c
S5	0.99±0.01^a	0.044±0.001^b	0.042±0.000^c

Values are means ± SD of triplicate determinations. [abc]Means with the same letters in each column are not significantly different (P > 0.05). See Table 1 for brief description of SO, SCH, SAE, SAA, S4, and S5. *n* = flow behavior index, K = consistency index.

3.3.6 Thermal Properties of the Unrefined and Refined Fish Oils

The DSC thermograms (Figures 3.1 and 3.2) show differences in melting points of unrefined and refined fish oil samples. The DSC thermogram shows four to five main distinct endothermic peaks for the unrefined and refined oil samples. The low melting points of the oils were attributed to triacylglycerol which contained unsaturated fatty acids (Tan and Che Man 2002). Oil samples with a higher degree of unsaturated fatty acids melt at lower temperatures, whereas those with a higher degree of saturated fatty acids melt at higher temperatures.

Physical properties of triglycerides are more complex than those of the individual fatty acids because they contain three fatty acid groups. All peaks for both the menhaden and salmon oil samples were not sharp, except M4, M5, and S5. For both oils, the raw oils (MO and SO) were the flattest comparing with all of the purified oil samples. This might be attributed to the presence of impurities, such as phospholipids, ketones, and other materials in the (Young, 1978; Richardson, 1978). Those impurities component components melt uncharacteristically compared to pure fatty acids. Fatty acids have their own melting points; therefore, sharp peaks were not

observed for the raw oils. Sathivel and others (2008) reported that the DSC thermograms of purified catfish oil showed sharper and narrower peaks than unpurified fish oil. The melting points of fish oil were sharper after each purification step that removed impurities from the oil. These results support the use of DSC to evaluate fish oil quality.

The thermal degradation of the unpurified and purified menhaden and salmon oils is given in Figures 3.3 and 3.4. Weight loss of all the oils increased with increased heating temperature between 200 and 500ºC. At 550ºC, MO, MCH, MAE, MAA, M4, and M5 oil samples retained 1.95%, 0.49%, 0.17%, 0.65%, 0.38%, and 0.03% of their initial weight, respectively. At 550ºC, SO, SCH, SAE, SAA, S4, and S5 oil samples retained 0.75%, 0.47%, 0.29%, -1.57%, -0.27%, and 0.12%, of their initial weight, respectively. M4, M5, S4, and S5 had higher degradation rate comparing with other oil samples. The presence of impurities in the oil samples reduces heat transfer to unpurified oils; therefore, less energy available to evaporate the volatiles. Sathivel and others (2003) reported that the weight loss of oils due to thermal decomposition is higher in purified oils than those of unpurified oils. This study indicates that TG analysis may be useful in evaluating the quality of fish oils.

3.4 Conclusions

The results indicated that activated earth was most effective on reducing primary oxidation compounds from menhaden and salmon oils comparing with chitosan and activated alumina from both menhaden and salmon oils. Activated alumina was very effective on removing free fatty acids (FFA) from both oils and neither chitosan nor activated earth was effective in reducing FFA. Chitosan exhibited the best function adsorbing the moisture in the fish oils. No difference among the IV and FAMEs of the unrefined and refined menhaden oils were observed, indicating that the adsorption process did not change the degree of unsaturation and composition of fatty acid profile on both oils. Comparing with chitosan and activated alumina,

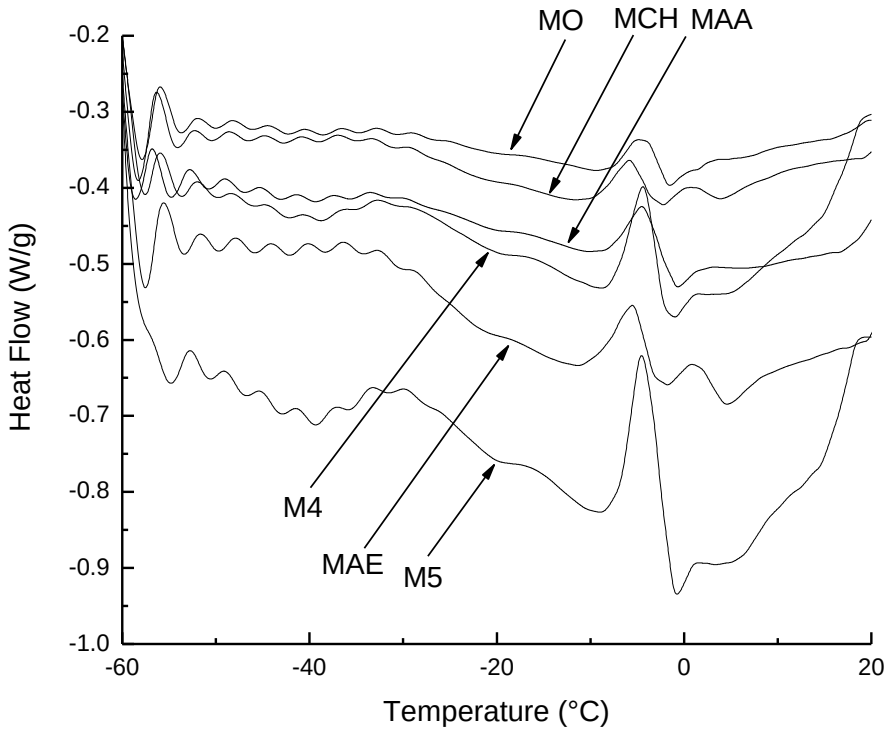


Figure 3.1 - DSC thermogram of MO, MCH, MAE, MAA, M4, and M5.

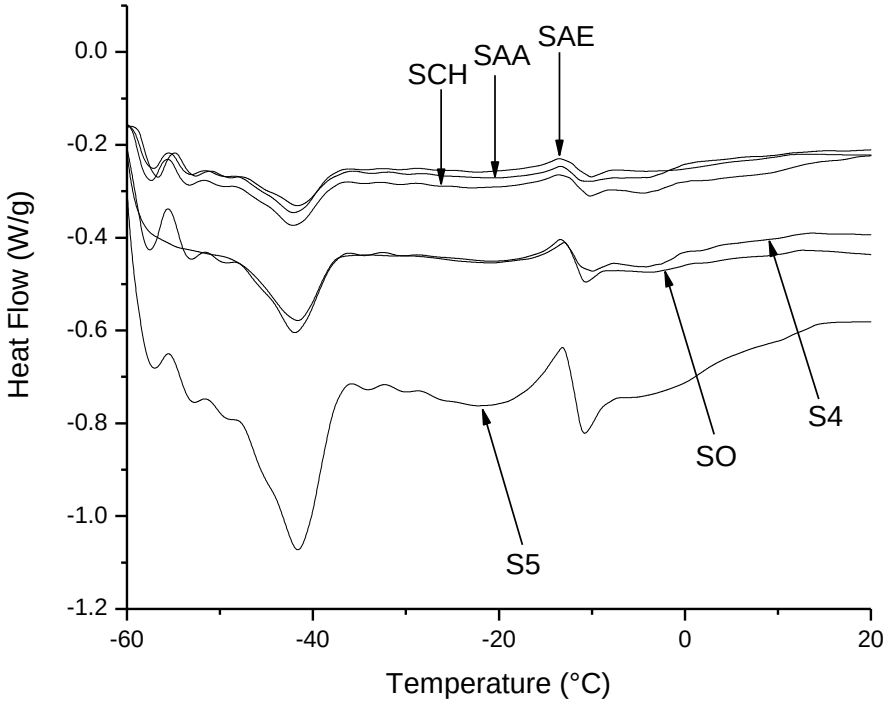


Figure 3.2 - DSC thermogram of SO, SCH, SAE, SAA, S4, and S5.

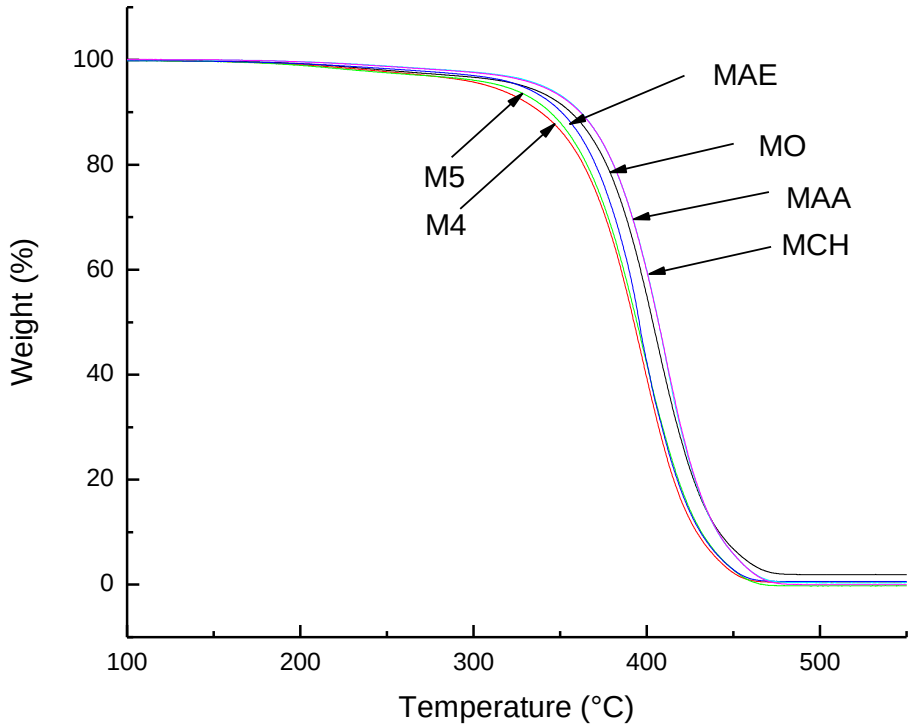


Figure 3.3 - Thermal degradation of MO, MCH, MAE, MAA, M4, and M5.

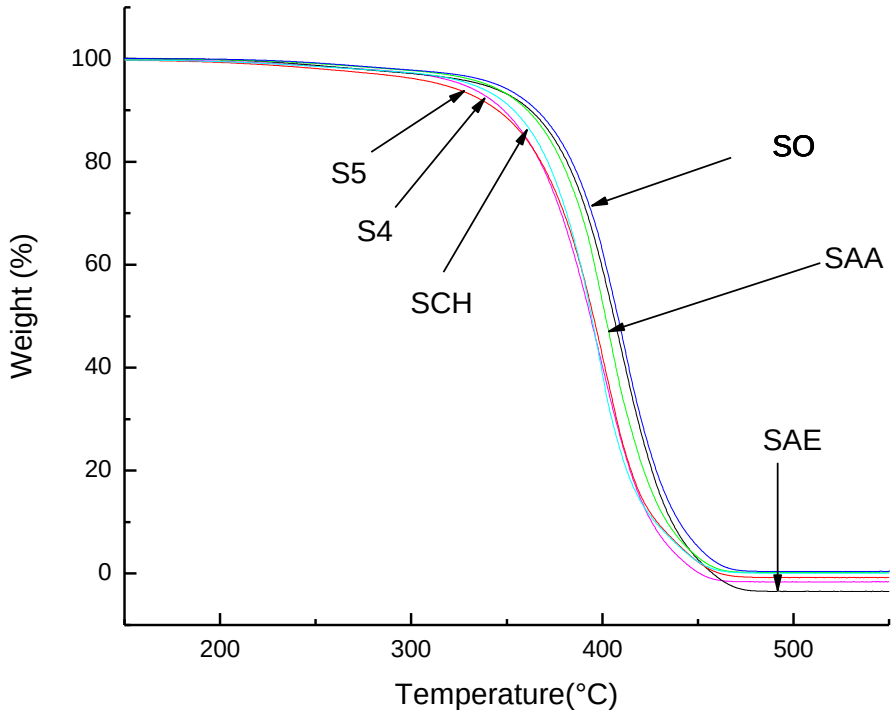


Figure 3.4 - Thermal degradation of SO, SCH, SAE, SAA, S4, and S5

activated earth was most effective reducing B, Fe, and Zn from the menhaden oil. For the salmon oil, activated earth effectively removed the Fe, Zn, Ca, S, and Na. Activated alumina removed most amounts of Ca and Mg from the menhaden fish oil. Chitosan had highest capacity adsorbing with K and reduced from 7.83 to 4.95ppm. MO and SO contained high contents of P (45.36 ppm and 38.60ppm) and they were reduced to 15.65 ppm and 17.88 ppm by activated earth. MO exhibited shear thinning properties, while purified menhaden oils and all the salmon oil samples are considered as Newtonian fluids at room temperature with n value very close to 1. All the oil samples became less viscous after the purification processes. All DSC peaks for unrefined and refined menhaden and salmon oil samples were not sharp, except M4, M5, and S5. The thermal degradation of the unpurified and purified menhaden and salmon oils showed that M4, M5, S4, and S5 had higher degradation rate comparing with other oil samples. This study indicates that DSC and TG analysis may be useful in evaluating the quality of fish oils. From this study, a batch adsorption process was developed for purification of menhaden and salmon oils in reducing PV, FFA, moisture, and heavy metal content and retaining the fatty acid compositions.

CHAPTER 4 PURIFYING FISH OILS WITH COLUMN ADSORPTION PROCESSES

4.1 Introduction

Adsorption could be an attractive alternative for purifying fish oils. It is a separation process during which specific components of one phase of a fluid are transferred onto the surface of a solid adsorbent (McCabe and others 1993). Use of inexpensive adsorbents with less oil loss and less lipid oxidation render the method cost effective. Lipid oxidation is reduced because of the mild conditions such as ambient temperature and atmospheric pressure that are employed in adsorption processes. As mentioned in the previous chapter a laboratory scale batch study utilizing an activated earth, activated alumina, and chitosan purification process to reduce hydroperoxides (PV), free fatty acids (FFA), moisture, and minerals in menhaden and salmon oils was conducted. The results showed that activated earth reduced PV, while chitosan and activated alumina reduced moisture and FFA, respectively. The combined method which included adsorbents chitosan, activated earth and activated alumina effectively reduced PV, FFA, and moisture of the unpurified fish oils.

In the laboratory experiments, certain aspects of the adsorption process were investigated by handling small amounts of raw materials to reduce the material constraints to a minimum. A proper laboratory scale study is very important because it gives information on the reaction mechanism, kinetics, and hydrodynamics, especially for a new reaction system (Dutta and Gualy 2000). The laboratory process should mimic as closely as possible the anticipated design, hydrodynamic conditions, and operation of a commercial process. Unfortunately, a close approach to the commercial system is not often possible in a lab-scale process (Inglezakis and Poulopoulos 2006). Therefore, it's necessary to develop a continuous pilot scale adsorption process.

During pilot-plant experiments industrial constraints are taken into account and problems that may happen in industrial production must be dealt with adequately. Pilot plant operation is a tool for understanding physical and chemical mechanisms involved in the process and for generating data. Mathematical models are usually used to transform findings of pilot-scale experiments to commercial production (Trambouze 1990). The scale of pilot plant production is very important because if it's too small there may be problems such as wall effects and slugging in the column, if it's too big, the cost could be too high (Knowlton 2000). The scale or size of the equipment in which the adsorption process takes place has different effects depending on the mechanism concerned, for example, chemical kinetics and thermodynamics are not affected by size, but heat transfer and mass transfer are both influenced by the size of the equipment (Inglezakis and Poulopoulos 2006). Huang and Sathivel (2010) reported that adsorption technology could potentially provide a simplified process for refining fish oil for human consumption. The results obtained in their study could be used to design and build an adsorption unit for purifying fish oils. However, to date there are few studies dealing with designing pilot-scale adsorption columns to purify fish oils. Therefore, the objectives of this study were to develop a continuous pilot scale adsorption purification process for unpurified menhaden and salmon oils, to study the mechanisms involved in this process, and to characterize both the unpurified and the resulting purified fish oils.

4.2 Material and Methods

4.2.1 Fish Oil Production and Purifying Methods

Unrefined menhaden fish oil (MO) extracted using a rendering process was obtained from a commercial source (Omega Protein Inc., Houston.TX). Unpurified salmon oil (SO) was produced from salmon processing byproducts including viscera, heads, skins, frame, and discarded fish obtained from a large commercial plant in Alaska. The unpurified salmon oil was

extracted and stored at -40ºC until used. Activated alumina was obtained from Zapp's Potato Chips Inc. (Gramercy, LA) and activated earth was obtained from the BASF Chemicals Division, Geismar, LA.

Unrefined menhaden or salmon oil samples (20 kg) was each mixed with 1 kg of activated earth and 1 kg of activated alumina and stirred for 0, 30, 60, 90, 120, or 150 min in a covered vessel. Nitrogen was injected into the container to replace the air. The resulting mixtures were pumped at 6.0 kg/min through a pilot scale adsorption unit that contained two 0.95 m tall and 0.24 m diameter adsorption columns lined with 5µm filter bags to filter out the adsorbents (Figure 4.1). Experiments were repeated three times. Unpurified and purified oil samples were analyzed for fatty acids profile, peroxide value (PV), free fatty acids (FFA), moisture content, minerals, rheological and thermal properties.

4.2.2 Peroxide Value (PV), Free Fatty Acids (FFA), and Moisture in Unpurified and Purified Fish Oils

The PV and FFA contents were measured as described in 2.2.1. The moisture content was measured according to the method described in 2.2.2. All of the analyses were repeated three times.

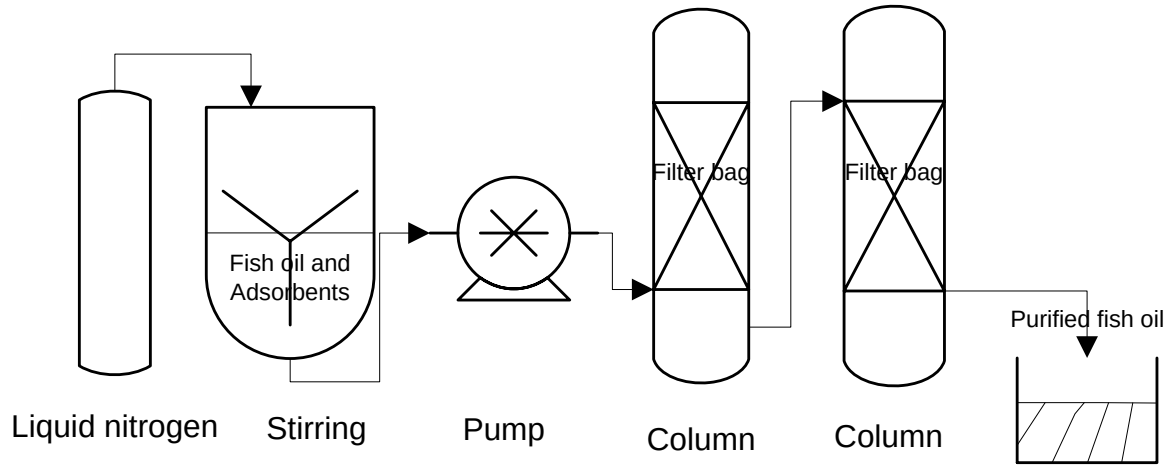


Figure 4.1 - Flow diagram of the column purification process by adsorbents

4.2.3 Adsorption Kinetics of the PV, FFA, and Moisture in Fish Oils by Activated Earth and Activated Alumina

A second- order kinetic model was used to describe the adsorption kinetics of the activated earth and alumina on PV, FFA, and moisture content in menhaden and salmon oils. Kinetic phenomenon by the reaction rate at which reactant A depletes can be modeled according to the generic model (Lima 2003):

$$-r_A = -\frac{dC_A}{dt} = kC_A^x \quad \text{(eq. 4.1)}$$

where r_A is the rate of reaction, dC_A/dt is the rate at which the concentration of reactant A is changing with time, k is the reaction rate constant, C_A is the concentration of the reactant A, and x is the order of reaction.

When x=2, the generic kinetics model becomes:

$$-\frac{dC_A}{dt} = kC_A^2 \qquad \text{(eq. 4.2)}$$

This equation states that the rate of the depletion of reactant A is proportional to the square of the concentration of A. Integration of the differential equation yields a second order reaction equation:

$$\frac{1}{c_A} = \frac{1}{c_{A0}} + kt \quad \text{(eq. 4.3)}$$

To determine the rate constant experimentally, $1/C_A$ vs. time was plotted and a linear line was obtained. The rate constant is the slope of the best fit line.

4.2.4 Fatty Acid Profile and Mineral Concentrations in Unrefined and Refined Fish Oils

Fatty acid composition and mineral content of oil samples was determined by the method described in 3.2.4.

4.2.5 Rheological Properties of the Unrefined and Refined Fish Oils

Rheological properties of the oil samples were measured according to 3.2.5.

4.2.6 Thermal Properties of Unrefined and Refined Fish Oils

Thermal stability of the oil samples were analyzed by thermal gravimetric analyzer following the procedure described in 3.2.6. The differential scanning calorimetric experiments were conducted as described in 3.2.6.

4.2.7 Statistical Analysis

Means and deviations of the data collected were reported. Multiple comparison was performed at the significant level of $P < 0.05$ using SAS version 8.2 (SAS Institute Inc., 2002). Tukey's studentized range tests were performed to detect significant differences among the different treatments.

4.3 Results and Discussion

4.3.1 Peroxide Value (PV), Free Fatty Acids (FFA), and Moisture Content of Unrefined and Refined Fish Oils

PV indicates the initial lipid oxidation of the fish oils. The unrefined menhaden oil had a peroxide value of 8.11±0.55 meq/Kg and immediately decreased to 5.01±0.14 meq/Kg after 30min of purification with activated earth and activated alumina. After 120min of adsorption, PV dropped to 3.34±0.07 meq/Kg. The initial PV of the unrefined salmon oil was 8.72±0.10 meq/Kg and it was reduced to 3.71±0.42 meq/Kg after adsorbing with activated earth and activated alumina for 120min. The oil with PV less than 5 meq/Kg can be considered as fresh oil (Gracey and others 1999), while an oil with a PV of 7.5 meq/Kg is unacceptable for human consumption (Robards and others 1988; Schnepf and others 1991). The results show that the mix of activated earth activated alumina can effectively adsorb primary oxidation products. Based on the results from the previous chapter, activated earth was an effective adsorbents for reducing PV and the adsorption principles of activated earth on the oxidization products could be related to hydrogen bonding, competition for adsorption sites; electrostatic field strength and intraparticles diffusion of molecules (Huang and Sathivel 2010). Prolonged purification (150min) elevated the PV of the

menhaden oil from 3.34±0.07 meq/Kg (purified for 120min) to 3.91±0.16 meq/Kg, and there's no significant change for PV of salmon oils after purification for 150min . This means that the adsorption ability of the activated earth decreased with time and once equilibrium obtained, it would not adsorb any oxidative compounds from the fish oils and more agitation could have caused oxidation.

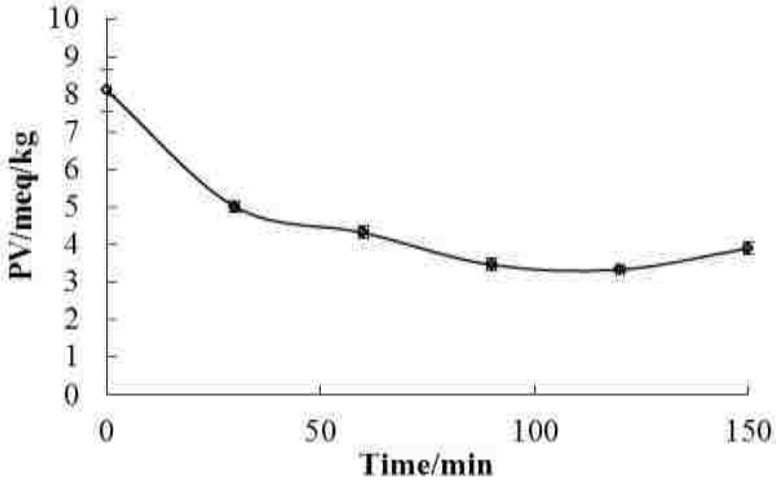


Figure 4.2 - PV of the menhaden oil purified with column purification method by time.

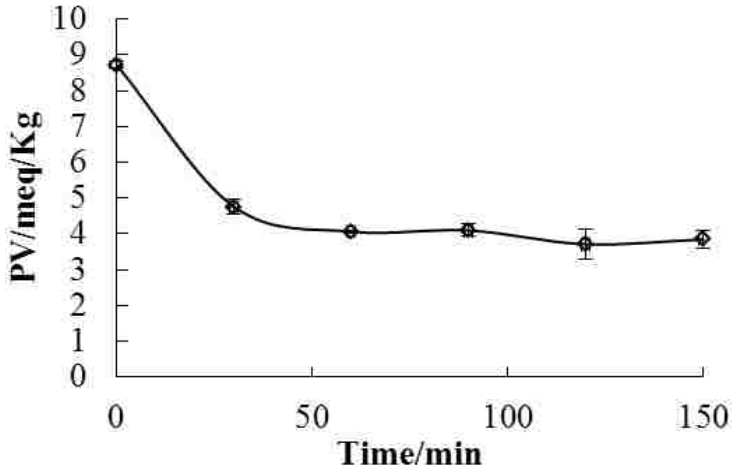


Figure 4.3 - PV of the salmon oil purified with column purification method by time.

Lipid rancidity could be caused by a number of effects such as hydrolyzed and oxidation rancidity. Polyunsaturated acids are easily oxidized producing aldehydes and ketones which cause unpleasant taste. Hydrolyzed rancidity forms free fatty acids due to the hydrolysis of

triglycerides. This process may be promoted by the reaction of oil with moisture (Iqbal and Bhanger 2007). FFA is an important measure of food rancidity. Reducing FFA is a very important goal in the fish oil purification process. Higher FFA indicates that fish oil will have a higher refining loss when purified. Activated earth and alumina decreased the free fatty acids of menhaden and salmon oils from 1.91±0.05% and 1.42±0.12% to 1.13±0.02% and 0.75±0.02%, respectively after 120min of adsorption (Figures 4.4 and 4.5). After adsorbing for 150min, the FFA of the menhaden decreased to 0.99±0.01%, while the FFA of salmon oil had no significant change. As indicated in chapter 3, activated alumina is very effective in reducing FFA because of the combination between aluminum oxides and the free fatty acids. The adsorption process reached equilibrium at 120min for salmon oil and was still effective for reducing FFA for menhaden oil.

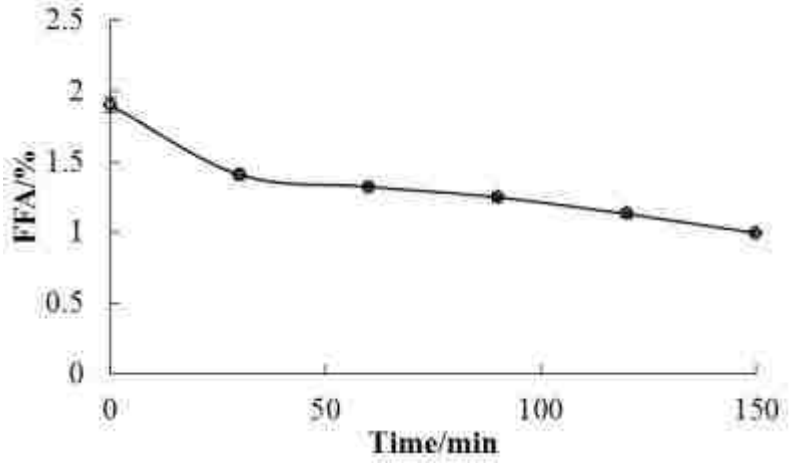


Figure 4.4 - FFA of the menhaden oil purified with column purification method by time.

Moisture can promote lipid hydrolysis and increase FFA concentration (Iqbal and Bhanger 2007) so it's necessary to remove the moisture in fish oil to prevent further rancidity. The initial moisture contents of menhaden and salmon oils were 4523.3±31.1ppm and 1906.3±23.4ppm. Activated earth and activated alumina mix decreased the moisture content to 670.3±16.2ppm and to 649.2±23.6ppm, respectively. The capacity of the adsorbents could be

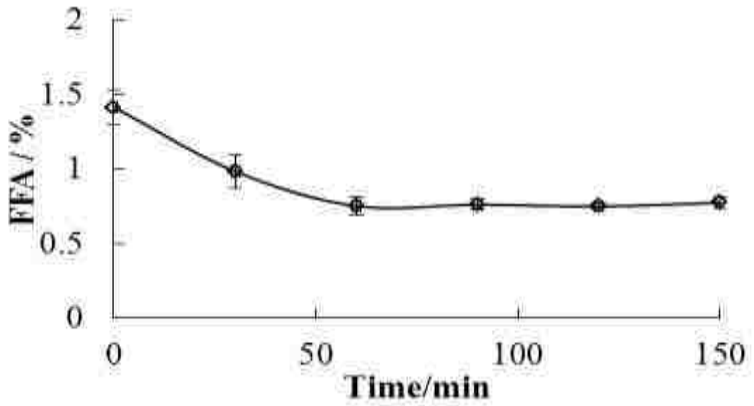


Figure 4.5 - FFA of the salmon oil purified with column purification method by time.

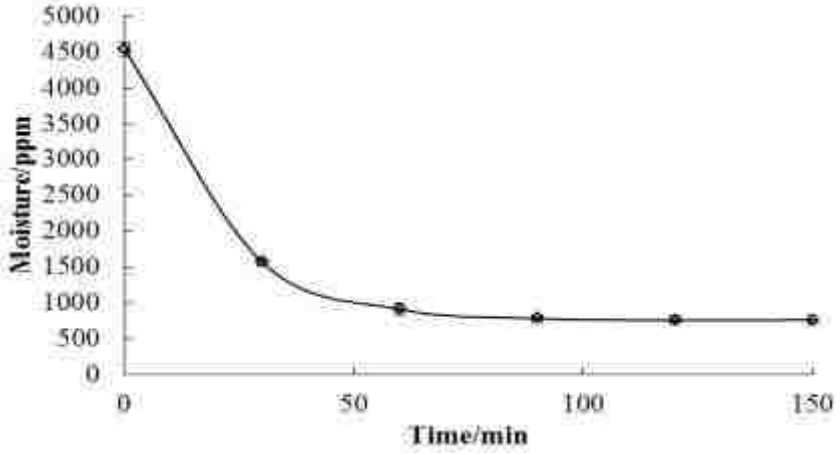


Figure 4.6 - Moisture content of menhaden oil purified with column purification method by time.

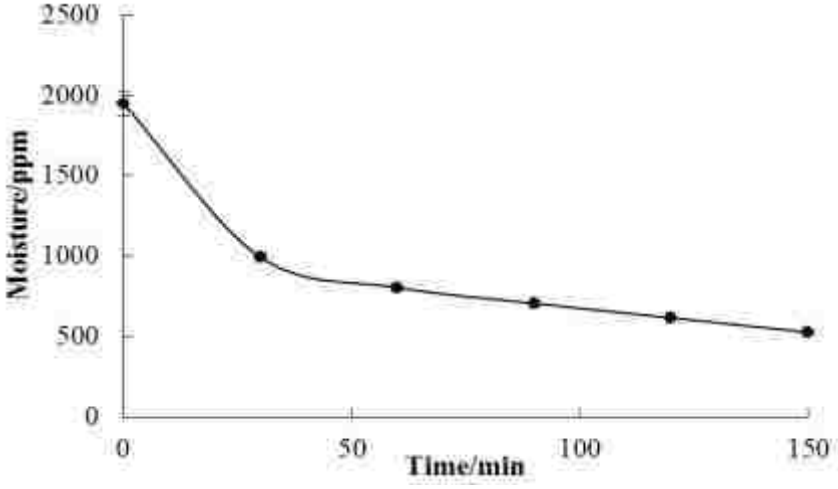


Figure 4.7 - Moisture content of salmon oil purified with column purification method by time.

attributed to the hydrogen bonding between adsorbents and fish oils (Huang and Sathivel 2010).

4.3.2 Adsorption Kinetics of PV, FFA, and Moisture of Fish Oils by Activated Earth and Activated Alumina

The adsorption of peroxides, free fatty acids, and moisture from the menhaden and salmon oils all best fit the second order kinetic model at certain time intervals (as shown in figures 4.8 to 4.13) . After the adsorption equilibrium was obtained, the peroxides, free fatty acids, and moisture contents kept stable or slightly increased. The adsorption of peroxides (PV) from menhaden oil followed the second kinetic model from time 0 to 120min with an adsorption rate constant (k) of 0.0015 and R^2 of 0.9477. And the adsorption of peroxides from salmon oil followed the second kinetic model from time 0 to 60min (k = 0.0022 and R^2 =0.935). The second kinetic model could be used to describe the removal of the free fatty acids from menhaden and salmon oils at the time of 0 to 150min and 0 to 60min, respectively. The adsorption of moisture by activated earth and activated alumina fit the second kinetic model up to 90 and 150min for menhaden and salmon oils. The concentration of peroxides, free fatty acids, and moisture in menhaden and salmon oils can be predicted based on the adsorption rate constants at any time that followed the second order kinetic model. Oliveira and Porto (2005) reported that the second order kinetic model was used for bleaching soybean and corn oils by bleaching earth (F180), which has given good agreement with the experimental data.

4.3.3 Fatty Acid Methyl Ester (FAME) Composition of Unrefined and Refined Fish Oils

One of the most important goals of the study was to retain the fatty acid composition of the fish oils. As shown in tables 4.1 and 4.2, no differences were found among the main fatty acids in the unrefined and refined menhaden and salmon oils. The reason is that these processes were conducted at mild conditions (room temperature under nitrogen), so there were almost no factors causing the oxidation of the unsaturated fatty acids. The EPA and DHA concentrations

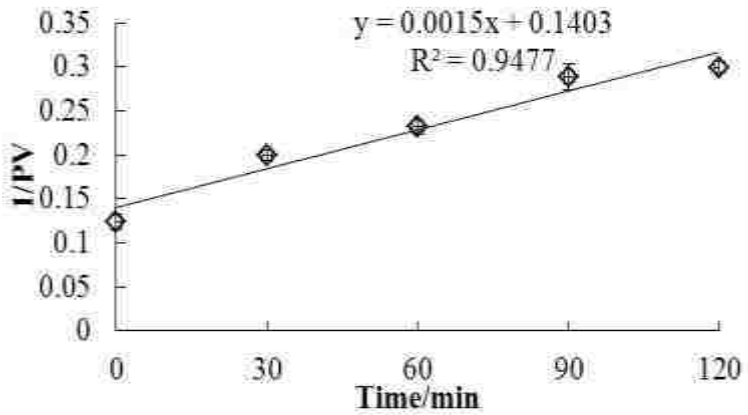


Figure 4.8 - Second-order kinetic plots of peroxide adsorption by activated earth and alumina in menhaden oil.

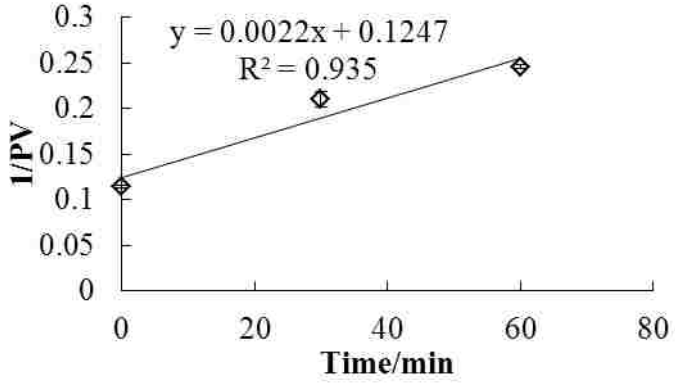


Figure 4.9 - Second-order kinetic plots of peroxide adsorption by activated earth and alumina in menhaden oil.

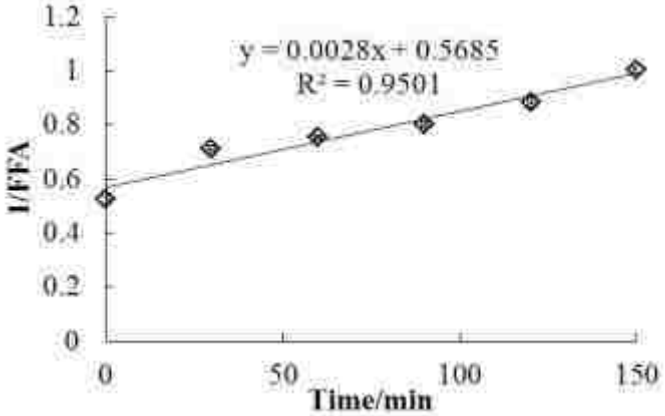


Figure 4.10 - Second-order kinetic plots of free fatty acid adsorption by activated earth and alumina in salmon oil.

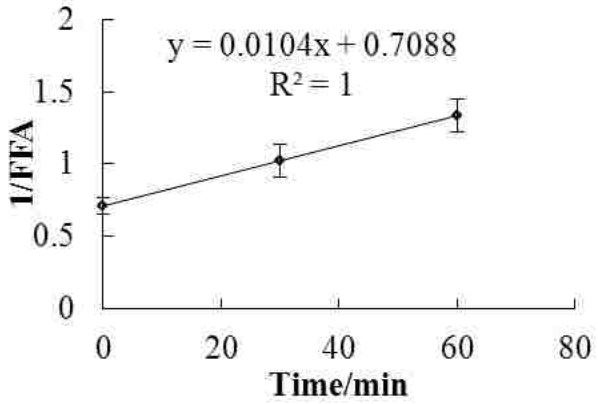


Figure 4.11 - Second-order kinetic plots of free fatty acid adsorption by activated earth and alumina in salmon oil.

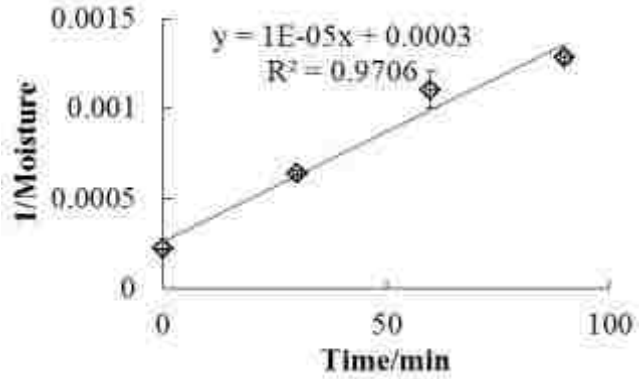


Figure 4.12 - Second-order kinetic plots of free fatty acid adsorption by activated earth and alumina in menhaden oil.

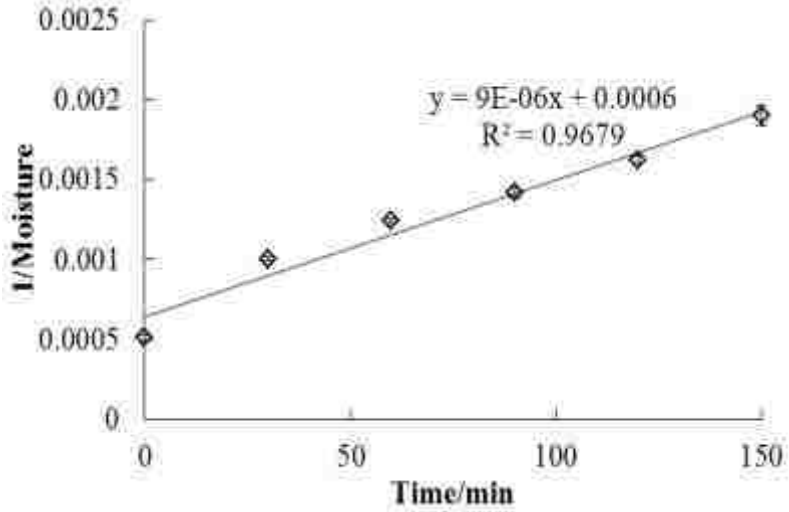


Figure 4.13 - Second-order kinetic plots of free fatty acid adsorption by activated earth and alumina in salmon oil.

ranged between 15.02-15.38% and 7.69-7.82% for menhaden oils and 9.82-9.83% and 12.65-12.68% for salmon oils, respectively.

Table 4.1 - Fatty acid methyl ester (%) of the unrefined and refined menhaden oils

FAMEs	UMO	PMO
C14	9.89±0.15[a]	9.94±0.06[a]
C16	18.77±0.52[a]	18.08±0.67[a]
C16:1n7	11.14±0.27[a]	10.95±0.34[a]
C18:1n9	8.02±0.01[a]	8.00±0.03[a]
C20:5n3	15.38±0.38[a]	15.02±0.55[a]
C22:6n3	7.82±0.26[a]	7.69±0.31[a]

Values are means ± SD of triplicate determinations. Fatty acids less than 5% are not reported. [a]Means with the same letters in each row are not significantly different (P > 0.05). UMO = unrefined menhaden oil; PMO = Purified menhaden oil by adsorbing with activated earth and alumina for 150min.

Table 4.2 - Fatty acid methyl ester (%) composition of the unrefined and refined salmon oils

FAMEs	USO	PSO
C16	14.27±0.33[a]	14.30±0.53[a]
C16:1n7	5.54±0.11[a]	5.52±0.09[a]
C18:1n9	16.83±0.34[a]	16.76±0.87[a]
C20:1n11	8.09±0.17[a]	8.05±0.52[a]
C20:5n3	9.83±0.42[a]	9.82±0.66[a]
C22:1n11	6.99±0.18[a]	6.95±0.03[a]
C22:6n3	12.68±0.25[a]	12.65±0.81[a]

Values are means ± SD of triplicate determinations. Fatty acids less than 5% are not reported. [a]Means with the same letters in each row are not significantly different (P > 0.05). USO = unrefined salmon oil; PSO = Purified salmon oil by adsorbing with activated earth and alumina for 150min.

4.3.4 Mineral Concentrations of the Unrefined and Refined Fish Oils

Metal, such as copper and iron, can catalyze oxidation of fish oil (Young 1986). Tables 4.3 and 4.4 list the mineral and heavy metal contents of unrefined and refined oil samples. The first step in the oil purification process is the removal of phospholipids which is usually carried

out by degumming process. It involves mixing oil with 1-3% water with agitation for 30-60min at 70ºC to hydrate the phosphatides, followed by centrifugation to separate the degummed oil from the gums (Brekke 1980). Degumming reduces the phosphorus content of the oil from 500-900 ppm to 12-170 ppm (Brown and Snyder 1985). The phosphorus present in fish oils may be attributed to the phospholipids in the oil which is the main components of the gum presents in oils. The column purification process decreased the phosphorus contents of the menhaden and salmon oils significantly ($P > 0.05$) from 45.36±1.36ppm and 38.60±2.26ppm to 16.42±0.37ppm and 17.85±1.14ppm, respectively.

Another complex that phosphorus can form is calcium-phosphate complexes (Young 1986). B, Fe, Zn, Al, Ca, Mg, Na, and Ar were the most abundant minerals in unrefined menhaden and salmon oils. After the column adsorption processes, most of these mineral contents decreased. The adsorbents decreased the iron contents of the menhaden and salmon oils from 19.15ppm and 5.90ppm to 7.56ppm and 1.06ppm, which was below the acceptable level (8 ppm) for iron (Bimbo 1998). After the column adsorption process for 150min, the B, Zn, Cr, Ca, Mg, K, Na, and Ar contents in menhaden and salmon oils all significantly decreased. Huang and Sathivel (2010) reported that the activated earth adsorption at 60 min reduced arsenic concentrations from 6.78 ppm to trace level (<0.2 ppm). The adsorbents increased the Al levels in the oil samples and may be caused by the alumina from the adsorbents.

Table 4.3 - Minerals and heavy metal concentration (ppm) of unrefined and refined menhaden oils

	UMO	**PMO**
Boron	30.6±1.13[a]	16.35±0.98[b]
Copper	<1.2	<1.2
Iron	19.15±0.78[a]	7.56±0.37[b]
Manganese	<1	<1
Zinc	4.50±0.32[a]	0.67±0.06[b]
Aluminum	5.34±1.27[b]	6.05±0.22[a]
Barium	<0.2	<0.2

(table 4.3 continued)

Cadmium	<0.2	<0.2
Chromium	1.80±0.08[a]	0.66±0.07[b]
Calcium	37.75±1.48[a]	15.34±1.03[b]
Cobalt	<0.2	<0.2
Magnesium	8.86±0.21[a]	3.64±0.52[b]
Lead	<0.2	<0.2
Molybdenum	<0.8	<0.8
Phosphorus	45.36±1.36[a]	16.42±0.37[b]
Potassium	7.83±0.46[a]	3.56±0.52[b]
Nickel	<0.4	<0.4
Sulphur	<22	<22
Selenium	<14	<14
Arsenic	18.55±1.62[a]	<4
Sodium	36.15±1.20[a]	18.43±0.37[b]

Values are means ± SD of duplicate determinations. [ab]Means with the same letters in each row are not significantly different (P > 0.05). UMO = unrefined menhaden oil; PMO = Purified menhaden oil by adsorbing with activated earth and alumina for 150min.

Table 4.4 - Minerals and heavy metal concentration (ppm) of unrefined and refined salmon oils

	USO	PSO
Boron	36.20±1.70[a]	27.65±0.76[b]
Copper	<0.2	<0.2
Iron	5.90±0.24[a]	1.06±0.34[b]
Manganese	<1	<1
Zinc	4.85±0.64[a]	1.55±0.23[b]
Aluminum	12.60±1.27[b]	14.35±0.88[a]
Barium	<0.2	<0.2
Cadmium	<0.2	<0.2
Chromium	1.47±0.08[a]	0.89±0.07[b]
Calcium	42.87±0.58[a]	20.17±0.64[b]
Cobalt	<0.2	<0.2
Magnesium	8.53±0.49[a]	4.78±0.57[b]
Lead	<1.2	<1.2
Molybdenum	<0.8	<0.8
Phosphorus	38.60±2.26[a]	17.85±1.14[b]
Potassium	26.18±2.23[a]	5.64±2.17[b]
Nickel	<0.4	<0.4

(table 4.4 continued)

Sulphur	71.9±0.70[a]	42.37±3.04[a]
Selenium	<0.5	<0.5
Arsenic	6.07±1.10[a]	<4
Sodium	59.60±5.66[a]	16.37±2.30

Values are means ± SD of duplicate determinations. [ab]Means with the same letters in each row are not significantly different (P > 0.05). USO = unrefined salmon oil; PSO = Purified salmon oil by adsorbing with activated earth and alumina for 150min.

4.3.5 Rheological Properties of the Unrefined and Refined Fish Oils

The flow behavior index (*n*), consistency index (*K*) values, and apparent viscosity of unrefined and refined fish oil samples were tested at 25 °C. The results are listed in Tables 4.5 and 4.6. The flow behavior index of MO (0.80) was much less than 1, which indicates that it exhibited shear thinning properties (Paredes and others 1989). The purified menhaden oils and all of the salmon oil samples are considered as Newtonian fluids at room temperature with *n* values very close to 1. The consistency index is an indicator of the viscous nature of the samples, and the higher *K* values indicate a more viscous consistency (Paredes and others 1989). This result is in agreement with the apparent viscosity of the oils. The interaction between oil and impurities may form an aggregated colloidal dispersion system and the oil exhibits higher viscosity (Teeter and Cowan 1956). This indicated that fewer impurities may present in the unrefined salmon oils.

Table 4.5 - Changes on rheological properties of the menhaden oils by adsorption time

Time/min	*n*	*K* (Pa.s^n)	Viscosity (Pa.s)
0	0.80±0.00[b]	0.46±0.00[a]	0.051±0.00[a]
30	0.99±0.00[a]	0.026±0.00[b]	0.030±0.00[b]
60	0.98±0.00[a]	0.025±0.00[b]	0.031±0.00[b]
90	0.98±0.00[a]	0.025±0.00[b]	0.030±0.00[b]
120	0.99±0.00[a]	0.024±0.00[b]	0.029±0.00[b]
150	0.99±0.00[a]	0.024±0.00[b]	0.029±0.00[b]

Values are means ± SD of triplicate determinations. [ab]Means with the same letters in each column are not significantly different (P > 0.05). *n* = flow behavior index, *K* = consistency index.

Table 4.6 - Changes on rheological properties of the salmon oils by adsorption time

Time/min	*n*	*K* (Pa.s^n)	Viscosity (Pa.s)
0	0.99±0.00^a	0.046±0.00^a	0.044±0.00^a
30	0.99±0.00^a	0.042±0.00^b	0.041±0.00^b
60	1.00±0.00^a	0.041±0.00^b	0.040±0.00^b
90	1.00±0.00^a	0.040±0.00^b	0.040±0.00^b
120	0.99±0.00^a	0.041±0.00^b	0.039±0.00^b
150	0.99±0.00^a	0.041±0.00^b	0.039±0.00^b

Values are means ± SD of triplicate determinations. ^{ab}Means with the same letters in each column are not significantly different (P > 0.05). *n* = flow behavior index and *K* = consistency index.

4.3.6 Thermal Properties of the Unrefined and Refined Fish Oils

The DSC thermogram (Figures 4.14 and 4.15) show several distinct endothermic peaks for the unrefined and refined oil samples. The low melting points of the oils were attributed to triacylglycerol which contained unsaturated fatty acids (Tan and Che Man 2002). Oil samples with a higher degree of unsaturated fatty acids melt at lower temperatures, whereas those with a higher degree of saturated fatty acids melt at higher temperatures. All peaks for the unrefined menhaden oil sample were not sharp. This might be attributed to the presence of impurities, such as phospholipids, ketones, and other materials in the oil (Young 1978; Richardson 1978). Those impurities melt uncharacteristically compared to pure fatty acids. Fatty acids have their own melting points; therefore, sharp peaks were not observed for the raw oil. After purification with the column process with activated earth and alumina, the peaks of the purified menhaden oil became sharper because of the removal of the impurities. Sathivel and others (2008) reported that the DSC thermograms of purified catfish oil showed sharper and narrower peaks than unpurified fish oil. The peaks of the salmon oils had no difference after purification by the

column adsorption. As indicated by the rheological results, fewer impurities may present in the unrefined salmon oils, which means when purifying the salmon oil less change occurred.

Figures 4.16 and 4.17 show the thermal degradation of unpurified and purified menhaden and salmon oils. Weight loss of all of the oils increased with increased heating temperature between 100 and 500ºC. PMO had higher degradation rate compared with UMO. No difference was found between the degradation curves of USO and PSO. The presence of impurities in the oil samples reduces heat transfer to unpurified oils; therefore, less energy is available to evaporate the volatiles. Sathivel and others (2003) reported that the weight loss of oils due to thermal decomposition was higher in purified oils than those of unpurified oils.

4.4 Conclusions

A pilot scale fast and economically viable purification process was developed in this study. The mix of activated earth and activated alumina can effectively adsorb primary oxidation products. The prolonged adsorption process (150min) elevated the PV of the menhaden oil. Activated earth and alumina decreased the free fatty acids of menhaden and salmon oils from 1.91±0.05% and 1.36±0.30% to 1.13±0.02% and 0.75±0.02%, respectively, after 120 min of adsorption. Activated earth and activated alumina mix decreased the moisture content of menhaden and salmon oils from 4523.3±31.1ppm and 1906.3±23.4ppm to 670.3±16.2ppm and 649.2±23.6ppm. The adsorption process did not affect the main fatty acids in the unrefined and refined menhaden and salmon oils. The column purification process decreased the phosphorus contents of the menhaden and salmon oils from 45.36±1.36ppm and 38.60±2.26ppm to 16.42±0.37ppm and 17.85±1.14ppm, respectively. After the column adsorption processes, most of the mineral contents decreased. MO exhibited shear thinning properties and all of the purified menhaden oils and all of the salmon oil samples were considered as Newtonian fluids at room temperature with *n* values very close to 1. Apparent viscosity of the oil samples decreased after

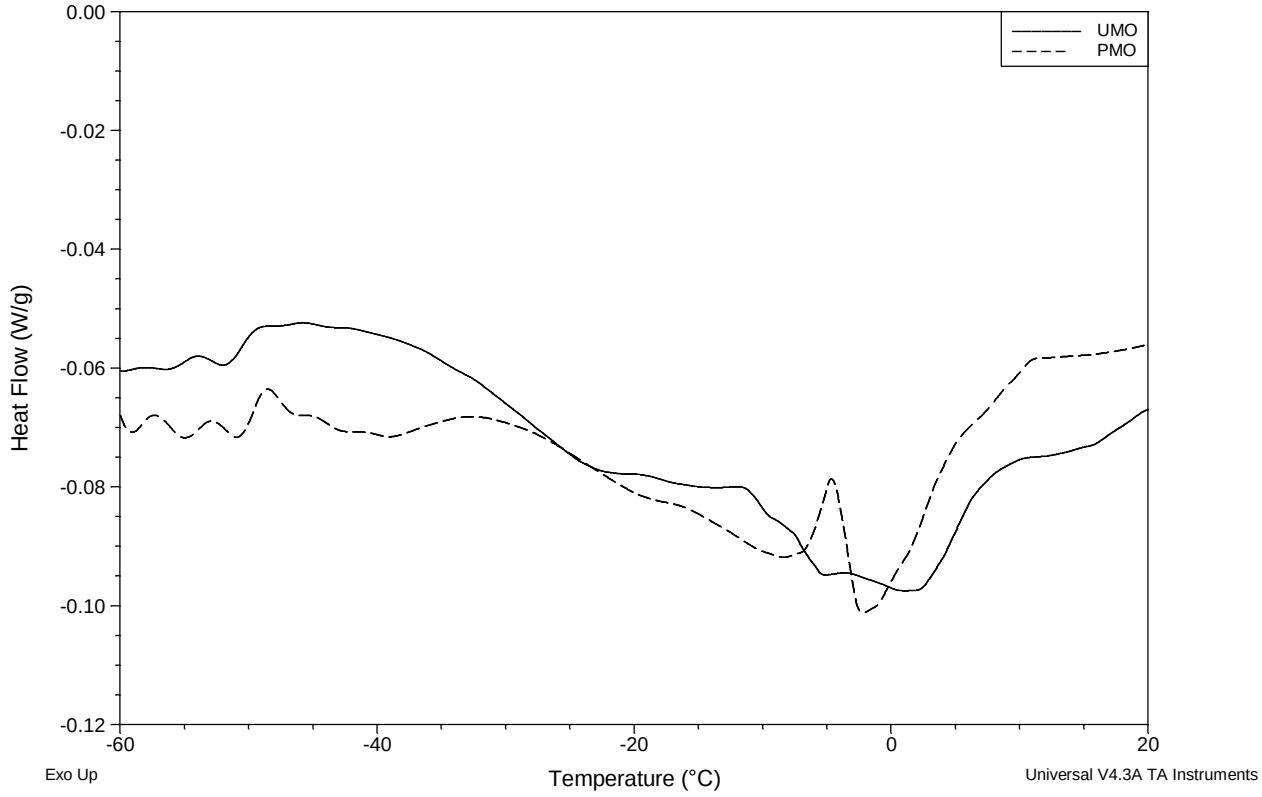


Figure 4.14 - DSC thermogram of UMO and PMO.

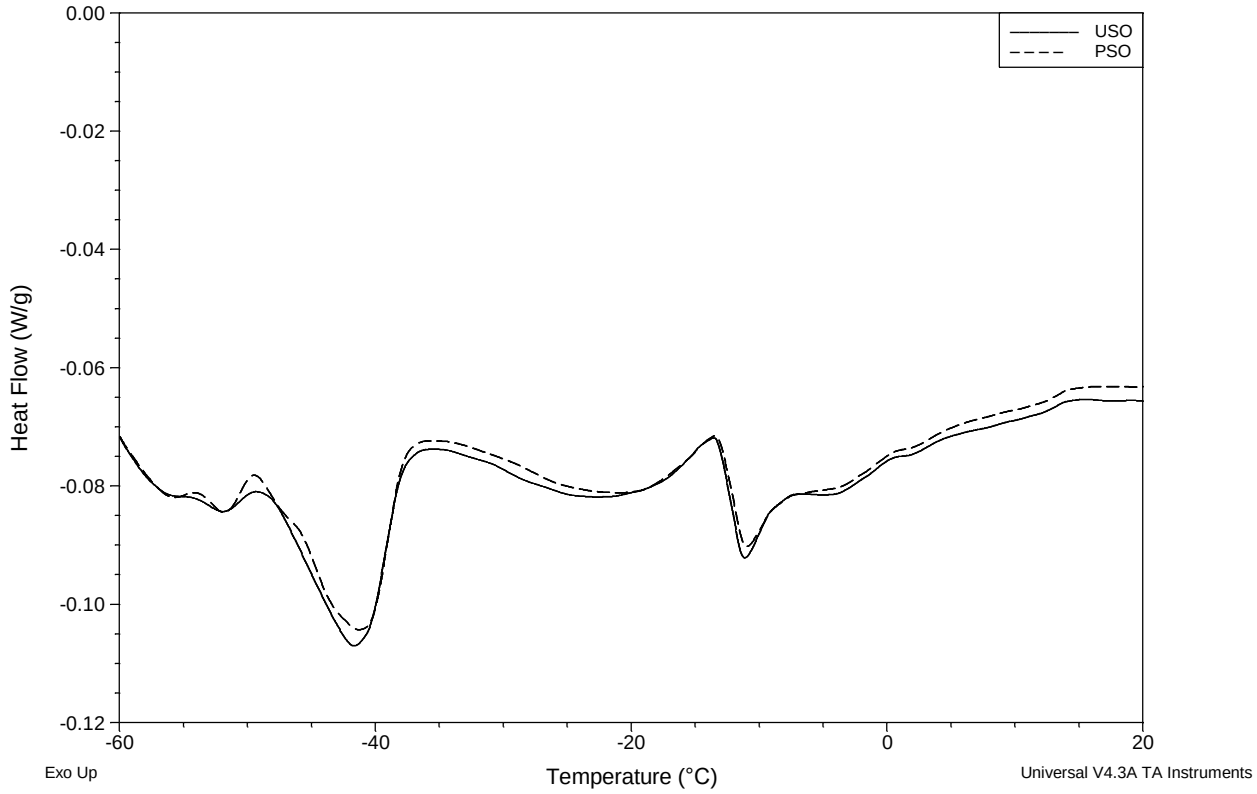


Figure 4.15 - DSC thermogram of USO and PSO.

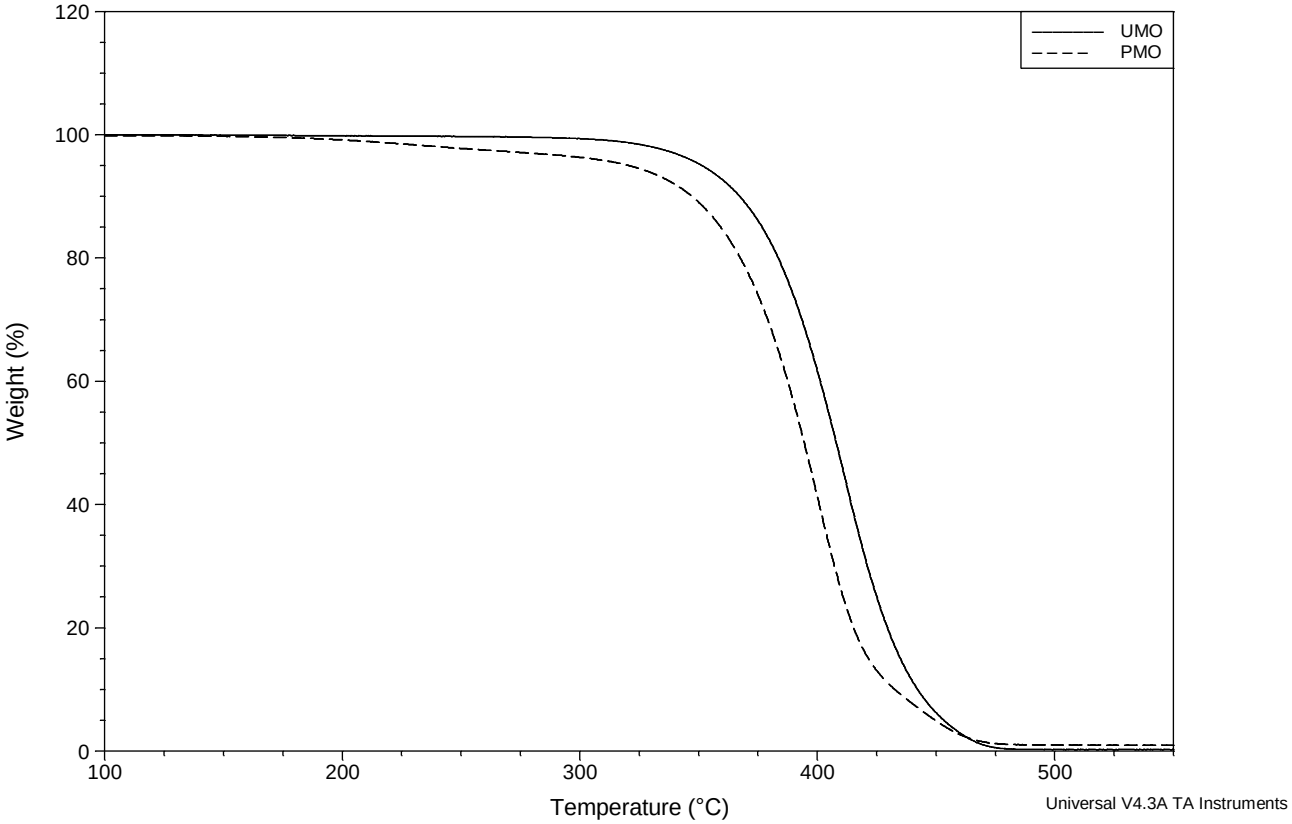


Figure 4.16 - Thermal degradation of UMO and PMO.

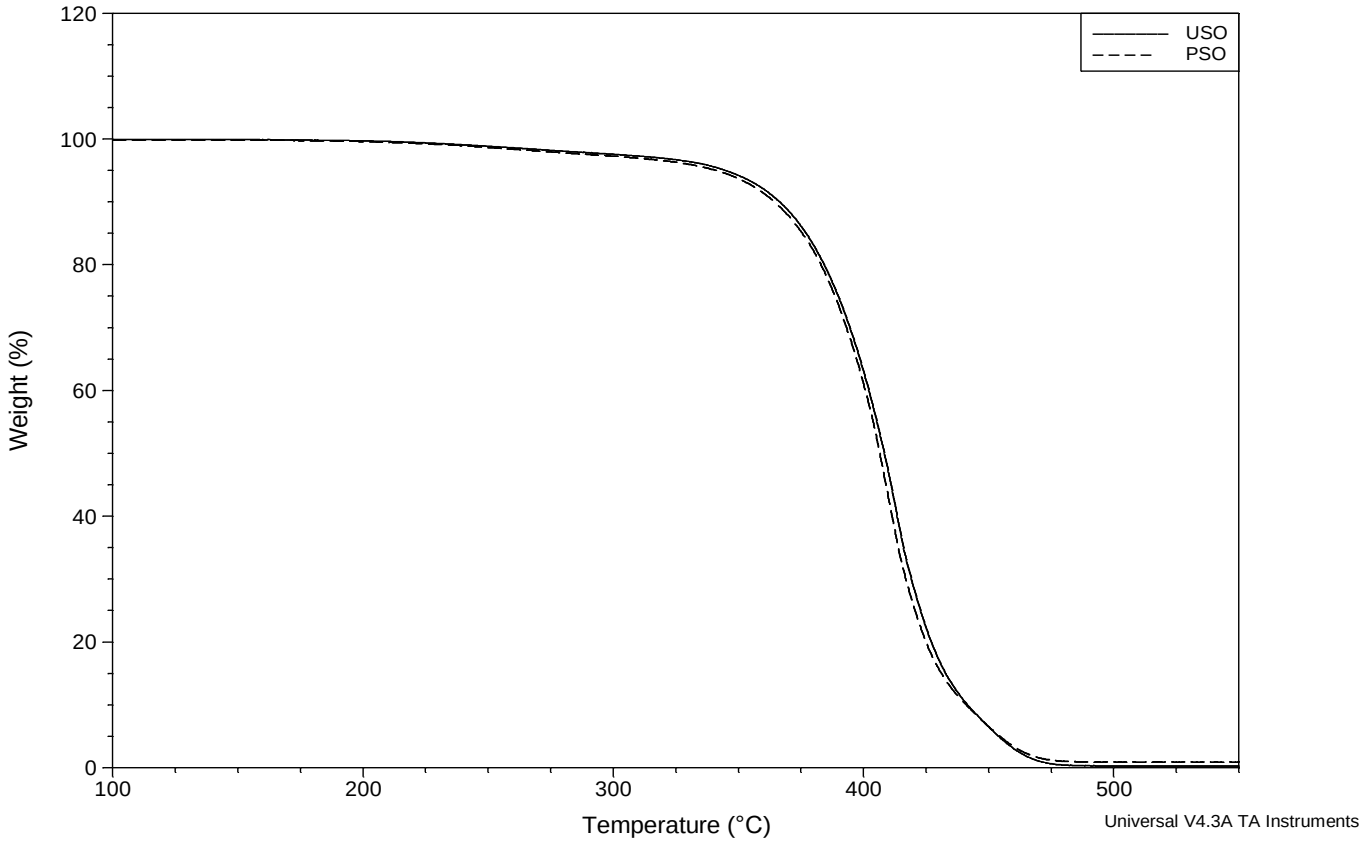


Figure 4.17- Thermal degradation of USO and PSO.

purification. After purification with activated earth and alumina for 120 min, the DSC endothermic peaks of the menhaden oil became sharper because of the removal of the impurities. The peaks of the salmon oils had no difference after purification by the column adsorption. PMO and PSO had higher thermal degradation rate comparing with UMO and USO. The study demonstrated that the column purification process could reduce the PV, FFA, and moisture contents from unrefined menhaden and salmon oils without affecting the fatty acid compositions.

CHAPTER 5 A NOVEL METHOD FOR PRODUCING PURIFIED FISH OILS ENRICHED WITH EICOSAPENTAENOIC (EPA) AND DOCOSAHEXAENOIC ACID (DHA)

5.1 Introduction

Polyunsaturated fatty acids (PUFA) in fish oils, especially eicosapentaenoic acid (C20:5n3, EPA) and docosahexaenoic acid (C20:6n3, DHA), have been shown to have a positive effect on preventing a variety of human diseases and disorders (Uauy and Valenzuela 2000; Wanasundara and Shahidi 1998; Benatti and others 2004; Horrocks and Yeo 1999). These fatty acids cannot be synthesized and can only be obtained by extraction from natural fats in which they naturally occur. Overconsumption of fish oils to obtain benefits from ω-3 PUFAs may increase the intake of cholesterol and other saturated fatty acids which could have deleterious health effects (Haagsma and others 1982; Shahidi and Wanasundara 1998).

It has been suggested that PUFA concentrates containing higher concentrations of EPA and DHA are much better than marine oils themselves because they allow daily intake of total lipids to remain as low as possible and, in particular, avoids intake of more saturated fatty acids (Wanasundara and Shahidi 1998). The market for fish oils enriched with ω-3 PUFAs is growing with the public awareness of the benefits from these enriched oils.

A variety of methods have been used to enrich marine oils with EPA and DHA. Many of the methods required extreme physical and chemical conditions and caused some degree of degradation of the fatty acids, formation of peroxides, and/or conversion of some of the cis-bonds to the trans- form. Furthermore, many materials (acetone, hexane, and other organic solvents) that are not on the Grnerally Regognized as Safe (GRAS) list of the U.S. Food and Drug Administration were used in previous enrichment studies. Fujita and others (1983) purified EPA by urea fractionation and distillation processes but some degree of cis-trans conversion

occurred, which is strictly undesirable for food or pharmaceutical use. Low temperature fractional crystallization is another commonly used method but usually carried out in organic solvents, such as acetone and different hydro carbons (Shinowara and Brown 1940). The salt solubility method mostly uses lithium soaps in acetone and alcohol because the lithium salts of polyenoic fatty acids are soluble in 95% acetone while less unsaturated acids are relatively insoluble (Marldey 1964). Supercritical fluid technology was used to separate a mix of fatty acid ethyl esters but the cost was very high (Eisenbach 1984). Other methods such as thin-layer chromatographic and gas chromatographic methods require undesirably high amounts of organic solvents. Most of the methods described above produce PUFA concentrate in the form of their corresponding alkyl esters. The acylglycerol form of PUFA is considered to be nutritionally more favorable than methyl or ethyl esters. Several studies revealed that alkyl esters of ω-3 fatty acids can impair intestinal absorption in laboratory animals (El-Boustani and others 1982; Hamazaki and others 1987; Lawson L.D. and Hughes 1988). From a marketing point of view, acylglycerols (mono-, di-, and triacylglycerols) are often promoted as being more "natural" than methyl or ethyl esters (Haraldsson and Hoskuldsson 1989).

Much attention has been given to microbial lipases to produce ω-3 PUFA concentrates in the form of acylglycerols by hydrolysis of marine oils (Tanaka and others 1992; Hoshino and others 1990; Shimada and others 1994; Yadwad and others 1991; Maehr and others 1994).). Lipases are one of the most important hydrolytic enzymes that can hydrolyze carboxyl esters of triglycerides into free fatty acids and partial acylglycerols. Compared to salt and solvent methods, the hydrolytic process happens at mild conditions, uses less energy and can avoid the formation of undesirable oxidation products, polymers, and the isomeric conversion of natural cis-PUFAs to deleterious trans-PUFAs. The reason that EPA and DHA can be concentrated by the lipase-assisted hydrolysis is because the 5 or 6 double bonds in EPA and DHA cause the

molecules to assume a bent, not linear configuration, so that they lie close to the ester bond and the lipase is less likely to hydrolyze the EPA and DHA ester bond (Bottino and others 1967). Many studies have been done to enrich fish oil with EPA and DHA in the form of glycerol (Sun and others 2002; Wanasundara and Shahidi 1998; Linder and others 2005). In most of the methods, fish oils were hydrolyzed with lipase as the catalyst and the FFA generated were neutralized with KOH or NaOH, followed by enriched oil separation by hexane. One disadvantage with these processes is that a high amount of organic solvents and chemicals were used to produce the oil enriched with EPA and DHA. The presence of organic solvents and chemicals might have negative health effects which necessitates their removal. These multi-step processes, including solvent and chemical removal are another disadvantage which includes the need for a considerable amount of equipment.

Moisture, dust, free fatty acids, phosphatides, oxidation products, pigments and trace elements (e.g. copper, iron, sulfur and halogens) were found in raw fish oils (Young and others 1994). Conventionally a series of purification steps including degumming, neutralization, bleaching, and distillation are required to remove these impurities. Fish oils, especially those containing higher concentrations of unsaturated fatty acids, are prone to oxidation during these processes because of duration of processing and extreme conditions employed. Adsorption processes could be an alternative way of purifying fish oils with low cost and oil loss. In this study, a novel enzymatic hydrolysis process was developed and used for enriching fish oils with EPA and DHA in form of acylglycerol. This method did not use chemicals to inactivate the enzyme or to neutralize the free fatty acids. In general, a multi-step process is used to produce enriched fish oils. In this study, a combined continuous two-step method (enrichment followed by purification) was developed for enriching fish oils with EPA and DHA.

5.2 Materials and Methods

5.2.1 Sample Preparation

Unrefined menhaden fish oil (MO) extracted using a rendering process was obtained from a commercial source (Omega Protein Inc., Houston.TX). Unrefined salmon oil (SO) was produced from processing salmon byproducts including viscera, heads, skins, frame, and discarded fish obtained from a large commercial plant in Alaska. The microbial lipase (*Candida Rugosa, CR*) was purchased from Sigma-Aldrich Co., St. Louis, MO.

5.2.2 Determination of Enzyme Activity

A commercially available microbial lipase (*Candida Rugosa, CR*) was selected in this study to hydrolyze the fish oils. CR lipase is extracted from yeast and non-specific to the fatty acid positions on triglycerides. The optimum temperature and pH are 30-50°C and 7.0. Enzyme activity of the lipase was determined by the enzymatic assay of lipase (EC3.1.1.3) from Sigma-Aldrich substituting menhaden or salmon oil for olive oil. Free fatty acids (FFA) released by the hydrolysis reaction (30 min) were titrated against 0.5 N sodium hydroxide and the pH changes were monitored by adding 0.1 mL thymolphthalein indicator solution (0.9% w/v). One unit of enzyme activity (U) was defined as the amount of enzyme that liberated 1 μmol of fatty acid in 1 h at 37°C.

5.2.3 Hydrolysis Reaction

The hydrolysis of fish oils by CR lipase and the separation of the EPA and DHA enriched fraction were carried out by the following procedure. Enzyme powder representing different amounts of lipase was dissolved in 25ml of phosphate buffer at pH 7.0 and then was mixed with 50g of MO or SO in an amber bottle. The air in the bottle was replaced by N_2, and the bottle was capped to minimize lipid oxidation. The hydrolysis reaction was maintained at 37°C in an incubator shaker (model 3525, LAB-LINE Instruments Inc., Melrose park, IL) at 250 rmp for a

certain period and lipase was separated by centrifugation at 10,000 rpm for 10min at 10ºC. Three layers were formed in the centrifuge tubes after centrifugation. The bottom layer was the aqueous phase, the middle layer was the enzyme powder and small amount of fish oil or impurities in the fish oil, and the upper layer was the desirable fish oil with high amounts of free fatty acid. The fish oils were collected and based on the previous study on oil purification, activated alumina (AA) was used to absorb the FFA from the fish oils. Sixty percent (w/w) of AA was added to the fish oil and agitated with a magnetic stir bar at 60°C for 1h. The AA, which was saturated by free fatty acids were separated with by centrifugation at 12,000 rpm for 10 min at 4°C. This step was repeated until all the FFA was removed from the oil. Final EPA and DHA enriched menhaden (MOE) or salmon oil (SOE) was flushed with N_2 and stored at -20°C until further use.

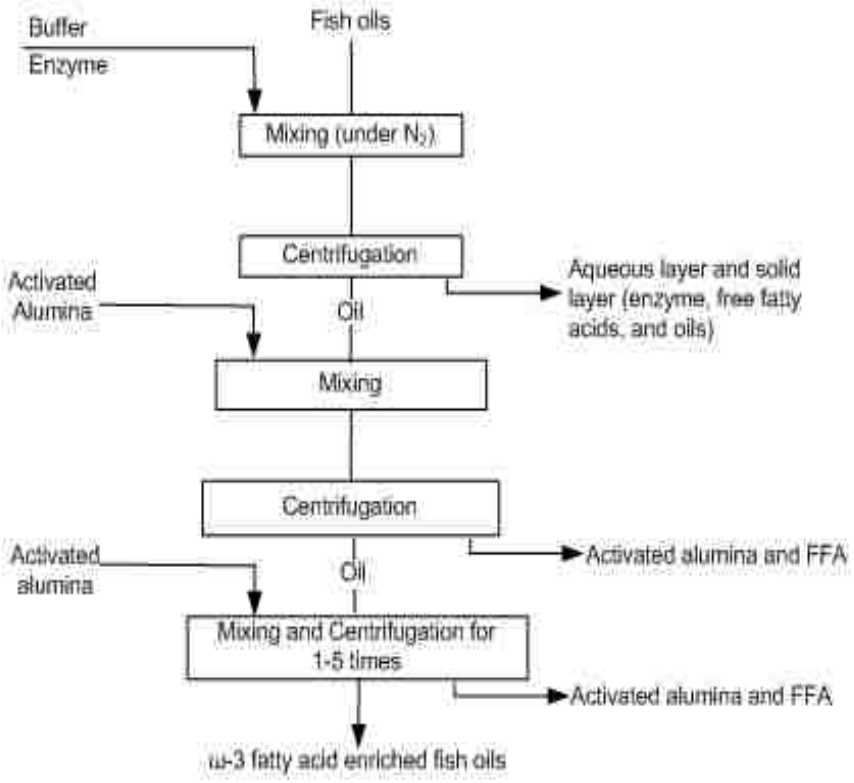


Figure 5.1 - enzymatic hydrolysis of menhaden or salmon oil and separation of acylglycerols and free fatty acids.

5.2.4 Kinetic Study of the Hydrolysis Process of the Fish Oils

The changes in the free fatty acids during the hydrolysis process were described by *Michaelis-Menten* equation (Holmberg 1982):

$$v = \frac{v_{\max} s}{K_m + s} \qquad \text{(Eq.5.1)}$$

where v is the reaction rate, s is the concentration of reactant; v_{max} is the maximum rate at infinite reactant concentration, and K_m is the *Michaelis* constant for the reactant.

This model can be used to describe the changes in FFA of the oils as a function of time because they have similar curve shape. So Eq. 5.1 can be modified as follow: s for t, where t is the hydrolysis time, v_{max} for F_E and K_m for t_H. F_E has units of hydrolysis percentage and t_H has units of time. As a result the empiric equation of *Michaelis-Menten* is formed as follow:

$$F(t) - F(0) = \frac{F_E * t}{t_H + t} \qquad \text{(Eq.5.2)}$$

F(t) is the FFA percentage at time t, F(0) is the FFA content in the raw oils, t is the hydrolysis time (h), F_E and t_H are constants.

From equation 5.2, when $t \to \infty$,

$$\lim_{t\to\infty}(F(t)) = \lim_{t\to\infty}[F_0 + \frac{F_E * t}{t_H + t}] = F_0 + F_E \qquad \text{(Eq.5.3)}$$

So F_E represents the increases of the FFA content at the end of the process with infinite time, t_H represents the time at which the process reach a FFA content corresponding to the half of the total change (F_E).

FFA of the fish oils were determined as described in 2.2.1. The model fitting to the experimental data was carried out using a curve fit program (CurveExpert 1.4, Copyright Daniel Hyams). The fit of the model was based on the correlation coefficient (R^2).

5.2.5 Determination of Degree of Hydrolysis (DH) in the Hydrolyzed Fish Oils

Degree of hydrolysis (DH) was determined by measuring the acid value of both raw and hydrolyzed oil as well as saponification value of raw oil according to American Oil Chemists' Society (AOCS) methods (AOCS 1998). Blanks (no enzyme) were determined at each treatment. DH was calculated according to the following equation:

$$DH\ (\%) = \frac{acid\ value\ (hydrolyzed\ oil) - acid\ value\ (raw\ oil)}{saponification\ value\ (raw\ oil) - acid\ value\ (raw\ oil)} \times 100 \quad \text{(Eq.5.4)}$$

where, acid value is expressed as the number of mg of KOH required to neutralize free fatty acids present in 1 g of oil; the saponification value is defined as the number of mg of KOH required to saponify 1 g of oil.

5.2.6 Analysis of Acylglycerol Composition of Hydrolyzed Fish Oils

Acylglycerol composition was analyzed at the laboratory of the W. A. Callegari Environmental Center, Louisiana State University, LA following the ASTM international standard method D-6584 with minor modification. A 20 mg sample was weighed into a 10 mL septa vial. Using microlitre syringes, exactly 100 μL of each internal standard and MSTFA were added. After shaking the vials, they were allowed to sit for 15 to 20 min at room temperature. Approximately 2 mL of hexane were added to the vial and shaken. One μL of the reaction mixture was injected into the cool on-column injection port of the gas chromatography. The initial temperature was 50°C and it was held for 1min, then the temperature was increased to 180°C at a rate of 15°C/min, and then 7°C/min to 230°C, finally 30°C/min to 380°C and held for 10 min. Helium was used as the carrier gas with a flow rate of 3 mL/min and a flame ionization detector was used to detect the peaks. The peaks were identified by comparing retention times with the standards. The mono-, di, and triglycerides were separated according to carbon numbers (CN). Monoglycerides consist of the four overlapping peaks with relative retention

times (RRT) of 0.76 and 0.83 to 0.86 with respect to the internal standard tricaprin. The grouping of 3 to 4 peaks with RRT of 1.05 to 1.09 (CN 34, 36, and 38) was attributed to diglycerides. Peaks with RRT of 1.16 to 1.31 (CN 52, 54, 56, and 58) were included in the calculation.

5.2.7 Changes during the Enrichment of EPA and DHA in the Fish Oils

The menhaden and salmon oils were hydrolyzed with lipase at different units (50, 250, 500, 1250, 2500, and 5000U) for 1h. The FFA and DH were determined after each hydrolysis treatment.

The menhaden oils were treated according to the procedure described by Figure 5.1 with 50, 250, and 1250 U of lipase for 3, 6, 12, and 24h, while salmon oils were hydrolyzed with 250, 500, and 2500 U of lipase for 3, 6, 12, and 24h. The DH and acylglycerol fractions (MG, DG, and TG) were determined after the hydrolysis reaction. The final oils with no FFA were collected for fatty acid composition analysis.

The enzyme amount and hydrolysis time during the process were optimized with regards to high EPA and DHA production. Menhaden and salmon enriched with EPA and DHA were produced at the optimum conditions and analyzed for the fatty acid composition, PV, FFA, TBARs, rheological properties, and color.

5.2.8 Fatty Acid Methyl Ester (FAMEs) Composition of the Fish Oils

Fatty acid composition of oil samples was determined by the method described in 3.2.4.

5.2.9 PV, FFA, TBARs, and Color of the Raw Fish Oil and Final Oil Enriched with EPA and DHA

PV and FFA of the fish oils were determined as described in 2.2.1.

A modification method according to Mei and others (1998) was employed for measuring the TBARs of emulsion samples. A thiobarbituric acid (TBA) solution was prepared by mixing 15 g of trichloroacetic acid, 0.375 g of TBA, 1.76 mL of 12 N HCl, and 82.9 mL of H2O. TBA

solution (100mL) was mixed with 3 mL of 2% butylated hydroxytoluene in ethanol, and 2 mL of this solution was mixed with 6mg of oil sample. The mixture was vortexed for 10 sec and heated in a boiling water bath for 15 min. After the mixture cooled down to room temperature, it was centrifuged at 3400 × g for 25 min. The absorbance of the supernatant was measured at 532 nm. Concentrations of TBARS were determined from standard curves prepared with 0-0.02 mmol/L 1, 1, 3, 3-tetraethoxypropane.

Color of the powders was determined using the chroma meter LABSCAN XE (Hunterlab, VA, USA). The data of colors were reported in CIELAB color scales (L* value is degree of lightness to darkness, a* value is degree of redness to greenness, and b* value is degree of yellowness to blueness). Chroma and hue angle value were calculated as following:

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} \qquad \text{(Eq.5.5)}$$

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \qquad \text{(Eq.5.6)}$$

5.2.10 Rheological Properties of the Raw Oils and Oils Enriched with EPA and DHA

Rheological properties of the oil samples were measured according to 3.2.5.

5.2.11 Statistical Analysis

Analysis of Variance (ANOVA) was conducted to evaluate the significance of observed differences among treatment means (SAS version 8.2, SAS Institute Inc., Cary, NC), followed by the post-hoc Tukey's studentized range test (SAS 2002).

5.3 Results and Discussion

5.3.1 Degree of Hydrolysis (DH) of the Fish Oils Hydrolyzed with Different Amount of Lipase for 1h

The purpose of this step was to optimize the lipase amount with respect lowering the cost of the process. DH of menhaden and salmon oils hydrolyzed with 50, 250, 500, 1250, 2500, 5000U of lipase for 1h are shown in Figure 5.2 and Figure 5.3. DH increased with the increase of lipase amount. With lipase of 50U, 250U, 500U, 1250U, and 2500U, there was a big difference

in the DH values of MO and SO, while when higher amounts of lipase were used (5000U), the difference was smaller. After hydrolysis with 2500U of lipase, the MO and SO had DH of 51.36% and 62.51%, respectively. To optimize the hydrolysis time based on the total EPA and DHA concentration in the final oil, the lipase amounts of 250, 500, and 5000U were selected for menhaden oil hydrolysis for up to 24h, and lipase amounts of 50, 250, and 1250U were selected for salmon oil hydrolysis for up to 24h.

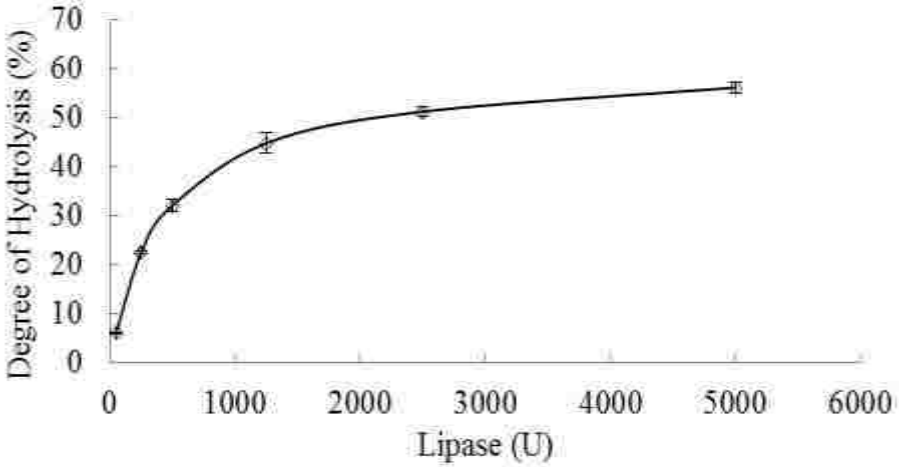


Figure 5.2 - Degree of hydrolysis (DH) of the menhaden oil hydrolyzed with different amount of lipase for 1h.

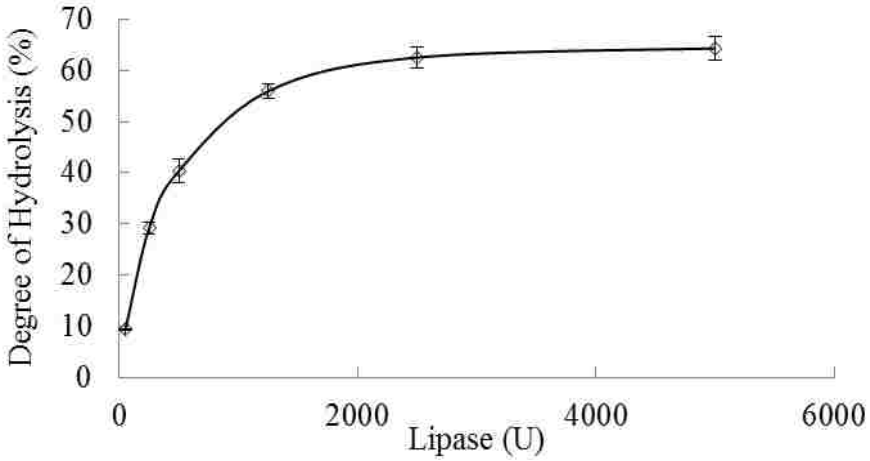


Figure 5.3 - Degree of hydrolysis (DH) of the salmon oil hydrolyzed with different amount of lipase for 1h.

5.3.2 Kinetics of the Hydrolysis Process of the Fish Oils

Figures 5.4 and 5.5 show the FFA contents as function of hydrolysis time with different enzyme activity for menhaden (250, 500, and 2500U) and salmon (50, 250, and 1250U)oils. The

results were fit using the *Michealis-Menten* model as function of hydrolysis time. F_E, t_H , and the correlation coefficient (R^2) were obtained for each oil at different enzyme activities. F_E represents the increases of the FFA content at the end of the process with infinite time, t_H represents the time at which the process reach a FFA content corresponding to the half of the total FFA change. Higher amount of enzymes caused higher F_E, which indicates that the enzyme amount could affect the final degree of hydrolysis. The FFA changes of menhaden oils hydrolyzed at 250, 500, and 2500 U lipase were 60.88, 65.86, and 80.62% at the end of the process, respectively, according to the *Michealis-Menten* model. Salmon oils hydrolyzed at 50, 250, and 1250U of lipase had FFA changes of 62.40, 66.84, and 82.58% at the end of the hydrolysis process. When more enzymes were applied, smaller t_H was obtained, which means that the half time of the hydrolysis reaction was affected by the enzyme amount. The t_H for both oils ranged among 1.13 to 2.34h except the salmon oil hydrolyzed with 50 U of lipase (t_H = 8.31h). This was caused by the comparatively extremely low level of lipase added to the salmon oil. R^2 for both oils at different enzyme activities were from 0.996-1.000. It means that the *Michealis-Menten* model is suitable to predict the kinetic of the hydrolysis of menhaden and salmon oils. And the parameters calculated from this study could be used to predict the changes on FFA contents at any time of hydrolysis.

Table 5.1 - F_E, t_H, and R^2 obtained from the Michealis-Menten model for menhaden oil hydrolysis

	F_E (%)	t_H (h)	R^2
250 U	60.88	2.34	0.999
500 U	65.86	1.55	0.996
2500 U	80.62	1.13	0.998

Table 5.2 – F_E, t_H, and R^2 obtained from the Michealis-Menten model for salmon oil hydrolysis

	F_E (%)	t_H (h)	R^2
50 U	62.40	8.31	0.999
250 U	66.84	1.61	0.998
1250 U	82.58	1.19	1.000

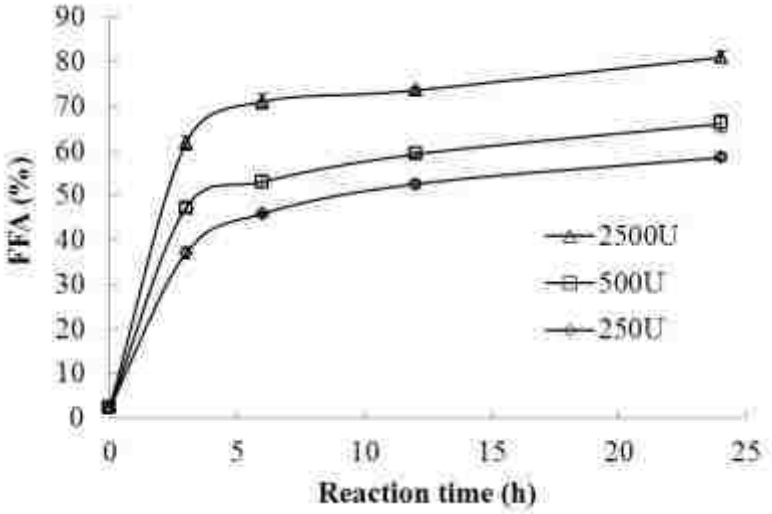


Figure 5.4 - Degree of hydrolysis (DH) of the salmon oil hydrolyzed with different amount of lipase for 1h.

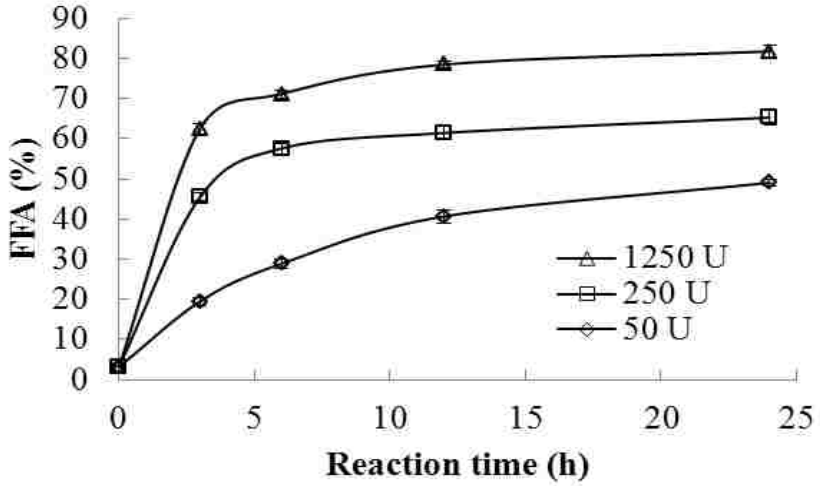


Figure 5.5 - Free fatty acid contents of the salmon oil hydrolyzed with different amount of lipase for 3, 6, 12, and 24h.

5.3.3 Degree of Hydrolysis (DH) of the Fish Oils Hydrolyzed with Different Amount of Lipase for 3, 6, 12, and 24h

The DH increased with the increase of the lipase amount from 250U to 2500U for menhaden oil, and a similar effect was found for the DH of the salmon oil with lipase from 50 to 1250U. Both menhaden and salmon oils were hydrolyzed rapidly during the initial 3h. Afterwards, hydrolysis was more gradual, and only minor increases were shown throughout the 6, 12, and 24h time periods. The highest DHs (81.64% and 81.47%) were shown by 2500 U and

1250 U lipase treatment at 24 h for MO and SO, respectively (Figures 5.6 and 5.7). Sun and others (2002) reported that the CR lipase hydrolyzed salmon viscera oil a DH of 70% at 80h with 800U/g oil. A DH of 70% was obtained for menhaden oil after hydrolyzing for 75h under 200 U/g of *Rhizopus oryzae* lipase (Wanasundara and Shahidi 1998), while the DH of sardine oil was 78.40% when *Candida rugosa* lipase was applied at 500 U for 9 h (Okada and Morrisesey 2007).

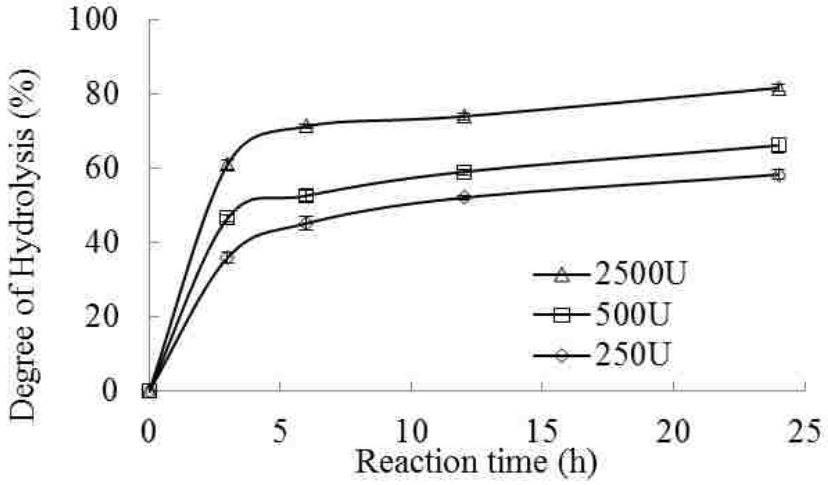


Figure 5.6 - Degree of hydrolysis (DH) of the menhaden oil hydrolyzed with different amount of lipase for 3, 6, 12, and 24h.

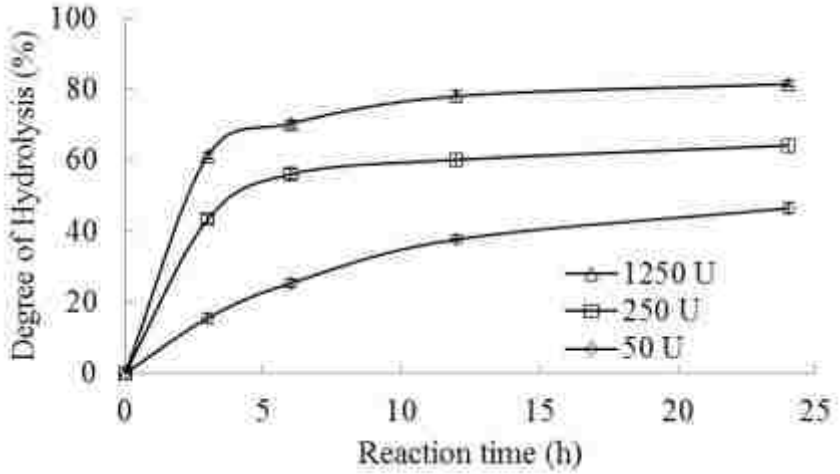


Figure 5.7- Degree of hydrolysis (DH) of the salmon oil hydrolyzed with different amount of lipase for 3, 6, 12, and 24h.

5.3.4 Changes during the Enrichment of EPA and DHA in the Fish Oils

Figure 5.8 shows the changes in C16:0, C16:1n7, EPA, DHA and EPA+DHA content in the final menhaden oil obtained after oil lipase-catalyzed hydrolysis with 250, 500, 2500 U/g oil

followed by FFA removal with the adsorption process. Different amounts of lipase caused changes in fatty acid composition in the menhaden oil. Ideally, enzymatic activity followed by FFA removal will increase EPA and DHA concentrations while reducing saturated and mono-unsaturated fatty acids in a period (Okada and Morrisesey 2007). The results showed that the contents of C16:0, C16:1n7, EPA, DHA and EPA+DHA of the menhaden oil were affected by enzyme amount and reaction time. The C16:0 content decreased significantly ($p > 0.05$) from 22.58% to 17.05% after 3h and gradually decreased to 13.09% after 24 h with 250 U lipase; the same pattern was observed with lipase at 500 and 2500U, except that C16:0 presented at slightly lower levels, ranging from 22.58% to 10.44% and 22.58% to 10.84%. Also, levels of 16:1n7 decreased significantly after 3h from 12.97% to 7.02%, 6.88%, and 7.60% with 250 U, 500 U and 2500 U, respectively; however the levels remained relatively constant for the rest of the hydrolysis reaction time (up to 24 h). With CR 250 U, EPA content significantly increased from 13.77% to 19.52% after 3 h and remained at relatively constant levels after 6 and12h (20.34% and 21.70%, respectively), but after 24h of hydrolysis, the EPA content decreased to 20.66%, which means the lipase started to facilitate hydrolysis of EPA at 24h with 250 U lipase. A similar tendency was observed with CR 500 U except that EPA was present at slightly higher levels, ranging from 13.77% to 22.01%. When 2500 U lipase applied, EPA levels reached the highest amount at 3h to 21.64% and started to drop down to 16.37%. This result indicated that a high amount of EPA was removed by the hydrolysis reaction. DHA levels also increased significantly after 3 h, from an original of 7.32% to 11.76% with 250 U, to 12.90% with 500U lipase, and to 17.26% with 2500U lipase. Gradual increases in DHA concentration from 11.76% to 14.34% with 250 U, 12.90% to 17.53% with 500U, and 17.26 to 22.55% with 2500 U occurred as hydrolysis continued to 24 h, and there was significant difference between 3 h and

longer reaction times. The highest total EPA and DHA fraction (39.54% and 39.95%) in the menhaden oil was found in the oil hydrolyzed with 2500 U lipase for 6 and 12h.

Figure 5.9 shows the changes in C16:0, C16:1n7, C18:1n9, EPA, DHA and EPA+DHA content in the final salmon oil obtained after lipase-catalyzed hydrolysis with 50, 250, 1250 U/g oil followed by FFA removal with adsorption process. Similarly to menhaden oil, different amounts of lipase caused changes in fatty acid composition of salmon oil. The results showed that the contents of C16:0, C16:1n7, C18:1n9, EPA, DHA and EPA+DHA of the salmon oil were affected by enzyme amount and reaction time. The C16:0 content decreased significantly ($p > 0.05$)from 14.58% to 12.34% after 3h and gradually decreased to 9.30% after 24 h with 50 U lipase; the same pattern was observed with lipase at 250 and 1250U, except that C16:0 was present at much lower levels, ranging from 14.58% to 6.64% and 14.58% to 6.47%. Levels of 16:1n7 decreased significantly after 3h from 7.30% to 4.78%, 3.50%, and 3.76% with 250 U, 500 U and 2500 U, respectively; and then the levels gradually decreased to 3.27%, 3.12% and 3.18%, respectively, with reaction time up to 24 h. The C18:1n9 content decreased significantly from 16.15% to 13.43% after 3h and gradually decreased to 9.34% after 24 h with 50U lipase; the same pattern was observed with lipase at 250 and 1250U, except that it was present at much lower levels, ranging from 16.15% to 7.66% and 16.15% to 7.67%, respectively. With lipase 50 U, EPA content increased from 11.55% to 14.15% gradually after 24 h. With lipase 250 U, EPA content significantly increased from 11.55% to 14.32% after 3 h and remained at relatively constant levels after 6 and12h (15.04% and 15.54%, respectively), but after 24h of hydrolysis the EPA content decreased to 14.99%, which means the lipase started to facilitate hydrolysis of EPA at 24h with 250 U lipase. A similar tendency was observed for EPA with lipase 1250 U except that the highest content (15.49%) was achieved at 6h of hydrolysis and decreased to 14.14% at 24h of hydrolysis. DHA content increased from 8.59% to 12.10% gradually after 24 h with lipase

50 U. With lipase 250 U and 1250 U, DHA levels of the salmon oils increased significantly after 3 h, from an original of 8.59% to 11.59% and 8.59% to 15.68%, respectively. Gradual increases in DHA concentration from 11.59% to 15.56% with 250 U up to 24h, while with 1250 U, the highest level (17.01%) of DHA occurred at 12h of hydrolysis and then dropped to 16.28% after 24h of hydrolysis, which indicated that the hydrolysis reaction was hydrolyzing the DHA from the acylglycerol at 24h from salmon oil with 1250 U lipase. The highest total EPA and DHA fraction (32.12%) in the salmon oil was found in the oil hydrolyzed with 1250 U lipase for 6h.

From the result, we can conclude that it was easier to hydrolyze EPA than DHA for the CR lipase in both menhaden and salmon oils. It is because EPA (C20:5n3) has less carbons and shorter a chain than DHA (C22:5n3) and thus much easier for the access of the lipase to ester bond which connects the fatty acids to glycerides. It was reported that most lipases including *Candida rugosa* discriminate against DHA more than EPA (Mukherjee and others 1993).

The changes in levels of MG, DG, and TG in the unhydrolyzed menhaden oil and final menhaden oils that were hydrolyzed for 3, 6, 12, and 24h with 250, 500, and 2500 U of lipase are shown in Figure 5.8 (A, B, and C). The unhydrolyzed menhaden oil contained 73.53% TG, 14.13% DG, and 12.34% MG. TG levels were significantly less in all final n - 3 PUFA concentrate compared to the original menhaden oil. TG levels were significantly reduced from 73.53% to 45.92% with 250 U lipase after 3 h and finally decreased to 18.50% after 24 h. DG levels significantly increased from 14.13% to 33.18% after 3h of hydrolysis with 250 U lipase and then gradually decrease to 14.99% after 24 h of hydrolysis. That's because while the TG were hydrolyzed to DG, DG were hydrolyzed to MG at the same time, which resulted in a big increase in the MG level from 12.34% to 66.51% after 24h. The lipase at 500 U showed a similar trend as at 250 U, the only difference was that the TG and DG levels both had more decrease (TG: from 73.53% to 7.97% at 24 h, DG: from 14.13% to 9.03% at 24h). MG levels also

increased more than at 250 U (from 12.34% to 83.00% at 24h). There was a much steeper change on the TG, DG, and MG levels when 2500 U of lipase was applied to the menhaden oil for hydrolysis. After 3h of hydrolysis, the TG level decreased from 73.53% to 12.32% and gradually decreased to 3.43% after 24h. DG level had a constant decrease with the reaction time and dropped to 4.66% after 24h. MG level was elevated dramatically from 12.34% to 76.34% after 3h hydrolysis and eventually increased to 91.90% after 24h.

The changes in levels of MG, DG, and TG in the unhydrolyzed salmon oil and final salmon oils that were hydrolyzed for 3, 6, 12, and 24h with 50, 250, and 1250U of lipase are shown in Figure 5.9 (A, B, and C). The unhydrolyzed salmon oil contained 77.17% TG, 3.17% DG, and 19.53% MG. TG levels were significantly less in all final n - 3 PUFA concentrate compared to the original salmon oil. TG levels were significantly reduced from 77.17% to 57.57% with 50 U after 3 h and finally decreased to 27.99% after 24 h. DG levels significantly increased from 3.17% to 23.98% after 3h of hydrolysis with 50 U lipase and then gradually increased to 57.38% after 24 h of hydrolysis, which means most of the TG was hydrolyzed to DG after 24h. The lipase at 250 U showed a different trend than at 50 U, TG level decreased from 77.17% to 23.42%, but the DG levels were significantly increased to 30.95% at 12h and then dropped to 20.70% after 24h of hydrolysis. Also MG increased dramatically from 19.66% to 55.87% after 24 h hydrolysis. There was a more obvious change in the TG, DG, and MG levels when using 1250U of lipase. After 3h of hydrolysis, the TG level decreased from 77.17% to 49.80% and then decreased to 9.51% after 24h. DG level decreased to 26.13% after 6 h of hydrolysis and then dropped to 17.08% after 24h. MG level was raised dramatically from 19.66% to 73.40% after 24h of hydrolysis.

The tri-, di-, and monoglycerols in sardine oil changed from 86.20%, 13.40%, and 0.51% to 65.94%, 32.33%, and 2.08% after hydrolyzed with CR lipase for 9h at 250 U and similar

glycerol levels were found when 500 U of CR lipase were used (Okada and Morrissey 2007). The difference between the results in this study and previous study may be caused by different amounts lipase, the fatty acid profile of the fish oils, and/or the conditions for the treatment.

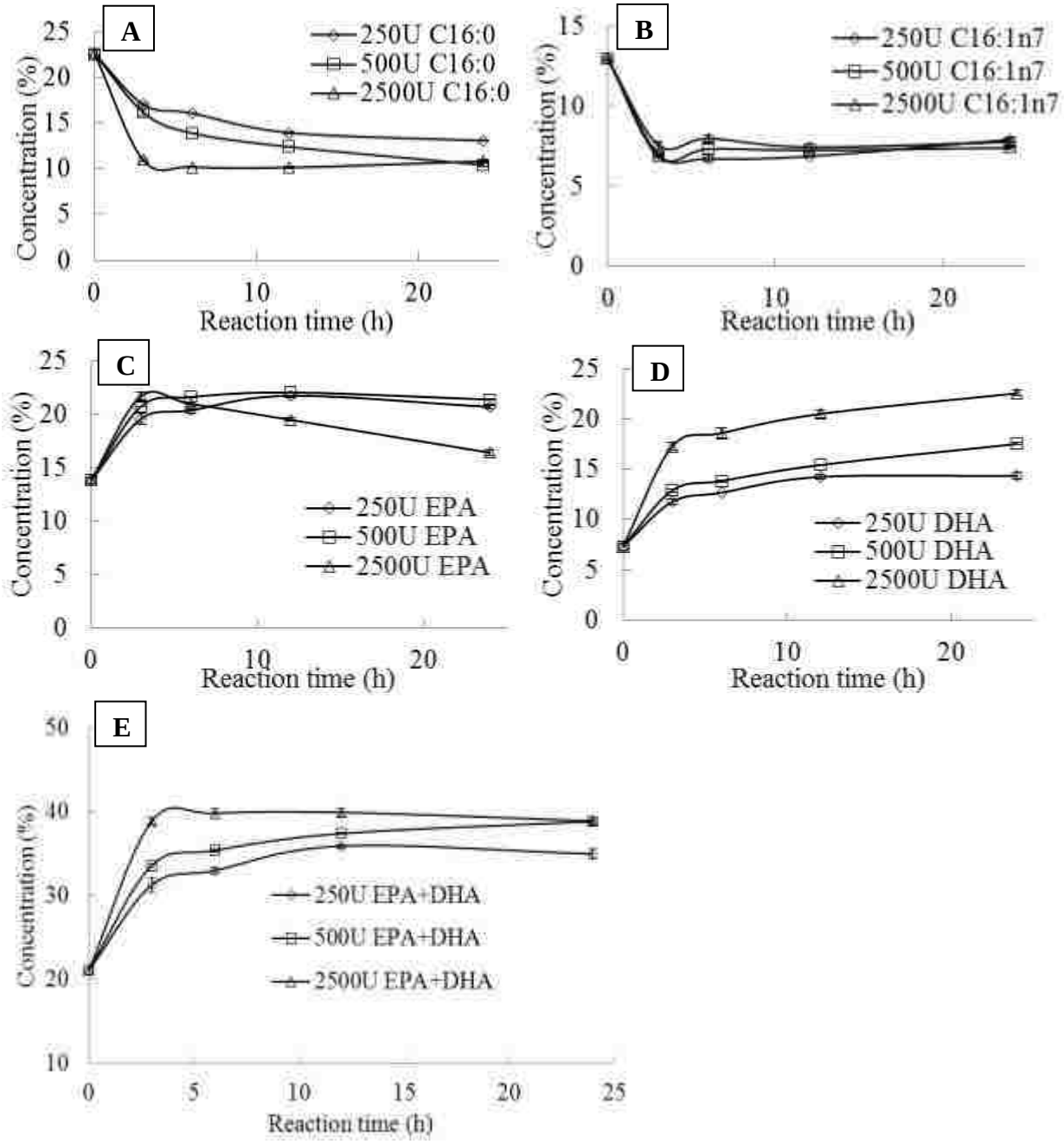


Figure 5.8 - Changes in C16:0, C16:1n7, EPA and DHA concentration (%) in final menhaden oils hydrolyzed with different enzyme activities for 3, 6, 12, and 24h. A, C16:0; B, C16:1n7; C, EPA; D, DHA; E, EPA+DHA.

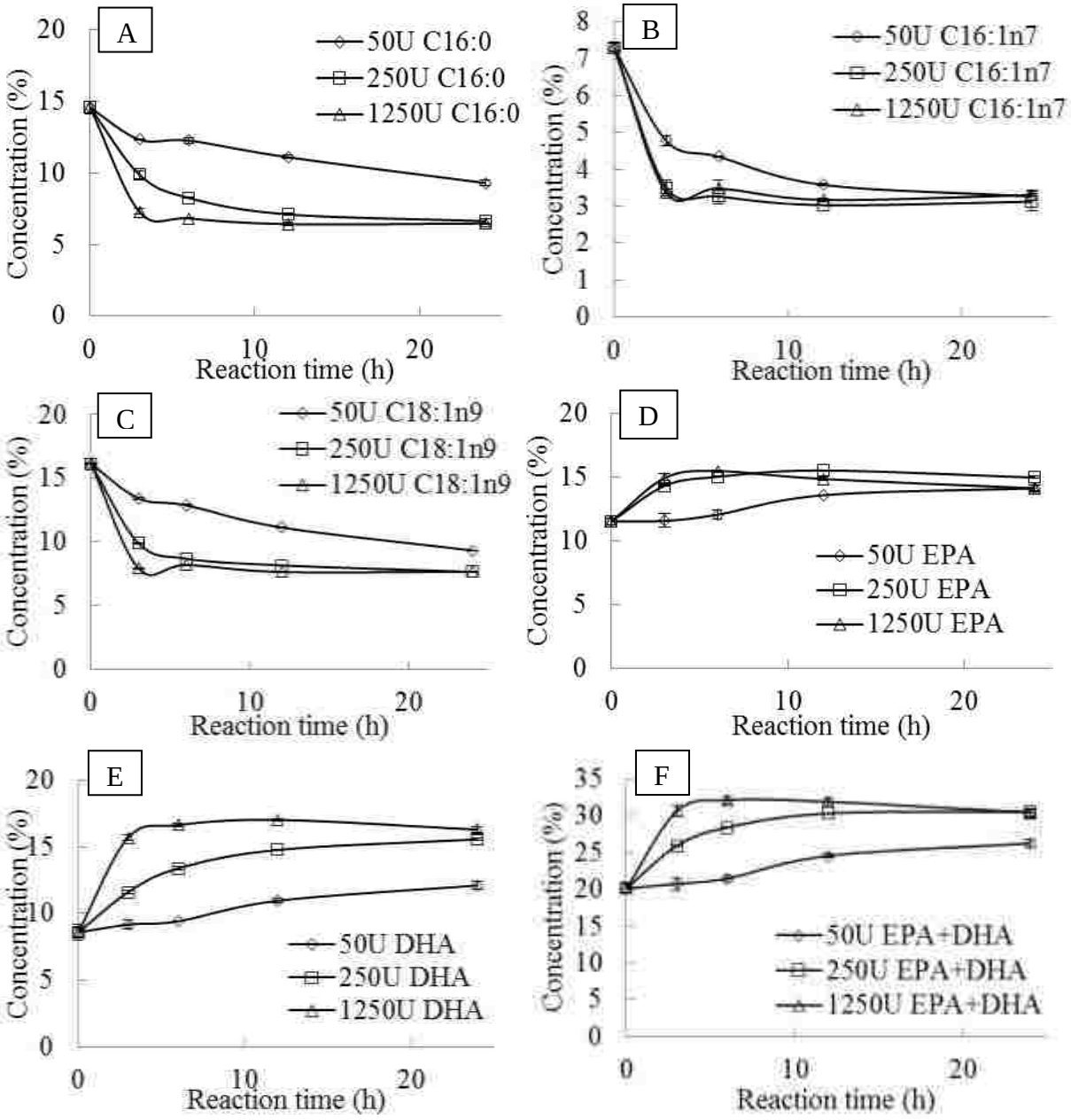


Figure 5.9 - Changes in C16:0, C16:1n7, C18:1n9, EPA and DHA concentration (%) in final salmon oils hydrolyzed with different enzyme activities for 3, 6, 12, and 24h. A, C16:0; B, C16:1n7; C, C18:1n9; D, EPA; E, DHA; F, EPA+DHA.

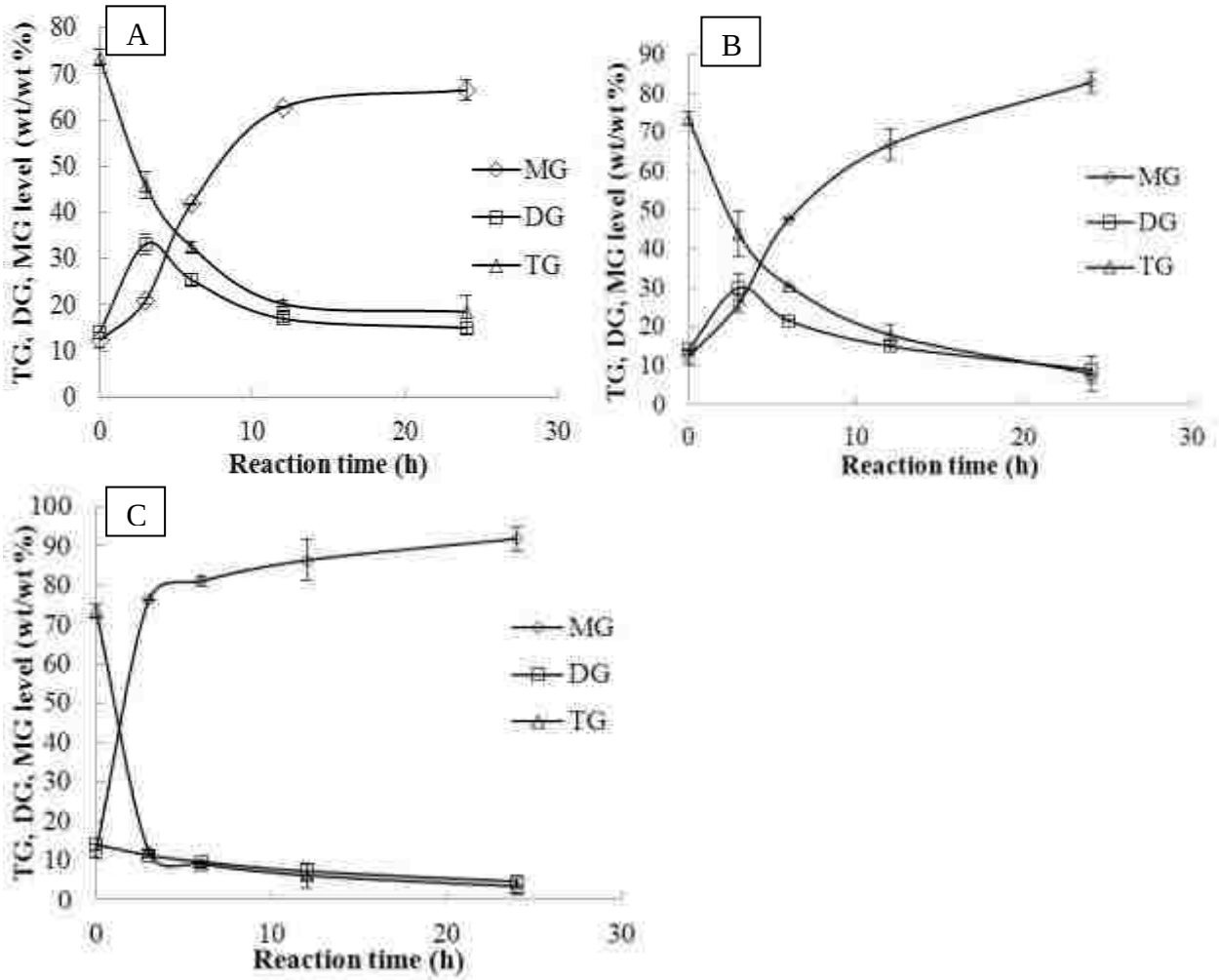


Figure 5.10 - Changes in TG, DG, and MG fractions of final menhaden oils hydrolyzed with different enzyme activities for 3, 6, 12, and 24h. A, 250U lipase; B, 500U lipase; C, 2500U lipase.

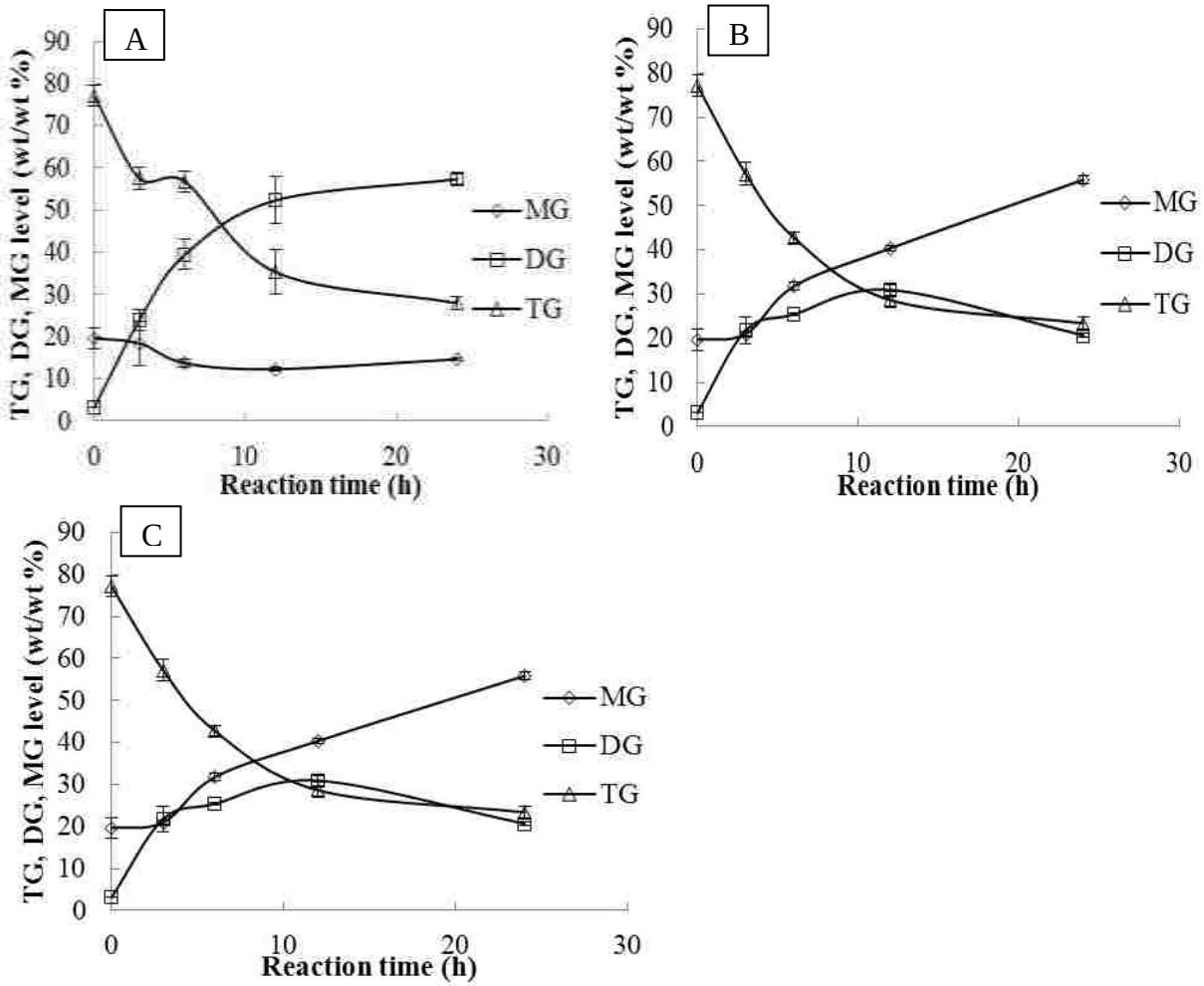


Figure 5.11 - Changes in TG, DG, and MG fractions of final salmon oils hydrolyzed with different enzyme activities for 3, 6, 12, and 24h. A, 50U lipase; B, 250U lipase; C, 1250U lipase.

5.3.5 PV, FFA, TBARs, EPA and DHA, and Color of the Raw and Final Fish Oils Enriched with EPA and DHA

Based on the previous steps, the optimum conditions for enrichment of EPA and DHA were selected for menhaden and salmon oils, which are 2500 U/6h and 1250 U/6h, respectively. These oils were characterized for the PV, FFA, TBARs, EPA, DHA, and color. The results are shown in Tables 5.3 and 5.4. After the hydrolysis and adsorption process, the PV, FFA, and TBARs values of menhaden oil all decreased from 11.06±0.75 meq/kg oil to 1.49±0.13meq/kg oil, from 2.66±0.07% to 0.67±0.06%, and from 0.89±0.01mmol/kg to 0.56±0.01mmol/kg, respectively. Similar tendency were observed for salmon oil, the process decreased the PV, FFA,

and TBARs of salmon oil from 14.71±1.26 meq/kg oil to 2.33±0.16meq/kg oil, from 3.14±0.07% to 0.66±0.05%, and from 1.26±0.02mmol/kg to 0.62±0.03mmol/kg, respectively. As indicated in previous study, the activated alumina was very effective in reducing FFA and also effective in reducing PV.

After the process, the menhaden oil had a darker (less L* value) and more yellowish and reddish color, while salmon oil became lighter and more yellowish and less red in color.

Table 5.3 – PV, FFA, TBARs, EPA and DHA, color of MO and MOE

	MO	MOE
PV (meq/Kg)	11.06±0.75[a]	1.49±0.13[b]
FFA (%)	2.66±0.07[a]	0.67±0.06[b]
TBARs (mmol/kg)	0.89±0.01[a]	0.56±0.01[b]
EPA (%)	13.77±0.27[b]	20.38±0.22[a]
DHA (%)	7.32±0.34[b]	18.47±0.68[a]
EPA+DHA (%)	21.09±0.61[b]	38.85±0.89[a]
L*	43.54±0.77[a]	40.24±0.06[b]
a*	12.11±0.06[b]	14.80±0.03[a]
b*	57.68±0.69[b]	66.96±0.11[a]
Chroma	58.93±0.68[b]	78.14±0.08[a]
Hue angle	68.58±0.12[b]	77.54±0.00[a]

MO = unrefined menhaden oil; MOE = menhaden oil concentrated with lipase at 2500 U/g oil for 6h. Each value is an average of three determinations with its SD. Values with the same superscript letters in each row are not significantly different ($p>0.05$)

Table 5.4 – PV, FFA, TBARs, EPA and DHA, color of SO and SOE

	SO	SOE
PV (meq/Kg)	14.71±1.26[a]	2.33±0.16[b]
FFA (%)	3.14±0.07[a]	0.66±0.05[b]
TBARs (mmol/kg)	1.26±0.02[a]	0.62±0.03[b]
EPA (%)	11.55±0.12[b]	16.27±0.11[a]
DHA (%)	8.59±0.03[b]	16.53±0.27[a]
EPA+DHA (%)	20.14±0.14[b]	32.80±0.38[a]
L*	42.94±0.07[b]	43.55±0.01[a]

(table 5.4 continued)

a*	14.38±0.08[a]	12.93±0.02[b]
b*	52.20±0.53[b]	55.35±0.08[a]
Chroma	54.14±0.53[b]	74.60±0.06[a]
Hue angle	56.84±0.08[b]	76.86±0.00[b]

SO = unrefined salmon oil; SOE= salmon oil concentrated with lipase at 1250 U/g oil for 6h. Each value is an average of three determinations with its SD. Values with the same superscript letters in each row are not significantly different ($p>0.05$)

5.3.6 Rheological Properties of the Raw and Final Fish Oils Enriched with EPA and DHA

The flow behavior index (*n*), consistency index (K) values, and apparent viscosity of the raw and concentrated oils at a shear rate of 200 s^{-1} were measured at 25°C. The results were listed in Tables 5.5 and 5.6. The flow behavior index the raw menhaden oil was 0.825, which was much less than 1, which indicates it exhibited shear thinning properties (Paredes and others 1989). The MOE, SO, and SOE all had *n* values close to 1, and are considered as Newtonian behavior fluids. The consistency index is an indicator of the viscous nature fluid, and higher K values indicate a more viscous consistency (Paredes and others 1989). After the enzyme process, both MOE and SOE had less K values, i.e., from 0.195 $Pa.s^n$ to 0.035 $Pa.s^n$ and 0.048$Pa.s^n$ to 0.041 $Pa.s^n$, respectively. This result was in consistent with the apparent viscosity of the oil samples, which also decreased after the enzyme treatment. The reduction in the viscosity of both menhaden and salmon oils was caused by removing impurities such as phospholipids and FFA from the oils.

Table 5.5 - Rheological properties of the MO and MOE

	n	K ($Pa.s^n$)	Viscosity (Pa s)
MO	0.825±0.009[b]	0.195±0.011[a]	0.077±0.001[a]
MOE	0.971±0.022[a]	0.035±0.004[b]	0.030±0.001[b]

MO = unrefined menhaden oil; MOE = menhaden oil concentrated with lipase at 2500 U/g oil for 6h. Each value is an average of three determinations with its SD. Values with the same superscript letters in each column are not significantly different ($p>0.05$)

Table - 5.6 Rheological properties of the SO and SOE

	n	K (Pa.s^n)	Viscosity (Pa s)
SO	0.982±0.005^a	0.048±0.001^a	0.044±0.001^a
SOE	0.987±0.002^a	0.041±0.001^b	0.038±0.000^b

SO = unrefined salmon oil; SOE= salmon oil concentrated with lipase at 1250 U/g oil for 6h. Each value is an average of three determinations with its SD. Values with the same superscript letters in each column are not significantly different ($p>0.05$)

5.4 Conclusions

The highest DHs (81.64% and 81.47%) of menhaden and salmon oils were obtained by 2500 U and 1250 U lipase treatment for 24 h, respectively. Menhaden oil treated with 2500 U lipase for 6 and 12h had the most total EPA and DHA fractions (39.54% and 39.95%) in the final oil, while the highest total EPA and DHA fractions (32.12%) in the salmon oil were found in the oil hydrolyzed with 1250 U lipase for 6h. The unhydrolyzed menhaden oil contained 73.53% TG, 14.13% DG, and 12.34% MG. After treated with 2500G lipase for 24h, the TG and DG levels of menhaden oil decreased to 3.43% and 4.66%, while MG level increased to 91.90%. The unhydrolyzed salmon oil contained 77.17% TG, 3.17% DG, and 19.53% MG. After being hydrolyzed with 1250U of lipase for 24h, the TG, DG, and MG levels changed to 9.97%, 17.03%, and 72.87%. This process not only optimized the lipase treatment for enrichment of EPA and DHA concentrations in menhaden and salmon oils but also indicated the kinetics of the process in which the lipase transformed most of the TG into MG. After the EPA and DHA enrichment, PV, FFA, and TBARs values of menhaden oil (MOE) decreased from 11.06±0.75 meq/kg oil to 1.49±0.13meq/kg oil, from 2.66±0.07% to 0.67±0.06%, and from 0.89±0.01mmol/kg to 0.56±0.01mmol/kg. Similar tendencies were observed for salmon oil, the process decreased the PV, FFA, and TBARs of salmon oil from 14.71±1.26 meq/kg oil to 2.33±0.16meq/kg oil, from 3.14±0.07% to 0.66±0.05%, and from 1.26±0.02mmol/kg to 0.62±0.03mmol/kg. The MOE, SO, and SOE all had n values close to 1, and are considered as Newtonian behavior fluids, while MO was non-Newtonian fluid. The apparent viscosity significantly decreased after the enzyme

treatment. This study developed a novel enzymatic method in combination with the adsorption technology to purify and enrich EPA and DHA in fish oils without organic solvent and chemicals.

CHAPTER 6 MICROENCAPSULATION OF FISH OILS CONTAINING ENRICHED EPA AND DHA

6.1 Introduction

Omega -3 PUFAs, especially EPA and DHA, has been shown to have beneficial effects in the prevention and treatment of cardiovascular diseases (Bucher and others 2002; Hooper and others, 2003). The EPA and DHA enriched menhaden and salmon oils having greater levels of PUFAs, less saturated fatty acids, are much better than menhaden and salmon oils themselves (Wanasundara and Shahidi 1998). However the EPA and DHA enriched fish oils are more susceptible to oxidative deterioration during processing, distribution and handling due to their high degree of unsaturation. Oxidation leads to the formation of unpleasant appearance, tastes and odors, and free radicals, which may have negative physiological effects and leads to the reduction of product's shelf life (Ahn and others 2008). Microencapsulation of oils in a polymeric matrix (wall) is an alternative that has been used by many researchers in order to protect unsaturated fatty acids against lipid oxidation, thus increasing their shelf life (Ahn and others 2008; Drusch and others 2009; Bae and Lee 2008).

In the case of foods, the most common procedure for microencapsulation is spray drying. Microencapsulation with spray drying involves four stages: preparation of the dispersion or emulsion; homogenization of the dispersion; atomization of the infeed emulsion; and dehydration of the atomized particles (Shahidi and Han 1993). The mixture to be atomized is prepared by dispersing the core material, which is usually of hydrophobic nature, into a solution of the coating agent with which it is immiscible. The dispersion must be heated and homogenized, with or without the addition of an emulsifier depending on the emulsifying properties of the coating materials because some of them have interfacial activities. The obtained

emulsion is then dried by the evaporation of the solvent, usually water, which leads to the formation of microcapsules.

Emulsification is a critical process for microencapsulation, in which many variables can influence the processing and the final characteristics of the product (Franco and others 1995). To improve the emulsification process, droplet size of the dispersed phase and its polydispersity, as well as knowledge of its viscous and viscoelastic properties should be measured, because all of them are related to the emulsion stability (Carrillo and Kokini 1988).

Proteins and peptides are reported for many benefits including stimulating muscle protein synthesis and accelerating muscle recovery (Suryawan and others 2008; Tischler and others 1982; Layman and Rodriguez 2009), as well as cardiovascular health benefits (Ballard and others 2009; Lin and others 2004; Anderson and others 1995). Egg protein contains abundant of leucine which can contribute to muscles' ability to use energy and help post-exercise muscle recovery (Layman and Rodriguez 2009). Whey protein has higher concentrations of branched chain amino acids (BCAA including leucine, isoleucine, and valine) so that when short-term glycogen stores are expended during exercise they can be utilized by muscle to provide energy (Kasperek and Snider 1987; Wagenmakers 1998). Soy protein has beneficial impacts on the risk factors for cardiovascular disease (Lin and others 2004, Anderson and others 1995). Collagen exhibits superior biocompatibility comparing with other natural polymer because collagen is one of the major components of extracellular matrix and plays important roles in various biological events. Peptides are widely accepted and produced because of the rapid digest speed and the health benefits to cardiovascular systems. Proteins are considered to be the most commonly single used class of emulsifying agent used in the food industry. They are the ideal ingredients for food processors because of the natural, non-toxic, cheap and widely available properties. Tonon and others (2011) compared the different mechanisms of protein and emulsifier in stabilizing food

emulsions: Proteins stabilize emulsions by forming a viscoelastic adsorbed layer and the extent of protein adsorption to the layer is influenced by surface hydrophobicity and charge. Emulsifiers are more surface-active than proteins and can form a compact adsorbed layer, which relies on charge repulsion to stabilize emulsions.

A blend of protein can create a "time-release" effect that could prolong the delivery time of the absorbed amino acids to muscle thus resulting in a faster muscle recovery (Paul 2009). In addition, consumption of a mix of proteins may have more benefits for sports performance and provides nutritional advantages over consuming only one type of protein. Therefore, the objective of the study was to develop a protein powder containing fish oil with enriched EPA and DHA targeting people seeking products having benefits on the cardiovascular system or/and managing weight by exercise.

6.2 Materials and Methods

6.2.1 Emulsion Preparation

The protein/peptide mix composed of whey protein (donated by Le Sueur Food Ingredient Co., Le Sueur, MN), egg protein (Jay Robb Enterprises Inc., Carlsbad, CA), soy protein (Whole Foods Market, Austin, TX), collagen (donated by Gelita AG, Sergeant Bluff, IA), gelatin (beefhide gelatin, donated by Gelita AG, Sergeant Bluff, IA), NOP-47 (donated by Glanbia Nutritionals Inc.,Carlsbad, CA), and peptopro (donated by DSM Nutritional Products, Ames, IA) at the same ratio (100g) was dissolved into water (850g) in a 1L beaker and stirred using a RCT B S1 magnetic stir (IKA Labrotechnik, Janke & Kunkel GmbH & Co., Staufen, Germany) at room temperature. After dissolving, the concentrated menhaden/salmon oil (50g) along with 200ppm (based on oil weight) of rosemary oil was added to the beaker and they were homogenized well using a hand blender (Cuisinart Inc., East Windsor, NJ, USA) for 2 min. An ultrasonic processor (500 Watt Model, Cole-Parmer Instrument Co. Vernon Hill, IL) was applied

for 5 min for further homogenization and production of stable emulsions (MOEE and SOEE). The emulsion containing menhaden or salmon oil without addition of rosemary oil was prepared as control (MOEEC and SOEEC) to compare the antioxidant effect of the rosemary oil (Hawaiian Gardens, CA).

6.2.2 Emulsion Stability, Moisture, Rheology Properties, and Microstructure of Emulsions Containing Enriched Fish Oils

Emulsifying stability was carried out according to the method of Min and others (2003) with minor modifications. The emulsion sample (5 g) was placed into a 10 mL centrifugal tube and stored at -20ºC for 2 days and then allowed to thaw at room temperature for 3 hr. The thawed sample was centrifuged at 15,000 g for 40 min at -2ºC and the amount of oil separated was measured. Oil recovery % was calculated as (weight of oil recovered/ 5 g of emulsion sample) × 100. Moisture content in the emulsions was analyzed in triplicate following the AOAC standard methods 930.15 (AOAC 1999).

Rheological properties were measured using an AR 2000 ex Rheometer (TA Instruments, New Castle, DE) fitted with plate geometry (steel plates with a 40-mm diameter and a gap of 200 μm). The sample was placed in the temperature-controlled parallel plate and allowed to equilibrate to 4, 10, and/or 25ºC. The shear stress was measured at shear rates ranging from 10 to 200 s^{-1}. The power law (Eq. 6.1) was used to analyze the flow properties of the emulsion samples.

$$\sigma = K\gamma^n \qquad \text{(Eq. 6.1)}$$

Where σ = shear stress (Pa.s), γ = shear rate (s^{-1}), K = consistency index ($Pa.s^n$), and n = flow behavior index. The logarithms were taken on both sides of Eq. 6.1, and a plot of log σ versus log γ was constructed. The resulting straight line yielded the magnitude of log K (i.e., intercept) and n (i.e., slope). The mean values of n, K, and apparent viscosity (at 200 s^{-1}) of

triplicate samples were reported. The frequency sweep test was conducted from 0.6283 to 62.82 rad/s at a constant temperature of 25ºC.

The storage modulus and loss modulus of MOEE and SOEE were obtained using Universal Analysis (TA instrument) software and were calculated using Eq. 6.2 and 6.3

$$G' = \left[\frac{\sigma_0}{\gamma_0}\right] \cos \delta \qquad \text{(Eq. 6.2)}$$

$$G'' = \left[\frac{\sigma_0}{\gamma_0}\right] \sin \delta \qquad \text{(Eq. 6.3)}$$

where G′ (Pa) is the storage modulus, G″ (Pa) is the loss modulus, tan δ is the loss tangent, σ is generated stress, and γ is oscillating strain.

The microstructure of the two emulsions was determined using a light microscope (Nikon Microphot-FXA, Nikon Instrument Inc., Japan). A drop of the emulsion was observed at an objective magnification of 80. Images of the emulsion structure were accessed using image processing software with a CCD camera.

6.2.3 Hydroperoxide, TBARs, and EPA, DHA Contents of the Fish Oils in Emulsion

The hydroperoxide values (PV) of emulsions were measured according to the method of Boon and others (2008). A 0.2g of the emulsion was added into 1.5 mL of isooctane-2-propanal (3:1 v:v) solution and vortexed for 30 s. The organic phase was separated by centrifugation at 3400 × g for 2 min. Then, 100 µL of organic phase was mixed with 2.8 mL of methanol-1-butanol (2:1 v:v) followed by adding 15 µL of 3.94 M thiocyanate solution and 15µL of ferrous iron solution (prepared by mixing equal volumes of 0.144 M $FeSO_4 \cdot H_2O$ and 0.132 M $BaCl_2$, centrifuged and collected the clear supernatant). The mixture was vortexed and allowed to react for 20 min at room temperature. The absorbance was measured at 510 nm using a spectrophotometer (Thermo Fisher Scientific, Vernon Hills, IL). Hydroperoxide content was determined using a cumene hydroperoxide standard curve.

Thiobarbituric acid-reactive substances (TBARS) of the emulsions were determined to evaluate the emulsion oxidation during processing. Emulsion containing fish oil without rosemary oil was used as control. A modified method according to Mei and others (1998) was employed for measuring the TBARs of emulsion samples. A thiobarbituric acid (TBA) solution was prepared by mixing 15 g of trichloroacetic acid, 0.375 g of TBA, 1.76 mL of 12 N HCl, and 82.9 mL of H_2O. TBA solution (100mL) was mixed with 3 mL of 2% butylated hydroxytoluene in ethanol, and 2 mL of this solution was mixed with 1 mL of emulsion sample. The mixture was vortexed for 10 sec and heated in a boiling water bath for 15 min. After the mixture cooled to room temperature, it was centrifuged at 3400×g for 25 min. The absorbance of the supernatant was measured at 532 nm. Concentrations of TBARS were determined from standard curves prepared with 0-0.02 mmol/L 1, 1, 3, 3-tetraethoxypropane.

Fatty acid composition (only EPA and DHA were reported) of oil samples was determined by the method described in 3.2.4.

6.2.4 Microencapsulation of Enriched Fish Oils

The emulsion was spray dried to produce microencapsulated menhaden and salmon oil powders (MOEP and SOEP) by a pilot plant scale FT80/81 Tall Form spray dryer (Armfield, Ringwood, UK). The parameters for the spray dryer including inlet and outlet air temperature, inlet and outlet air velocity, inlet and outlet air relative humidity, internal diameter of ambient air intake pipe and exhaust air pipe, and mass flow rate of emulsion are listed in Tables 7.1 and 7.2(Appendix). The velocity and temperature of inlet ambient and outlet air were recorded using an anemometer (Anemomaster Model 6162, Kanomax Inc. Japan). The relative humidity of inlet and outlet air was measured using an Omega 4-in-1 multifunctional anemometer (Omega Engineering, Stamford, CT). The emulsion, powder, and dust were analyzed for moisture content. The resulting powders were stored at 4^oC, and the storage time did not exceed four days.

The drying procedure was carried out in triplicate. The estimated production rate was determined and compared with actual production rate; the evaporation rate of the emulsion and energy required to increase ambient temperature to inlet temperature (150°C) were determined as described in Appendix 7.1.2-7.1.4.

6.2.5 Moisture, Water Activity (a_W), Microencapsulation Efficiency (ME), and Color of the Microencapsulated Fish Oils Powders

The spray dried powders stored at 4 °C were analyzed for moisture, water activity, color, and microencapsulate efficiency. The moisture content was determined using a CEM SMART System 5 microwave moisture/solids analyzer (CEM Corporation, Matthews, NC). A calibrated Rotronic water activity meter (AwQUICK, Rotronic Instrument Corp., Huntington, NY) was used to measure the water activity of the powders at room temperature. The amount of surface oil (OS) was determined to calculate the ME as described by Tan and others (2005) with a slight modification. Surface oil was determined by stirring 5 g of MFMO with 50 mL hexane in a covered 100 mL beaker at 25 °C for 10 min. The suspension was then filtered and the residue rinsed thrice by passing 20 ml of hexane each time. The surface oil was determined gravimetrically by weight difference after 60°C air drying for 30 min. The total oil (O_T), which included both the encapsulated oil and surface oil, was determined according to the method described by (Shahidi and Wanasundara 1995). Five gram of powder was dispersed in 25 ml of a 0.88% (w/v) KCl solution. Then 50 ml of chloroform, 25 ml of methanol and a few crystals of tert-butylhydroquinone (TBHQ) were added. The mixture was then homogenized using a high speed mixer for 5 min at 8,000 rpm. The mixture was transferred to a separatory funnel and the chloroform layer was collected and then chloroform was evaporated using a rotary evaporator at 50°C to recover the oil.

The ME was calculated as follows:

$$ME = (O_T - O_S)/O_T \times 100\% \qquad \text{(Eq.6.5)}$$

Color of the powders was determined using the chroma meter LABSCAN XE (Hunterlab, VA, USA). The equipment was calibrated using a black tile first and then a white tile. The data of colors were reported in CIELAB color scales (L* value is degree of lightness to darkness, a* value is degree of redness to greenness, and b* value is degree of yellowness to blueness). Chroma and hue angle value were calculated as following:

$$\text{Chroma} = (a^{*2} + b^{*2})^{½} \qquad \text{(Eq.6.6)}$$

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \qquad \text{(Eq.6.7)}$$

6.2.6 Hydroperoxide, TBARs and EPA, DHA Content of Fish Oils in Powders

The hydroperoxide values (PV) of microencapsulated fish oil powders were measured according to the method of Boon and others (2008). A 0.5 g sample of microencapsulated powder was dispersed in 5 mL of distilled water. Then the dispersion was vortexed for 5 min to allow complete dispersion. A 0.3 mL aliquot of this dispersion was added into 1.5 mL of isooctane-2-propanal (3:1 v:v) solution and vortexed for 30 s. The organic phase was separated by centrifugation at 3400 × g for 2 min. Then, 100 µL of organic phase was mixed with 2.8 mL of methanol-1-butanol (2:1 v:v) followed by adding 15 µL of 3.94 M thiocyanate solution and 15µL of ferrous iron solution (prepared by mixing equal volumes of 0.144 M $FeSO_4 \cdot H_2O$ and 0.132 M $BaCl_2$, centrifuged and collected the clear supernatant). The mixture was vortexed and allowed to react for 20 min at room temperature. The absorbance was measured at 510 nm using a spectrophotometer (Thermo Fisher Scientific, Vernon Hills, IL). Hydroperoxide content was determined using a cumene hydroperoxide standard curve.

TBARS content were tested to estimate the lipid oxidation of the microencapsulated powder. The microencapsulated powder was reconstituted as 10 % (w/w) emulsion by mixing with distilled water and vortexing for 5 min. The measurement of TBARS was as described in

6.2.3. The oil was extracted following the method to extract the total oil in the powder and analyzed for the EPA and DHA content using the method described in 6.2.3.

6.2.7 Scanning Electron Microscopy (SEM) Image and Particle Size of the Microencapsulated Fish Oil Powders

The morphology of the microencapsulated fish oil powders were evaluated by a scanning electronic microscopy (SEM) (JSM-6610LV, JEOL Ltd., Japan) using an acceleration voltage of 5 kV. The samples were mounted on aluminum SEM stubs, coated with gold: palladium (60:40) in an Edwards S150 sputter coater (Edwards High Vacuum International, Wilmington, MA) and imaged with SEM.

The particle size distribution was measured with a Microtrac S3500 light scattering system (MicroTrac, Largo FL) in wet operation. Isopropyl alcohol was used as carrying fluid. The system uses three fixed 780 nm solid state lasers with computer controlled single lens alignment and the measurement capability was from 0.24 to 2800 microns. During the measurement, a certain amount of powder samples was placed in the test chamber with circulating isopropyl alcohol. A period of 10 second ultra sound mixing at 20 watts was used before each test. The sample was pumped through sample cell at 40% of the maximum flow rate. Light was scattered from the tri-lasers from low to high angles (0-163 degrees). The whole light scatter pattern was collected. The volume distribution of the particle size was calculated using modified MIE-scattering technique.

6.2.8 Amino Acid Profile of the Protein Powders

Amino acid profiles of the protein powder was determined by the AAA Service Laboratory Inc., Boring, OR. Powder sample was hydrolyzed with 6N HCl and 2% phenol at 110ºC for 22 h. Amino acids were quantified using a Beckman 6300 analyzer with post-column ninhydrin derivatization. Tryptophan and cysteine content were not determined. Only the most

common 16 amino acids and hydroxyproline and hydroxylysine were analyzed in this study. Due to the increased cost, determination of tryptophan and cysteine were not performed.

6.2.9 Statistical Analysis

Mean values from the three separate experiments or replicate analysis are reported with standard deviation. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA) (SAS Version 9.2, SAS Institute Inc., Cary, NC, U.S.A), followed by post hoc Tukey's studentized range test.

6.3 Results and Discussion

6.3.1 Emulsion Stability, Moisture, Rheology Properties, and Microstructure of Emulsions Containing Enriched Fish Oils

No oil was recovered from MOEE and SOEE stored at -20^{o}C for 2 days, which demonstrated both of the menhaden and salmon emulsion samples were stable. The stability of the emulsion is related to many factors, such as droplet size, wall materials, and oil and wall ratio. The smaller droplet size has a positive effect on stabilizing the emulsions (Jafari and others 2008). Emulsion stability can influence the amount of encapsulated oil, microsphere quality and functionality (Lin and others 1995; Sheu and Rosenberg 1995; Kim and others 1996). It's important to produce stable emulsions before they are spray dried to obtain microspheres (Tan and others 2005). The emulsions composed of different protein/peptides in this study are very stable as proteins can be used to stabilize foam and emulsions alone. Kiokias and others (2007) reported that the electrostatic repulsion between the protein molecules at the interface prevented flocculation of the droplets and eventually stabilized the emulsions.

The moisture contents in MOEE and SOEE were 85.59±0.03% and 85.63±0.08%, respectively. The water ratio preparing the emulsion was 85%, and the extra water in the emulsion is from the moisture in the proteins and a trace amount from the oils. The flow behavior index (*n*), consistency index (*K*) values, and apparent viscosity of MOEE and SOEE at

a shear rate of 200 s^{-1} were tested at 4, 10 and 25 °C. The results were listed in Table 6.1 and 6.2. The flow behavior index of both emulsions at the tested temperatures was much lower than 1, which indicates that they both exhibited shear thinning properties at these temperatures (Paredes and others 1989). Both Newtonian behavior and shear thinning non-Newtoniam behavior were reported for emulsions consisting of different oil and wall materials (Tonon and others 2011, Yanez-Fernandez and others 2008, Bae and Lee 2008). All of the *n* values increased with temperature. The consistency index (*K*) for both of the emulsions had higher values at lower temperature. The consistency index is an indicator of the viscous nature of the emulsion system, and higher *K* values of these emulsion samples indicate a more viscous consistency (Paredes and others 1989). This result was consistent with the apparent viscosity of the emulsions. Both of the emulsions exhibited more than 9-fold higher apparent viscosity at 4°C comparing with 25°C. This effect could be caused by collagen which has good water binding capacity. Zhang and others (2006) found that the viscosity of collagen was greatly influenced by temperature, while gelatin and collagen hydrolysates had only slight difference in viscosity when the temperature changed. The higher viscosity reduces the rate at which particle sediment or cream, resulting in better emulsion stabilization and thus avoiding droplets coalescence (McClements 2005).

Table 6.1 - Rheological properties of the emulsions containing concentrated menhaden oil at different temperatures

Temperature	*n*	*K* (Pa.s^n)	Apparent viscosity (Pa s)
25ºC	0.736±0.014^a	0.012±0.000^c	0.049±0.002^c
10ºC	0.538±0.010^b	0.088±0.007^b	1.335±0.178^b
4ºC	0.245±0.003^c	0.116±0.008^a	4.580±0.355^a

Each value is an average of three determinations with its SD. Values with the same superscript letters in each column are not significantly different ($p>0.05$)

Table 6.2 - Rheological properties of the emulsions containing concentrated salmon oil at different temperatures

Temperature	*n*	*K* (Pa.s^n)	Apparent viscosity (Pa s)

(table 6.2 continued)			
25ºC	0.727±0.018[a]	0.015±0.000[c]	0.062±0.007[c]
10ºC	0.399±0.075[b]	0.097±0.004[b]	3.095±0.378[b]
4ºC	0.220±0.017[c]	0.127±0.009[a]	5.760±0.203[a]

Each value is an average of three determinations with its SD. Values with the same superscript letters in each column are not significantly different (p>0.05)

The G' (an elastic or storage modulus) and G" (a viscous or loss modulus) of the emulsions (MOEE and SOEE) were determined as a function of angular frequency at 25 ºC (Figures 6.1 and 6.2). G' measures the energy recovered per cycle of sinusoidal shear deformation and G" is an estimate of energy dissipated as heat per cycle (Rao 1999). The results showed a gradual increase in both the loss modulus and the storage modulus with increasing frequency. G' was larger than G" throughout the tested range of frequency (6.283 to 62.83 rad/s) for both emulsions, which indicated that they behaved as viscoelastic fluids. Moschakis and others (2005) reported that viscoelastic fluid can retard the rearrangement of macroscopic phase separation and formed stable emulsions.

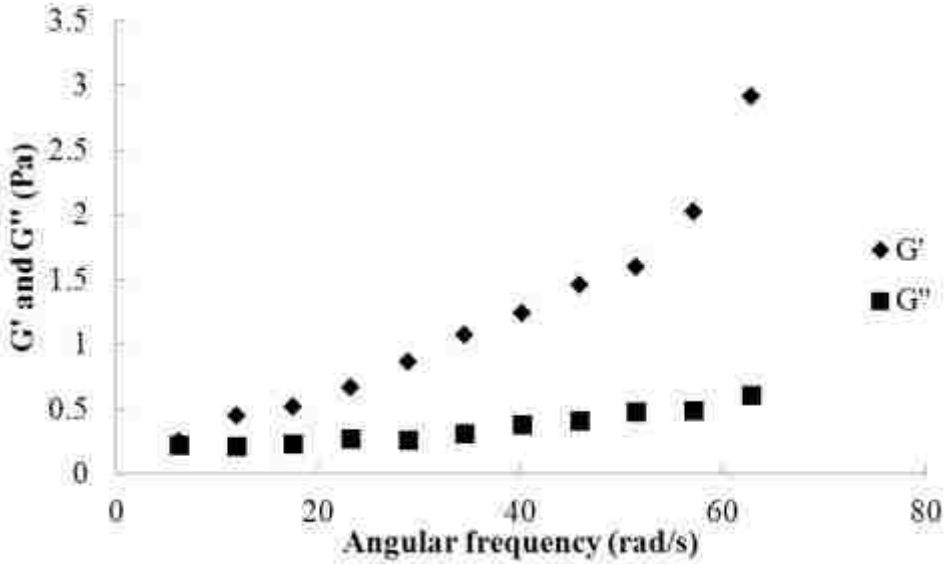


Figure 6.1 - Viscoelastic properties of emulsions containing EPA and DHA enriched menhaden oil. G' = storage modulus; G'' = loss modulus.

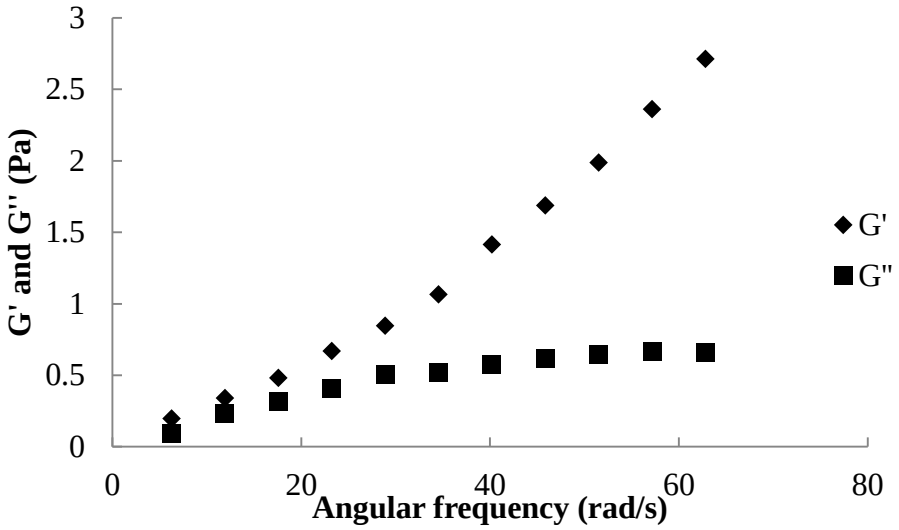


Figure 6.2 - Viscoelastic properties of emulsions containing EPA and DHA enriched salmon oil. G' = storage modulus; G'' = loss modulus

The oil droplets were observed under light microscopy for both emulsions (Figure 6.3 and 6.4). The droplets were well distributed in the emulsion system as small spheres and most of the size ranges from 1-10μm, which is in agreement with most commercial emulsion droplet sizes (0.1-10 μm) (Walstra 1983). There are many factors affecting the physical properties of the emulsion such as preparation methods, operating conditions, viscosities and ultimately affect the droplet formation from the emulsions (Iqbal and others 2011). A higher energy input could produce smaller droplets and emulsions with a smaller droplet size distribution (Forgiarini and others 2001). The energy input was not reported in this study. According to Tonon and others (2011), higher oil concentrations can led to higher mean diameters of emulsion droplets.

6.3.2 Hydroperoxides, TBARs, and EPA, DHA Contents of the Fish Oils in Emulsions

Hydroperoxide contents (PV) and TBARs values characterize the lipid oxidation by quantifying the hydroperoxide amount because of autoxidation and decomposition of hydroperoxides, respectively. MOEE and SOEE, which had rosemary oil as an antioxidant,

Figure 6.3 - Light Microscopy image of the MOEE. Magnification: 80; MOEE = Emulsion containing enzyme treated menhaden oil.

Figure 6.4 - Light Microscopy image of the SOEE. Magnification: 80; SOEE = Emulsion containing enzyme treated salmon oil

exhibited significantly lower hydroperoxides (1.65±0.17 and 2.68±0.52 mmol/kg) and TBARs (0.73±0.00 and 0.68±0.00 mmol/kg) than the control emulsions (2.03±0.22 and 3.56±0.27 mmol/kg for hydroperoxides and 0.95±0.04 and 0.98±0.03 mmol/kg for TBARs). Many factors can influence the lipid oxidation in emulsions such as water soluble pro-oxidants and antioxidants, increased surface area and the presence of an interfacial membrane Coupland and

McClements 1996. Furthermore, the homogenization time, temperature, amplitude could also be affecting the lipid oxidation. In the menhaden and salmon emulsions, rosemary oil played an important role inhibiting the lipid oxidation. The antioxidant activity of rosemary extracts is associated with the presence of several phenolic diterpenes such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol (Frankel 1996; Thorsen and others 2003; Che-Man and Jaswir 2000) and antioxidant activity is based on their structure, hydrogen-donating potential and ability to chelate metal ions. Rosmanol had greater antioxidant activity than carnosol and both were more effective than α-tocopherol, BHT and BHA (Nakatani and Inatani 1981) .

There was no difference in the EPA and DHA content between the oils from control emulsions without rosemary and that from MOEE and SOEE containing rosemary oil. The higher TBARs in the control emulsions were caused by the decomposition of the hydroperoxides in the fish oil (menhaden and salmon had PV of 1.49meq/kg and 2.33meq/kg, respectively) before emulsification process. The emulsion having rosemary oil contains phenolic compounds and may reduce the hydroperoxides in the oils and cause less decompositions. The emulsification process without rosemary oil retained EPA and DHA but caused a higher oxidation.

Table 6.3 – TBARs, and EPA, DHA contents of the menhaden oil in emulsion

	MOEEC	MOEE
Hydroperoxide (mmol/kg oil)	2.03±0.22[a]	1.65±0.17[b]
TBARs (mmol/kg)	0.95±0.04[a]	0.73±0.00[b]
(table 6.3 continued)		
EPA (%)	19.68±0.18[a]	20.13±0.18[a]
DHA (%)	18.54±0.46[a]	18.37±0.73[a]
EPA+DHA (%)	38.22±0.64[a]	38.50±0.91[a]

MOEEC = Emulsion containing enzyme treated menhaden oil without rosemary oil; MOEE = Emulsion containing enzyme treated menhaden oil with rosemary oil. Each value is an average

(table 6.3 continued)
of three determinations with its SD. Values with the same superscript letters in each row are not significantly different (p>0.05)

Table 6.4 TBARs, and EPA, DHA contents of the salmon oil in emulsion

	SOEEC	SOEE
Hydroperoxide (mmol/kg oil)	3.56±0.27[a]	2.68±0.52[b]
TBARs (mmol/kg)	0.98±0.03[a]	0.68±0.00[b]
EPA (%)	16.24±0.14[a]	16.38±0.02[a]
DHA (%)	16.18±0.23[a]	16.14±0.13[a]
EPA+DHA (%)	32.42±0.37[a]	32.52±0.15[a]

SOEEC = Emulsion containing enzyme treated salmon oil without rosemary oil; SOEE = Emulsion containing enzyme treated salmon oil with rosemary oil. Each value is an average of three determinations with its SD. Values with the same superscript letters in each row are not significantly different (p>0.05).

6.3.3 Moisture, Water Activity (a_W), Yield, Microencapsulation Efficiency (ME), and Color of the Microencapsulated Fish Oil Powders

Moisture content in the MOEP and SOEP were 4.02±0.25% and 3.99±0.04%, respectively. These results were a little higher than the maximum moisture specification for most dried powder in the food industry which is between 3-4% (Klinkesorn and others 2006). Klinkesorn and others (2006) reported a moisture range of 1-3% with inlet temperature from 165 to 180°C. The higher moisture content may be caused by the inlet temperature (150 °C), the moisture content in the emulsion (85-86%), and the spray dryer performance. Kelly and others (2002) found that the moisture content of spray dried products was highest when operated at lowest temperature. The water activity was 0.189±0.001 and 0.186±0.002 for the MOEP and SOEP, which is much lower than the result (0.3) reported by Klaypradit and Huang (2008) and is comparable to the result (0.1-0.25) reported by Klinkesorn and others (2006).

The yields of the MOEP and SOEP were 64.37±2.31% and 63.33±1.85%, respectively, which is comparable to those obtained by Tan and others (2005). The low yield was due to microspheres sticking to the inner wall of the spray dryer components. The amount of surface oil

on MOEP and SOEP were 0.086±0.013 and 0.086±0.011g/g powder, respectively. These results are higher than those (3.0-3.5g/100 g powder) reported by Klinkesorn and others (2006). The presence of the surface oil can influence adversely the physical properties of spray dried powders, such as flowability, bulk density and dispersibility and induces more rapid lipid oxidation (Granelli and others 1996; Keogh and others 2001). It should be noted that the surface oil extracted with hexane can also induce an extraction of encapsulated oil to some extent from the inner surface of microcapsules, thereby resulting in a lower ME than an actual content (Bae and Lee 2008). The ME for the two fish oil powders were 70.32±3.55% (MOEP) and 71.38±3.16 (SOEP) and these values were lower than reported values (85-87%) by Klinkesorn and others (2006). The higher ME means less surface oil on the microcapsules, and consequently, less sticking to the spray dryer resulting in a higher yield (Tan and others 2005). The properties of wall and core materials as well as the emulsion characteristics and drying parameters are the factors that can affect the microencapsulation efficiency. The MOEP and SOEP both exhibited very light yellow color.

Table 6.5 - Moisture, OS, OT, microencapsulation efficiency (ME), and color of MOEP

Properties	MOEP
Powder moisture (%)	4.02±0.25
Water activity (a_W)	0.189±0.001
Surface oil O_S (g/g powder)	0.086±0.013
Total oil O_T (g/g powder)	0.289±0.008
ME (%)	70.32±3.55
(table 6.5 continued)	
Yield (%)	64.37±2.31
L*	83.83±0.16
a*	-0.093±0.006
b*	9.83±0.10
Chroma	9.83±0.11
Hue angle	13.90±0.15

(table 6.5 continued)
MOEP =Powder containing enzyme treated menhaden oil. Each value is an average of three determinations with its SD.

Table - 6.6 Moisture, OS, OT, microencapsulation efficiency (ME), and color of SOEP

Properties	SOEP
Powder moisture (%)	3.99±0.04
Water activity (a_W)	0.186±0.002
Surface oil O_S (g/g powder)	0.086±0.011
Total oil O_T (g/g powder)	0.300±0.006
ME (%)	71.38±3.16
Yield (%)	63.33±1.85
L*	86.06±0.34
a*	0.21±0.01
b*	6.79±0.13
Chroma	6.80±0.13
Hue angle	9.61±0.18

SOEP = Powder containing enzyme treated salmon oil. Each value is an average of three determinations with its SD.

6.3.4 TBARs and EPA, DHA Contents of the Fish Oils in Microencapsulated Powders

The hydroperixodes of the MOEP vs control powder and SOEP vs control powder were 2.55±0.37 vs 2.06±0.08 mmol/kg oil and 4.87±0.16 vs 2.98±0.24 mmol/kg oil, respectively. The TBARs of the MOEP vs control powder and SOEP vs control powder were 1.12±0.03 vs 0.79±0.03 and 1.25±0.07 vs 0.72±0.03, respectively. The powders with rosemary oil exhibited lower hydroperixide contents and TBARs values comparing with control, which means the rosemary oil was effective in reducing lipid oxidation. Lipid oxidation of microencapsulated oil is caused by oxidation of both surface and encapsulated oils (Hogan and others 2003). Surface oil is prone to be oxidized because it's not "protected" by the wall materials. Therefore, lipid oxidation of the powders can be affected by microencapsulation efficiency (ME), which is related to the solid and oil concentration in the emulsion. Lower solid content and higher oil

concentration can lead to higher oxidation, which is related to lower encapsulation efficiency at this condition (Tonon and others 2011). In addition, rosemary oil helped retain the EPA+DHA of the menhaden and salmon oil in the microencapsulated powders at 38.41 and 32.53%, while the controls had only 34.14 and 30.95%, respectively.

Table - 6.7 TBARs, and EPA, DHA contents of the menhaden oil in powder

	Control	MOEP
Hydroperoxides (mmol/kg oil)	2.55±0.37[a]	2.06±0.08[b]
TBARs (mmol/kg)	1.12±0.03[a]	0.79±0.03[b]
EPA (%)	17.86±0.18[b]	19.93±0.27[a]
DHA (%)	16.28±0.73[b]	18.49±0.81[a]
EPA+DHA (%)	34.14±0.91[b]	38.41±1.08[a]

Control = Powders containing enzyme treated menhaden oil without rosemary oil; MOEP = Powder containing enzyme treated menhaden oil with rosemary oil. Each value is an average of three determinations with its SD. Values with the same superscript letters in each row are not significantly different ($p>0.05$).

Table 6.8 - TBARs, and EPA, DHA contents of the salmon oil in powder

	Control	SOEP
Hydroperoxides (mmol/kg oil)	4.87±0.16[a]	2.98±0.24[b]
TBARs (mmol/kg)	1.25±0.07[a]	0.72±0.00[b]
EPA (%)	14.88±0.14[b]	16.14±0.14[a]
DHA (%)	15.07±0.23[b]	16.40±0.05[a]
EPA+DHA (%)	30.95±0.37[b]	32.53±0.20[a]

Control = Powders containing enzyme treated salmon oil without rosemary oil; SOEP = Powder containing enzyme treated salmon oil with rosemary oil. Each value is an average of three determinations with its SD. Values with the same superscript letters in each row are not significantly different ($p>0.05$).

6.3.5 Scanning Electron Microscopy (SEM) Image and Particle Size of the Microencapsulated Fish Oil Powders

Scanning electron micrographs (SEM) showed that the spray-dried particles were spherical with surface indentations and no apparent surface cracks (Figures 6.5 and 6.6). The surface indentations were due to rapid particle shrinkage during the early stage of the drying process and high inlet temperature (Kim and Morr 1996). The continuous surface with no cracks

can offer good protection of the encapsulated oil (McNamee and others 1998). Microstructures of spray-dried microcapsules have been shown to be affected by wall composition and properties, core and wall ratio, drying parameters (atomization conditions, drying temperatures, and feed properties), and storage conditions (Sheu and Rosenberg 1998; Tan and others 2009). Both of the powders exhibited some extent of agglomeration, which could be related to the presence of the surface oil (Vega and Roos 2006).

Most of the particle sizes for the two powders ranged between 20 to 40μm (Figures 6.7 and 6.8). The result agrees with the scanning electron micrographs that shows various sizes of microparticles. Tonon and others (2011) reported a higher range of particle size (0.1 to 477 μm) for the powders produced by gum arabic. Solid content of the emulsion, which is related to viscosity, is the main factor affecting the particle size (Tonon and others 2011). The higher the feed viscosity, the larger are the particle size during spray drying (Masters 1991). Inlet temperature is another factor affecting particle size. Higher temperature leads to faster drying rates and structure is formed at the early stage, thus, does not allow the particles to shrink during drying as does slow drying (Reineccius 2004). Feeding solids (wall materials) have a similar effect in that the particles dry quickly if they are high in solids and cannot shrink as much (Masters 1991).

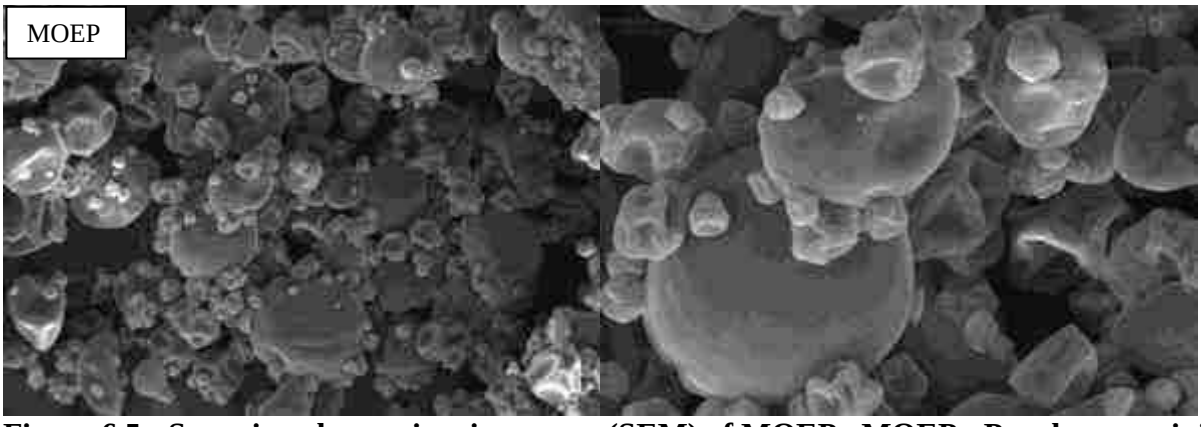


Figure 6.5 - Scanning electronic microscopy (SEM) of MOEP. MOEP =Powder containing enzyme treated menhaden oil.

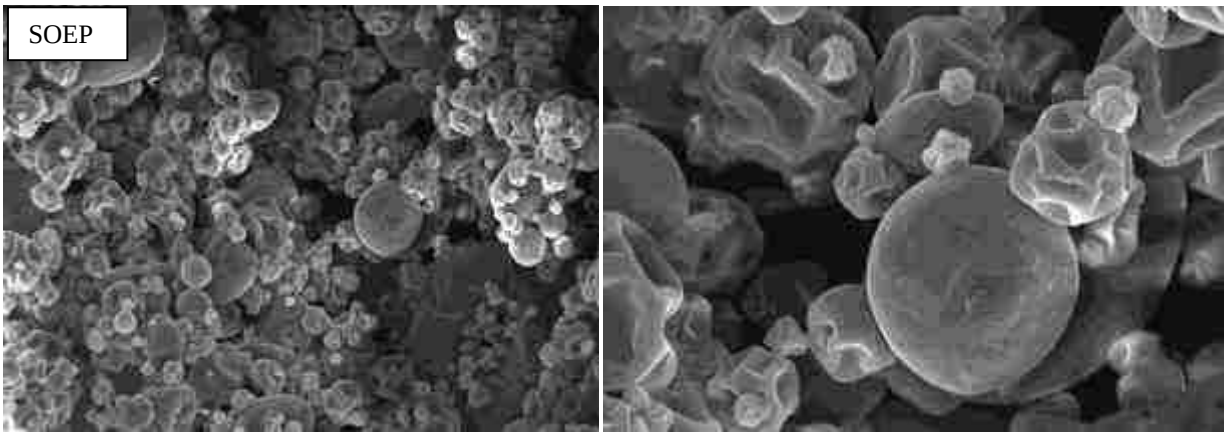


Figure 6.6 - Scanning electronic microscopy (SEM) of SOEP. SOEP =Powder containing enzyme treated salmon oil.

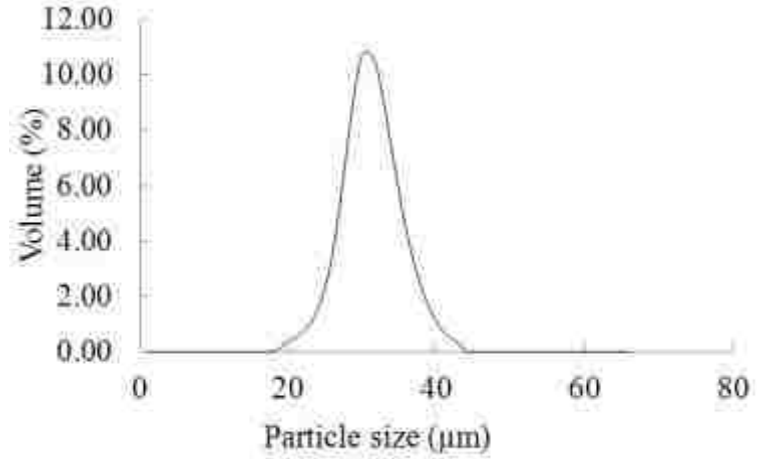


Figure 6.7 - Particle size distribution of powder containing enzyme treated menhaden oil.

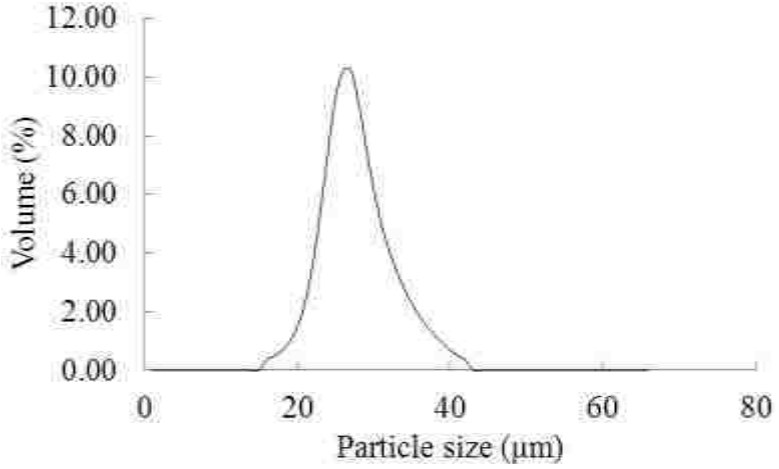


Figure 6.8 - Particle size distribution of powder containing enzyme treated salmon oil.

6.3.6 Amino Acid Profile of the Protein Powders

The protein powder contains all of the essential amino acids (EAA) except cysteine which was not determined in this analysis. Leucine was the main EAA found in the protein powder (42.10±1.70 mg/g protein) followed by lysine (34.08±1.41 mg/g protein) and valine (24.11±1.00 mg/g protein). According to Tarnopolsky (2004), the branched-chain amino acids (isoleucine, leucine, and valine) are preferentially oxidized compared with other amino acids during exercise. Layman and Rodriguez (2009) observed that leucine is a critical element in regulating muscle protein synthesis and may be the key amino acid for optimizing skeletal muscle mass and muscle recovery from both resistance and endurance exercise. In addition, leucine was reported to be an inhibitor of protein degradation in skeletal muscle (Nakashima and others 2007; Tischler and others 1982) and liver (Meijer and Dubbelhuis 2004). The powder is expected to improve performances of exercise due to the high leucine content.

Table - 6.9 Amino acid composition of the protein powder

Amino acid	Concentration (mg/g protein)
Hydroxyproline	16.73±0.86
Aspartic acid	41.60±1.69
Threonine[a]	19.85±0.83
Serine	20.22±0.94
Glutamic acid	68.88±2.85
Proline	36.56±1.62
Glycine	34.57±1.54
Alanine	29.15±1.23
Valine[a]	24.11±1.00
Methionine[a]	2.68±0.15
Isoleucine[a]	21.29±0.94
Leucine[a]	42.10±1.70
Tyrosine[a]	15.02±0.68
Phenylalanine[a]	19.44±0.86
Histidine[a]	9.16±0.42
Hydroxylysine	1.56±0.09
Lysine[a]	34.08±1.41
Arginine	26.34±1.17

Values are means of triplicate determinations. [a]Essential amino acid. Cysteine and Tryptophan were not determined.

6.4 Conclusions

Both the menhaden and salmon emulsion samples were stable. The n values of both emulsions decreased with the decreasing of the temperature. The consistency index (K) for both of the emulsions had higher values at lower temperature, which is in consistent with the apparent viscosity of the emulsions. Both of the emulsions exhibited more than 9-fold higher apparent viscosity at 4°C comparing with 25°C. G' was larger than G" throughout the tested range of frequency (6.283 to 62.83 rad/s) for both emulsions, which indicated that they behaved as viscoelastic fluids. The droplets were well distributed in the emulsion system as small spherical and most of the size ranges at about 1-10μm for both emulsions. MOEE and SOEE, which have rosemary oil as antioxidant, exhibited significantly lower TBARs (0.73±0.00 and 0.68±0.00 mmol/kg) than the control emulsion (0.95±0.04 and 0.98±0.03 mmol/kg). Addition of rosemary oil didn't affect the EPA and DHA compositions in both menhaden and salmon oil emulsions. MOEP and SOEP had yields of 64.37±2.31% and 63.33±1.85% and ME of 70.32±3.55% and 71.38±3.16%. The moisture contentt in the MOEP and SOEP were 4.02±0.25% and 3.99±0.04%, and the water activity was 0.189±0.001 and 0.186±0.002 for the MOEP and SOEP. Menhaden and salmon oil powders containing 200ppm rosemary oil had EPA+DHA levels of 38.41 and 32.53%, while the controls had only 34.14 and 30.95%, respectively. Scanning electron micrographs (SEM) showed that the spray-dried particles were spherical with surface indentations and no apparent surface cracks. Most of the particle sizes for the two powders ranged between 20 to 40μm. Leucine was the main EAA found in the protein powder (42.10±1.70 mg/g protein). This study demonstrated that the protein powder containing menhaden and salmon oils could be effectively produced by microencapsulation technology. These powders could provide health benefits for humans in prevention of cardiovascular disease and in weight management.

CHAPTER 7 SUMMARY AND CONCLUSIONS

The ω-3 PUFA especially EPA and DHA from fish oils may have a lot of potential benefits such as reduced risk of cardiovascular diseases, hypertension and atherosclerosis, inflammatory and autoimmune disorders. Menhaden (MO) and salmon oils (SO) are good sources of EPA and DHA but the high amounts of impurities may decrease product quality or processing efficiency. Therefore an economically viable purification method is needed to remove impurities from unpurified menhaden and salmon oils. The market for fish oils enriched with ω-3 PUFAs is growing with the public awareness of the benefits from these oils. Microencapsulation technology could be an effective method producing fish oil powder which delays the oxidation of the fish oils. The objectives of the study were to characterize physical, thermal, rheological properties of unpurified menhaden oil and salmon oil, to develop an economically viable purification process, to design a process to enrich the EPA and DHA contents in fish oils and to develop a protein powder containing fish oil with enriched EPA and DHA.

Unrefined menhaden oil at temperatures ranging from 5 to 25°C showed non-Newtonian fluid behavior, while Newtonian behavior was evident at 30°C. Unrefined salmon oil exhibited Newtonian fluid behavior at temperatures ranging from 5 to 30°C. The 6 week storage study showed that the rate of hydroperoxide formation of the oil stored at 4 °C was lower than that of the oil stored at room temperature and at 50°C. Changes in the magnitude of apparent viscosity and lipid oxidation of the unrefined menhaden and salmon oil with temperature could be well described by the Arrhenius equation.

Activated earth, activated alumina, and chitosan were effective in reducing PV, FFA, and moisture from menhaden and salmon oils, respectively. The batch adsorption process did not change the composition of fatty acid profile in both oils. Phosphorus contents of menhaden and

salmon oils were reduced from 45.36 ppm and 38.60ppm to 15.65 ppm and 17.88 ppm by activated earth, respectively. Activated earth was most effective in reducing B, Fe, and Zn in the menhaden oil. For the salmon oil, activated earth effectively removed the Fe, Zn, Ca, S, and Na. MO exhibited shear thinning properties, while purified menhaden oils and all of the salmon oil samples were considered as Newtonian fluids at room temperature. All DSC peaks for unrefined and refined menhaden and salmon oil samples were not sharp, except M4, M5, and S5. The thermal degradation of the unpurified and purified menhaden and salmon oils showed that M4, M5, S4, and S5 had higher degradation rate compared with other oil samples. A batch adsorption process was developed in this study to purify menhaden and salmon oils in reducing PV, FFA, moisture, and heavy metal content and retaining the fatty acid compositions. DSC and TG can be applied to evaluate the quality of the unrefined and refined fish oils.

A pilot scale, fast and economically viable purification process was developed in this study. The results showed that the mix of activated earth and activated alumina can effectively adsorb primary oxidation products, free fatty acids and moisture from the MO and SO. The column adsorption process did not affect the main fatty acids in the unrefined and refined menhaden and salmon oils. The column purification process decreased the phosphorus contents of the menhaden and salmon oils from 45.36±1.36ppm and 38.60±2.26ppm to 16.42±0.37ppm and 17.85±1.14ppm, respectively. After the column adsorption processes, most of the mineral contents decreased. The study demonstrated that the column purification process could reduce the PV, FFA, and moisture contents from unrefined menhaden and salmon oils without affecting the fatty acid compositions.

The menhaden and salmon oils were treated with lipase hydrolysis and EPA and DHA were enriched. Menhaden oil treated with 2500 U lipase for 6 and 12h had the highest total EPA and DHA fractions (39.54% and 39.95%) in the final oil, while the highest total EPA and DHA

fraction (32.12%) in the salmon oil was found in the oil hydrolyzed with 1250 U lipase for 6h. After treatment with 2500G lipase for 24h, the TG and DG levels of menhaden oil decreased from 73.53% and 14.13% to 3.43% and 4.66%, while MG level increased from 12.34% to 91.90%. The unhydrolyzed salmon oil contained 77.17% TG, 3.17% DG, and 19.53% MG. After hydrolysis with 1250U of lipase for 24h, the TG, DG, and MG levels changed to 9.97%, 17.03%, and 72.87%. The process transformed most of the TG into MG. After the EPA and DHA enrichment processed, PV, FFA, and TBARs values of menhaden oil (MOE) decreased from 11.06±0.75 meq/kg oil to 1.49±0.13meq/kg oil, from 2.66±0.07% to 0.67±0.06%, and from 0.89±0.01mmol/kg to 0.56±0.01mmol/kg, respectively. The process decreased the PV, FFA, and TBARs of salmon oil from 14.71±1.26 meq/kg oil to 2.33±0.16meq/kg oil, from 3.14±0.07% to 0.66±0.05%, and from 1.26±0.02mmol/kg to 0.62±0.03mmol/kg, respectively.

At the end of the research, the oils enriched with EPA and DHA were microencapsulated with a variety of proteins and a fish oil protein powder was developed. Both the menhaden and salmon emulsion prepared with 7 proteins were very stable. The droplets were well distributed in the emulsion system as small spheres and most of the size ranges at about 1-10μm for both emulsions. MOEE and SOEE, which had rosemary oil as an antioxidant, exhibited significantly lower TBARs than the control emulsion. Addition of rosemary oil didn't affect the EPA and DHA compositions in both menhaden and salmon oil emulsions. Total contents of EPA and DHA in menhaden and salmon oil powders containing 200ppm rosemary oil were higher than that of the controls. Scanning electron micrographs (SEM) showed that the spray-dried particles were spherical with surface indentations and no apparent surface cracks. Most of the particle size for the two powders ranged between 20 to 40μm. This study demonstrated that the protein powder containing menhaden and salmon oils can be effectively produced by

microencapsulation technology. These powders could provide health benefits for humans in prevention of cardiovascular disease and weight management.

REFERENCES

ADFG. 2007. Alaska Commercial Salmon Harvests and Exvessel Values 2006. <www.cf.adfg.state.ak.us/geninfo/finfish/salmon/catchval/blusheet/06exvesl.php>

Afseth K. N., Wolda P. J., and Segtnana H. V. 2006. The potential of Raman spectroscopy for characterisation of the fatty acid unsaturation of salmon. Analytica Chimica Acta 572 (1), 85-92

Ahn J., Grün I.U., and Fernando L.N. 2002. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. Journal of Food Science 67 (4). 1364-1369.

Anderson J.W., Johnstone B.M., and Cook N. 1995. Meta-analysis of the effects of soy protein intake on serum lipids. New England Journal of Medicine 333. 276-282.

Ang C.Y.W. and Lyon B.G., 1990. Evaluation of warmed-over flavor during chill storage of cooked broiler breast, thigh, and skin by chemical, instrumental and sensory methods. Journal of Food Science 55(3). 644-648.

Anonymous. 1999. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Soprevvivenza nell'Infarto miocardico. Lancet 354. 447-455.

AOCS. 1998. Official Methods and Recommended Practices of the American Oil Chemists' Society. Champaign, IL: American Oil Chemists' Society.

Aruoma O.I. 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. Journal of the American Oil Chemists' Society. 75. 199-212.

Association of Official Analytical Chemists (AOAC). 1999. Official methods of analysis, 16th edn. AOAC International, Arlington.

Babovic N., Zizovic I., Saicic S., Ivanovic J., and Petrovic S., 2010. Oxidative stabilization of sunflower oil by antioxidant fractions from selected lamiaceae herbs. Chemical Industry and Chemical Engineering Quarterly 16(4). 287-293.

Bae E. K. and Lee S. J. 2008. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. Journal of Microencapsulation. 25. 549-560.

Balassa L. and Fanger G.O. 1971. Microencapsulation in the food industry. CRC Critical Reviews in Food Technology 2. 245–62.

Ballard K. D., Bruno R. S., Seip R. L., Quann E. E., Volk B. M., Freidenreich D. J., Kawiecki D. M., Kupchak B. R., Chung M. Y., Kraemer W. J. and Volek J. S. 2009. Acute ingestion of a novel whey-derived peptide improves vascular endothelial responses in healthy individuals: a randomized, placebo controlled trial. Journal of Nutrition. 8. 34.

Barbut S., Josephson, D.B., Maurer A.J. 1985. Antioxidant properties of rosemary oleoresin in turkey sausage. Journal of Food Science. 50(5). 1356-1359.

Basaga H., Geban O. and Tekkaya C. 1994. The Effect of the Inquiry Teaching-Method on Biochemistry and Science Process Skill Achievements. Biochemical Education. 22. 29-32.

Benatti P., Peluso G., Nicolai R., Calvani M. 2004. Polyunsaturated fatty acids: biochemical, nutritional and epigenetic properties, Journal of the American College of Nutrition. 23. 281–302.

Bera D., Lahiri D., Nag A. 2004. Kinetic studies on bleaching of edible oil using charred sawdust as a new adsorbent. Journal of Food Engineering. 65. 33-36.

Berthold A., Cremer K., and Kreuter J. 1998. Collagen microparticles: carriers for glucocorticosteroids. European Journal of Pharmaceutics and Biopharmaceutics 45(1). 23-29.

Bhattacharya AB, Sajilata MG, Singhal RS. 2008. Lipid profile of foods fried in thermally polymerized palm oil. Food Chemistry 109. 808-812.

Bimbo A.P. and Cowther J.B. 1991. Fish oils: Processing beyond crude oil. Infofish International 6. 20-25.

Boki K., Shinoda S. and Ohno S. 1989. Effects of filtering thought bleaching media on decrease of peroxide value of autoxidized soybean oil. Journal of Food Science. 54. 20-25.

Bottino N.R., Vandenburg G.A., Reiser R. 1967. Resistance of certain long-chain polyunsaturated fatty acids of fish oils to pancreatic lipase hydrolysis. Lipids. 2(6). 489-493.

Bourre J., Dumont O., Piciotti M., Clement J., Chaudiere J., Bonneil M., Nalbone G., Lafont H., Pascal G. and Durand G. 1991. Essentiality of omega-3 fatty acids for brain structure and function. World Review of Nutrition and Dietetics 66. 103–117.

Boza J.J., Moennoz D., Vuichoud J., Jarret A.R., Gaudard-de-Weck D., and Ballèvre O. 2000. Protein hydrolysate vs free amino acid-based diets on the nutritional recovery of the starved rat. European Journal of Nutrition. 39 (6). 237-243.

Breivik H., Haraldsson G.G., Kristinsson B. 1997. Preparation of highly purified concentrates of eicosapentaenoic acid and docosahexaenoic acid. Journal of the American Oil Chemists' Society. 74. 1425-1429.

Brekke O.L. 1980. Oil degumming and soybean lecithin. In: D.L. Erickson et al., Editors, Hand Book of Soy Oil Processing, Utilization, St. Louis: American Soy bean Association; and Champaign, III. American Oil Chemists’ Society Champaign, Illinois.

Brown G. H. and Snyder E. H. 1985. Adsorption of soy oil phospholipids on silica. Journal of the American Oil Chemists' Society 62. 753-756.

Brule M.R. and Corbett R.W. 1984. What makes critical-solvent processes work? Hydrocarbon Processing. 63 (6). 73-77.

Bucher H.C., Hengstler P., Schindler C., and Meier G. 2002. n-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. American Journal of medicine. 112. 298-304.

Buckley D.J., Morrissey P.A., Gray J.I. 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. Journal of Animal Science. 73(10). 3122-3130.

Buhri, A.B. and Singh, R.P. 1994. Thermal properties measurements of fried foods using differential scanning calorimeter. In Developments in Food Engineering, (eds. T. Yano, R. Matsumoto & K. Nakamura), pp. 201-203. Glasgow, Blackie Academic & Professional, UK.

Burns J., Gardner P.T., Matthew D., Duthie C.G., Lean M.E., and Crozier A. 2001. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. Journal of Agricultural and Food Chemistry. 9. 5797–5808.

Calder P.C. 2004. n - 3 fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clinical Science. 107. 1-11.

Campbell B., Kreider R.B., and Ziegenfuss T. 2007. International Society of Sports Nutrition position stand: protein and exercise. Journal of International Society of Sports Nutrition 4. 8.

Carr R.A. 1978. Refining and degumming systems for edible fats and oils. Journal of the American Oil Chemists' Society 55. 765-771.

Carrillo A. R. and Kokini J. L. 1988. Effect of Egg-Yolk and Egg-Yolk + Salt on Rheological Properties and Particle-Size Distribution of Model Oil-in-Water Salad Dressing Emulsions. Journal of Food Science. 53.1352-1359.

Cenacchi T., Bertoldin T., Farina C., Fiori M. G., and Crepaldi G. 1993. Cognitive decline in the elderly: a double-blind, placebo controlled multicenter study on efficacy of phosphatidylserine administration. Aging and Clinical Experimental Research. 5. 123-133.

Chakraborty K. and Raj P. R. 2009. Selective enrichment of n-3 polyunsaturated fatty acids with C18-C20 acyl chain length from sardine oil using Pseudomonas fluorescens MTCC 2421 lipase. Food Chemistry. 114. 142-150.

Chapman D.M. 1994. Benefits and limitations of a novel chlorophyll adsorbent. Journal of the American Oil Chemists Society 71 (4). 397-400.

Che-Man Y.B. and Jaswir I. 2000. Effect of rosemary and sage extracts on frying performance of refined, bleached and deodorized (RBD) palm olein during deep-fat frying. Food Chemistry. 69. 301-307.

Chrastil J. 1982. Solubility of solids and liquids in supercritical gases. Journal of Physical Chemistry. 86. 3016-3021.

Chang S.S. 1967. Processing of fish oils. In Fish Oils, (M.E. Stansby, ed.), pp. 206-221, Chapman and Hall, New York.

Chapman D.M. 1994. Benefits and limitations of a novel chlorophyll adsorbent. Journal of the American Oil Chemists Society. 71. 397-400.

Chapman D.M. and Pfannkoch E.A. 1992. Thermodynamic Limitations for Pigment Adsorption during the bleaching of Triglyceride oils, Journal of the American Oil Chemists Society 69. 1009 - 1014.

Choe E, Min DB. 2005. Chemistry and reactions of oxygen species in foods. Journal of Food Science 70. 142-159.

Coupland J. N. and McClements D. J.1996. Lipid oxidation in food emulsions. Trends Food Science Technology. 7. 83-91.

Cowan J.C. 1976. Degumming, refming, bleaching and deodorization theory. Journal of the American Oil Chemists' Society. 3. 344-346.

Cunnane S.C. 2000. The conditional nature of the dietary need for polyunsaturates: a proposal to reclassify ‘essential fatty acids’ as ‘conditionally indispensible’ or ‘conditionally dispensible’ fatty acids. British Journal of Nutrition. 84. 803-812.

Demiral I. and Ayan E.A. 2011. Pyrolysis of grape bagasse: effect of pyrolysis conditions on the product yields and characterization of the liquid product. Bioresource technology 102 (4). 3946-3951.

Diaz M.N., Frei B., Vita J.E. and Keaney J.F., 1997, Antioxidants and atherosclerotic heart disease. Journal of Medicine and Nutrition England. 337. 408–416.

Dinamarca E., Garrido P., and Valenzuela A. 1990. Simple high vacuum distillation equipment for deodorizing fish oil for human consumption. Lipids. 25.170-171.

Drusch S., Rätzke K., Shaikh M.Q., Serfert Y., Steckel H. and Scampicchio M., Voigt I., Schwarz K., and Mannino S. 2009. Differences in free volume elements of the carrier matrix affect the stability of microencapsulated lipophilic food ingredients. Food Biophysics. 4 (1). 42–48.

Du D., Sato M., Mori M., Park E.Y. 2006. Repeated production of fatty acid methyl ester with activated bleaching earth in solvent-free system. Process Biochemistry. 41. 1849-1853.

Dutta S. and Gualy R. 2000. Chemical Engineering Progress, 10, 37.

Eisenbach W. 1984. Supercritical fluid extraction: A film demonstration. Berichte Der Bunsen-gesellschaft Physical Chemistry 88. 882-887.

Elson C. M. and Ackman R. G. 1978. Trace metal content of herring oil at various stages of pilot-plant refining and partial hydrogenation. Journal of the American Oil Chemists' Society 55. 616-618.

El-Boustani S., Colette C., Monnier L., Descomps B., Paulet C.A., and Mendey F. 1987. Enternal absorption in man of eicosapentaenoic acid in different chemical forms. Lipids. 22. 711-714.

El-Mashad H.M., Zhang R., and Avena-Bustillos R. 2008. A two-step process for biodiesel production from salmon oil. Biosystems Engineering. 99. 220-227.

Fennema O. R. 1996. Food Chemistry, 3rd edition, Marcel Dekker, Inc. TX.

Fiorillo J. 2005. Are fish oil supplements in threat? Intrafish 3. 18-20.

Fleming H.L. 1991. Adsorption on aluminas - current applications. Adsorption and its applications in industry and environmental protection 120. 561-583.

Ford E.S. and Mokdad A.H. 2008. Epidemiology of obesity in the Western Hemisphere. Journal of Clinical Endocrinology and Metabolism 93. S1-S8.

Forgiarini A., J. Esquena C. Gonzalez and C. Solans. 2001. Formation of nano-emulsions by low-energy emulsification methods at constant temperature. Langmuir 17. 2076-2083.

Franco J.M., Guerrero A. and Gallegos C. 1995. Rheology and processing of salad dressing emulsions. Rheologica Acta 34. 513-524.

Frankel E.N. 1996 Antioxidants in lipid foods and their impact on food quality. Food Chemistry 57. 51-55.

Frankel E.N. 1993. Formation of headspace volatiles by thermal decomposition of oxidized fish oil vs. oxidized vegetables oils. Journal of the American Oil Chemists' Society 70. 767-772.

Freeman M.P. 2000. Omega-3 fatty acids in psychiatry: a review. Annals of Clinical Psychiatry 12. 159-165.

Gavin A.M. 1978. Edible oil deodorization. Journal of the American Oil Chemists' Society 55. 783-791.

Gharsallaoui A., Roudaut G., Chambin O., Voilley A., Saurel R. 2007. Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Research International 40. 1107-1121.

Gracey J., Collims D.S., Huey R. 1999. Food poisoning and meat microbiology. In: Gracey J, Collims DS, Huey R (eds) Meat hygiene, 10th edn. Saunders, Philadelphia, PA, pp 407.

Granelli K., Faldt P., Appelqvist L.A., Bergenstahl B. 1996. Influence of surface structure on cholesterol oxidation in model food powders. Journal of the Science of Food and Agriculture. 71. 75-82.

Guillen M.D. and Goicoechea E. 2009. Oxidation of corn oil at room temperature: Primary and secondary oxidation products and determination of their concentration in the oil liquid matrix from H-1 nuclear magnetic resonance data. Food Chemistry 116. 183-192.

Gulf States Marine Fisheries Commission. (2002). The Menhaden Fishery of the Gulf of Mexico, United States (Number 99). Ocean Springs, Mississippi: Author.

Haag M. 2003. Essential fatty acids and the brain. Canadian Journal of Psychiatry 48. 195-203.

Hamazaki T., Hirai A., Terano T., Sajiki J., Kondo S., Fujita T., Tamura Y., and Kumagai A. 1982. Effects of orally administrated ethyl ester of eicosapentaenoic acid on pig-like substance production by rat aorta. Prostaglandins 23. 557–567.

Hamilton R.J., Kalu C., Prisk E., Padley F.B., Pierce H. 1997. Chemistry of free radicals in lipids. Food Chemistry 60. 193-199.

Haraldsson G.G. and Hoskuldsson P.A. 1989. The preparation of triglycerides highly enriched with ω3 polyunsaturated fatty acids via lipase catalysed interesterification. Tetrahedron letters 30.1671-1674.

Hardy R. and Keay J.N. 1967. The isolation of the polyunsaturated acids of the fish oils as their methyl esters by preparative scale gas chromatography. Journal of Chromatography 27. 474-479.

Hooper L., Summerbell C.D., Higgins J.P.T., Thompson R.L., Clements G., Capps N., Davery Smeth G., Riemersma R.A., and Ebrahim S. 2003. Reduced or modified dietary fat for preventing cardiovascular disease (Cochrane review). In The Cochrane Library, issue 4. Chichester, UK: John Wiley and Sons, Ltd.

Hogan S.A., McNamee B.F., O'Riordan E.D., and O'Sullivan M. 2001. Microencapsulating properties of whey protein concentrate 75. Journal of Food Science 66. 675-80.

Holmberg A. 1982. On the practical identifiability of microbial growth models incorporating *Michaelis – Menten* type nonlinearities. Mathematical Biosciences 62. 23- 43.

Hooper L., Summerbell C.D., Higgins J.P.T., Thompson R.L., Clements G., Capps N., Davery Smeth G., Riemersma R.A., and Ebrahim S. 2003. Reduced or modified dietary fat for preventing cardiovascular disease (Cochrane review). In The Cochrane Library, issue 4. Chichester, UK: John Wiley and Sons, Ltd.

Horrocks L.A. and Yeo Y.K. 1999. Health benefits of docosahexaenoic acid (DHA). Pharmacological Research 40. 211-225.

Hoshino T., Yamane T., and Shimuzu S. 1990. Selective hydrolysis of fish oil by lipase to concentrate ω3 polyunsaturated fatty acids. Agricultural and Biological Chemistry 54. 1459-1467.

Hu F.B., Cho E., Rexrode K.M., Albert C.M., and Manson J.E. 2003. Fish and long-chain omega-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic women. Circulation 107. 1852-1821.

Huang J., Sathivel S. 2008. Thermal and rheological properties and the effects of temperature on the viscosity and oxidation rate of unpurified salmon oil. Journal of Food Engineering 89. 105-111.

Huang J. and Sathivel S. 2010. Purifying salmon oil using adsorption, neutralization, and a combined neutralization and adsorption process. Journal of Food Engineering 96. 51-58.

Huang X., Bu H., Jiang G., Zeng M. 2011. Cross-linked succinyl chitosan as an adsorbent for the removal of Methylene Blue from aqueous solution. International Journal of Biological Macromolecules 49. 643– 651.

Hui Y.H. 1996. Banley's Industrial Oil and Fat Products. 5th Edn. John Willey and Sons Inc USA. 4. 192-196.

Hymore F.K. 1996. Effects of some additives on the performance of acid activated clays in the bleaching of palm oil. Applied Clay Science 10. 379-385.

Iannotta N., Oliviero C., Ranieri G.A. and Uccella, N. 2001. Determination of the oil content in olives by the DSC technique. European Food Research Technology 212. 240-243.

IFFO.net: http://www.iffo.net/intranet/content/archivos/63.pdf.

Inglezakis V.J. and Poulopoulos S.G. 2006. Adsorption, ion exchange and catalysis design of operations and environmental applications. In Inglezakis V.J. and Poulopoulos S.G., editors. Reactors Scale-up. Amsterdam, The Netherlands: Elsevier.

Iqbal J., Petersen S. and Ulrich J. 2011. Emulsion Solidification: Influence of the Droplet Size of the Water-in-Oil Emulsion on the Generated Particle Size. Chemical Engineering and Technology 34. 530-534.

Iqbal S. and Bhanger M.I. 2007. Stabilization of sunflower oil by garlic extract during accelerated storage. Food Chemistry 100. 246-254.

Ito N., Hirose M., Fukishima S., Isuada H., Shirai T. and Tatematsu M., 1986. Studies on antioxidants: Their anticarcinogenic and modifying effects on chemical carcinogenesis. Food and Chemical Toxicology 24. 1099-1102.

Itolman R.T. 1966. Progress in the Fats and Other Lipids, Holman, R.T., Lundberg, W.O. and Malkin, T. (eds.), vol. 9, pp. 1-11, London: Pergamon Press. General introduction to polyunsaturated acids.

Jaeger K.E. and Reetz M.T. 1998. Microbial lipases form versatile tools for biotechnology. Trends in Biotechnology 16. 396-403.

Jafari S.M., Assadpoor E., He Y., and Bhandari B. 2008. Encapsulation efficiency of food flavours and oils during spray drying. Drying Technology 26(7). 816-835.

Kaisersberger E. 1989. DSC investigations of thermal characterization of edible fats and oils. Thermochimica Acta 151. 83-90.

Karel M. 1992. Kinetics of lipid oxidation. In Schwarzberg H.G. and Hartel R.W., editors. Physical chemistry of foods. New York, NY: Marcel Dekker Inc.. p651-668.

Kasperek G.J. and Snider R.D. 1987. Effect of exercise intensity and starvation on activation of branched-chain keto acid dehydrogenase by exercise. American Journal of Physiology 252. E33-E37.

Kaushik V. and Roos, Y.H. 2007. Limonene encapsulation in freeze-drying of gum arabic-sucrose–gelatin system. LWT Food Science and Technology 40. 1381-1391.

Kelly J., Kelly P. M. and Harrington D. 2002. Influence of processing variables on the physicochemical properties of spray dried fat-based milk powders. Lait. 82. 401-412.

Keogh M.K., O'Kennedy B.T. 1999. Milk fat microencapsulation using whey proteins. International Dairy Journal 9. 657-663.

Kim Y.D., Morr C.V., Schenz T.W. 1996. Microencapsulation properties of gum Arabic and several food proteins: liquid orange oil emulsion particles. Journal of Agricultural and Food Chemistry 44. 1308-1313.

Kiokias S., Dimakou C., Oreopoulou V. 2007. Effect of heat treatment and droplet size on the oxidative stability of whey protein emulsions. Food Chemistry 105. 94-100.

Klaypradit W., and Huang Y.W. 2008. Fish oil encapsulation with chitosan using ultrasonic atomizer. LWT-Food Science and Technology 41. 1133-1139.

Klinkesorn U., Sophanodora P., Chinachoti P., Decker E.A., and McClements D.J. 2006. Characterization of spray-dried tuna oil emulsified in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. Food Research International 39. 449-57.

Knowlton T.M. 2000. Oil and Gas Science and Technology- Rev. IFP, 55, 209.

Kristinsson H.G. and Rasco B.A., 2000. Fish protein hydrolysates: Production, biochemical and functional properties. Critical Review of Food Science and Nutrition 40 (1). 43-81.

Kronhout D., Bosschieter E.B., Coulander C. 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. New England Journal of Medicines 312.1205-1209.

Kwon T.W., Snyder H.E., and Brown H.G. 1984. Oxidative stability of soybean oil at different stages of refining. Journal of the American Oil Chemists' Society 61. 1843-1846.

Kurt S. and Kilincceker O. 2011. Performance optimization of soy and whey protein isolates as coating materials on chicken meat. Poultry Science 90 (1). 195-200l

Labuza T.P. 1971. Kinetics of lipid oxidation in foods. CRC Critical Reviews. Food Science and Technology 2. 355-405.

Lai L.S. and Chao S.J. 2000. A DSC study on the gel-sol transition of a starch and hsian-tsao leaf gum mixed system. Journal of Agricultural and Food Chemistry 48. 3267-3274.

Lara A.V. and Park E.Y. 2004. Potential application of waste activated bleaching earth on the production of fatty acid alkyl esters using *Candida Cylindracea* lipase in organic solvent system. Enzyme and Microbial Technology 34. 270-277.

Lawson L.D. and Hughes B.G. 1988. Human Absorption of Fish Oil Fatty Acids as Triacylglycerols, Free Fatty Acids or Ethyl Esters. Biochemical and Biophysical Research Communications 152. 328-335.

Layman D.K. and Rodriguez N. R. 2009. Egg protein as a source of power, strength, and energy. Nutrition Today 44 (1). 43-48.

Li Y. and Watkins B.A. 2002. Analysis of fatty acids in food lipids. In: Wrolstad RE, editor. Current protocols in food analytical chemistry. New York: John Wiley & Sons Inc. p D1.2.1-15.

Lima M. 2003. Biological reaction kinetics. In: Heldman D.R. editor. Encyclopedia of Agricultural, Food, and Biological Engineering. New York: Marcel Dekker, Inc. P75-79.

Lin C.C., Lin S.Y., and Hwang L.S. 1995. Microencapsulation of squid oil with hydrophilic macromolecules for oxidative and thermal stabilization. Journal of Food Science 60. 36-39.

Lin Y., Meijer G.W., Vermeer M.A., and Trautwein E.A. 2004. Soy protein enhances the cholesterol-lowering effect of plant sterol esters in cholesterol-fed hamsters. Journal of Nutrition 134. 143-148.

Linder M., Fanni J., and Parmentier M. 2005. Proteolytic extraction of salmon oil and PUFA concentration by lipases. Marine biotechnology 15. 70-76.

Lopez A.D. 1993. Assessing the burden of mortality from cardiovascular diseases. World Health Statistics Quarterly 46. 91- 96.

Lopez-Duarte A.L. and Vidal-Quintanar R.L. 2009. Oxidation of linoleic acid as a marker for shelf life of corn flour. Food Chemistry 114. 478-483.

Maehr H., Zenchoff G., and Coffen D.L. 1994. Enzymic enhancement of n-3 fatty acid content in fish oils. Journal of the American Oil Chemists' Society 71. 463-467.

Marldey K.S. 1964. Fatty Acids, Their Chemistry, Properties, Production, and Uses, Markley, KS (ed.), 2nd edition, Part 3, pp. 1983-2123, New York: Interscience Publishers. Technique of separation. A. Distillation, salt solubility, low-temperature crystallization.

Martin A.D. and Gilbert D. 1968. Enzyme changes accompanying liver enlargement in rats treated with 3-tert-butyl-4-hydroxyanisole. Journal of Biochemistry 106. 22-23.

Masters K. 1991. Spray Drying Handbook; Longman, Harlow, U.K.

Matouba E., Mabiala B. , Nzikou J.M. , Linder M., Fanni J., Desobry S. and Parmentier M. 2008. Rheological and thermal properties of salmon oil after Aspergillus oryzae lipolysis. Journal of Engineering and Applied Sciences 3(8). 656-663.

Matsuno R. and Adachi S. 1993. Lipid encapsulation technology –Techniques and applications to food. Trends in Food Science and Technology 4. 256-261.

Maxwell R.J., Marmer W.N. 1983. Systematic protocol for the accumulation of fatty-acid data from multiple tissue samples - tissue handling, lipid extraction and class separation, and capillary Gas-Chromatographic analysis. Lipids 18(7). 453-459.

McCabe W., Smith J.C., Harriott P. 1993. Unit Operations of Chemical Engineering, 5th Edition, McGraw-Hill, New York.

McClements D. J. 2005. Food emulsions: Principles, practice, and techniques (2nd ed). Boca Raton: CRC Press.

McHugh M. 1986. Recent Developments in Separation Science, Vol. 9, edited by N.N. Li and J.M. Calo, CRC Press, Boca Raton, FL. 79.

McNamee B.F., O'Riordan E.D., O'Sullivan M. 1998. Emulsification and microencapsulation properties of gum Arabic. Journal of Agricultural and Food Chemistry 46. 4551-4555.

Mei, L.Y., McClements D.J., Wu J.N. and Decker E.A. 1998. Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl. Food Chemistry 61. 307-312.

Meijer A.J., Dubbelhuis P.F. 2004. Amino acid signaling and the integration of metabolism. Biochemical and Biophysical Research Communications 313. 397-403.

Mischoulon D. and Fava M. 2000. Docosahexaenoic acid and omega-3 fatty acids in depression. Psychiatric Clinics of North America 23. 785-794.

Molina G. E., Robles M. A., Gimenez G. A., and Ibanez G. M.J. 1996. Gram-scale purification of eicosapentaenoic acid (EPA, 20:5n-3) from wet Phaeodactylum tricornutum UTEX 640 biomass. Journal of Applied Phycology 8. 359-367.

Moschakis T., Murray B.S., and Dickinson E. 2005. Microstructural evolution of viscoelastic emulsions stabilized by sodium caseinate and xanthan gum. Journal of Colloid and Interface Science 284. 714-728.

Mucha M., and Ludwiczak S. 2005. Isotherms of water sorption in biomaterials based on chitosan. Advances in Chitin Science, vol. VIII. 85-90.

Mukherjee K. D., Kiewitt I., Hills M. J. 1993. Substrate specificities of lipases in view of kinetic resolution of unsaturated fatty acids. Applied Microbiology and Biotechnology 40. 489-493.

Nakashima K., Yakabe Y., Ishida A., Yamazaki M., and Abe H. 2007. Suppression of myofibrillar proteolysis in chick skeletal muscles by a-ketoisocaproate. Amino Acids 33. 499-503.

Nakatani, N. and R. Inatani. 1981. Structure of Rosmanol, a New Antioxidant from Rosemary (Rosmarinus-Officinalis L). Agricultural and Biological Chemistry 45. 2385-2386.

Nemets B., Stahl Z., and Belmaker R.H. 2002. Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. American Journal of Psychiatry 159. 477-479.

Nilsson W.B., Gauglitz Jr. E.J., Hudson J.K., Stour V.F., and Spinelli J. 1988. Fractionation menhaden oil ethyl esters using supercritical fluid CO2. Journal of the American Oil Chemists' Society 65(1). 109-117.

Nishi T., Hara H., Hira T. and Tomita F. 2001. Dietary protein peptic hydrolysates stimulate cholecystokinin release via direct sensing by rat intestinal mucosal cells. Experimental Biology and Medicine 226. 1031-1036.

NOAA. 2011. Forecast for the 2011Gulf and Atlantic menhaden purse-seine fisheries and review of the 2010 fishing season.
http://www.st.nmfs.noaa.gov/st1/market_news/menhaden_forecast_2011.pdf

O'Brien C.M., Chapman D., Neville D.P., Keogh M.K., and Arendt E.K. 2003. Effect of varying the microencapsulation process on the functionality of hydrogenated vegetable fat in short dough biscuits. Food Research International 36. 215-221.

Okada T. and Morrissey T. M. 2007. Production of n-3 polyunsaturated fatty acid concentrate from sardine oil by lipase-catalyzed hydrolysis. Food Chemistry 103. 1411-1419.

Oliveira C. G. D. and Porto L. M., 2005. A kinetic model for bleaching vegetable oils. Journal of the American Oil Chemists Society 82. 537-542.

Otten J.H.J. and Meyers L. (editors). 2006. Dietary Reference Intakes: The Essential Guide to Nutrient Requirements. Washington, DC: The National Academies Press.

Paredes M.D.C., Rao M.A., and Bourne M. 1989. Rheological characterization of salad dressing. 2: effect of storage. Journal of Texture Studies 20. 235-250.

Paul G.L. 2009. The rationale for consuming protein blends in sports nutrition. Journal of American College and Nutrition 44 (1). 43-48.

Proctor A. 1996. Novel adsorbents for edible oil processing. Institute of Food Techinologists (1996 annual meeting).

Pullarkat R.K. and Reha H. 1976. Fatty-acid compsotion of rat brain lipids. Determined by support-coated open-tubular gas chromatography. Journal of Chromatography Science 14. 25-28.

Rao M.A. 1999. Rheological of fluids and semisolids. In: Principal and applications. An Publishers, Inc. Gaitherburg, MD.

Redding B.K., Butcher J., Garison B., Schmucher W.S., and Arden E. Patent: WO/1992/011084.

Reidelberger R. D. 1994. Cholecystokinin and control of food intake. Journal of Nutrition 124. S1327-S1333.

Reineccius, G. A. 2004. The spray drying of food flavors. Drying Technology. 22. 1289-1324.
Richardson L.L. 1978. Use of bleaching clays in processing edible oils. Journal of the American Oil Chemists' Society 55. 777-780.

Richardson L.L. 1978. Use of bleaching clays in processing edible oils, Journal of the American Oil Chemists' Society 55. 777-780.

Ritter B., Schulte J. and Schulte E. 2001. Detection of coating waxes on apples by differential scanning calorimetry. European Food Research and Technology 212. 603-607.

Robards K, Kerr A.F., and Patsalides E. 1988. Rancidity and its measurement in edible oils and snack foods: a review. Analyst 113. 213-224.

Robles M.A., Grima E.M., Gimenez G.A., and Gonzalez M.J.I. 1998. Downstream processing of algal polyunsaturated fatty acids. Biotechnology Advances 16 (3). 517-580.

Rodriguez N.R., Di Marco N.M., and Langley S. 2009. American College of Sports Medicine position stand. Nutrition and athletic performance. Medicine and Science in Sports and Exercise 41. 709-731.

Romeu-Nadal M, Chavez-Servin JL, Castellote AI, Rivero M, Lopez-Sabater MC. 2007. Oxidaiton stability of the lipid fraction in milk powder formulas Food Chemistry 100, 756-763.

Ruxton C.H.S., Calder P.C., Reed S.C., and Simpson M.J.A. 2005. The impact of long-chain n-3 polyunsaturated fatty acids on human health. Nutrition Research Reviews. 18. 113-129.

SAS. 2002. SAS/STAT User's Guide, version 8.2; SAS Institute: Cary, NC.

Santana R.C., Perrechil F.A., Sato A.C.K., Cunha R.L. 2011. Emulsifying properties of collagen fibers: Effect of pH, protein concentration and homogenization pressure. Food Hydrocolloids 25. 604-612.

Sathivel, S., Prinyawiwatkul, W., Negulescu, I.I., King, J.M. and Basnayake, B.F.A. 2003. Effects of purification process on the rheological properties of catfish oil. Journal of the American Oil Chemists' Society 80. 829-832.

Sathivel S. 2005. Thermal and flow properties of oils from salmon head. Journal of the American Oil Chemists' Society 2, 147-151.

Sathivel S,, Huang J,, Prinyawiwatkul W. 2008a. Thermal Properties and Applications of the Arrhenius Equation for Evaluating Viscosity and Oxidation Rates of Unrefined Pollock Oil. Journal of Food Engineering 84, 187-193.

Sathivel S., Prinyawiwatkul W., Negulescu I.I., King J.M. 2008b. Determination of Melting Points, Specific Heat Capacity and Enthalpy of Catfish Visceral Oil during the Purification Process. Journal of the American Oil Chemists' Society 85, 291-296.

Schnepf M., Spencer G., and Carlat J. 1991. Chemical and sensory characteristics of stored menhaden oil/soybean oil blends. Journal of the American Oil Chemists' Society 68, 281-284.

Scholfield C.R. 1979. Silver nitratehigh performance liquid chromatography of fatty methyl esters. Journal of the American Oil Chemists’ Society 56 (4). 510-511.

Shahidi F. and Wanasundara P. K. J. P. D. 1995 Cyanogenic Glycosides of Flaxseed. Abstracts of Papers of the American Chemical Society 210. 245-AGFD.

Shahidi F., Wanasundara P.K.J.P.D., and Wansundara U.N. 1997. Changes in edible fats and oils during processing. Journal of Food Lipids 4, 199-231.

Shahidi F. and Han X.Q. 1993. Encapsulation of food ingredients. Critical Review in Food Science and Nutrition 33. 501-547.

Shantha N.C. and Ackman, R.G. 1991. Silica gel thin-layer chromatographic method for concentration of longer chain polyunsaturated fatty acids from food and marine lipids. Canidian Institute of Food Science and Technology Journal 24. 156-160.

Sheftall IV W. 2009. The gulf menhaden: Brevoortia patronus. Part one: species profile. Lagniappe Volume 22, No. 3. 1-2.

Sheu T. Y. and Rosenberg M. 1998. Microstructure of microcapsules consisting of whey proteins and carbohydrates. Journal of Food Science 63. 491-494.

Sheu T.Y. and Rosenberg M. 1995. Microencapsulation by spray drying ethyl caprylate in whey protein and carbohydrate wall systems. Journal of Food Science 60, 98-103.

Shiau S.Y. 2002. Tilapia, Oreochromis spp. nutrient requirements and feeding of finfish for aquaculture. In: Webster CD, Lim CE(eds), Cabi Publishing, New York, pp 273–292.

Shimada Y., Maruyama K., Okazaki S., Nakamura M., Sugihara A., and Tominagal Y. 1994. Enrichment of polyunsaturated fatty acids with Geotrichum candidum Lipase. Journal of the American Oil Chemists' Society 71. 951-954.

Shinowara G.Y. and Brown J.B. 1940. Studies on the chemistry of the fatty acids. VI. The application of crystallization methods to the isolation of arachidonic acid, with a comparison of the properties of this acid prepared by crystallization and by debromination. Observations on the structure of arachidonic acid. Journal of Biological Chemistry 134. 331-340.

Song Y., Qiao W., Yoon S. 2005. Toluene adsorption on various activated carbons with different pore structures. New Carbon Materials 20. 294-298.

Soobratte M.A., Neergheen V.S., Luximon-Ramma A., Aruoma O.I., and Bahorun T., 2005. Phenolics as potential antioxidant therapeutic agents : Mechanisms and actions. Mutation Research/fundamental and Molecular Mechanisms of Mutagenesis 579. 200-213.

Subramanian R., Nakajima M., Yasui A., Nabetani H., Kimura T., and Maekawa T. 1999. Evaluation of Surfactant-Aided Degumming of Vegetable Oils by Membrane Technology, Journal of the American Oil Chemists Society 76. 1247-1253.

Sun T., Pigott G.M., and Herwig R.P. 2002. Lipase-assisted concentration of n-3 polyunsaturated fatty acids from viscera of farmed Atlantic salmon (Salmo salar L.). Journal of Food Science 67 (1). 130-136.

Suryawan A., O'Connor P.M.J., Bush J.A., Hanh V.N., Davis A.T. 2008. Differential regulation of protein synthesis by amino acids and insulin in peripheral and visceral tissues of neonatal pigs. Amino Acids 37 (1). 55-63.

Tan C.P. and Che Man Y.B. 2002. Differential scanning calorimetric analysis of palm oil, palm oil based products and coconut oil: effect of scanning rate variation. Food Chemistry 76. 89-102.

Tan C.P., Che Man Y.B., Selemat J., Yusoff M.S.A. 2001. Application of Arrhenius kinetics to evaluate oxidative stability in vegetable oils by isothermal differential scanning calorimetry. Journal of the American Oil Chemists Society 78. 1133-1137.

Tan L.H., Chan L.W. and Heng P.W.S. 2005. Effect of oil loading on microspheres produced by spray drying. Journal of Microencapsulation 22. 253-259.

Tanaka Y., Hirano J., and Funada T. 1992. Concentration of docosahexaenoic acid in glyceride by hydrolysis of fish oil with Candida cylindracea lipase. Journal of the American Oil Chemists' Society 69. 1210-1214.

Teeter H.M. and Cowan J.C. 1956. Viscometric properties of higher fatty acids and their derivatives. Journal of the American Oil Chemists' Society 33. 163-168.

Thorsen M.A. and Hildebrandt K.S. 2003. Quantitative determination of phenolic diterpenes in rosemary extracts – aspects of accurate quantification. Journal of Chromatography A 995. 119-125.

Tipton K.D. and Witard O.C. 2007. Protein requirements and recommendations for athletes: relevance of ivory tower arguments for practical recommendations. Clinical Journal of Sports Medicine 26. 17-36.

Tischler M.E., Desautels M., and Goldberg A.L. 1982. Does leucine, leucyltransfer RNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? Journal of Biological Chemistry 257. 1613-1621.

Tonon R. V., Grosso C. R. F. and Hubinger M. D. 2011. Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. Food Research International 44. 282-289.

Trambouze P. 1990. Chemical Engineering Progress, 86, 23.

U.S. Dairy Export Council.
http://www.usdec.org/Products/content.cfm?ItemNumber=82509&navItemNumber=82259

U.S. Food and Drug Administration. 1999. Food labeling health claims: soy protein and coronary heart disease. Food and Drug Administration, HHS. Final rule. Federal Register 64. 57700-33.

Uauy R. and Valenzuela A. 2000. Marine oils: the health benefits of n -3 fatty acids. Nutrition 6(7/8). 680-684.

Vega C. and Roos Y.H. 2006. Spray-dried dairy and dairy-like emulsions – compositional considerations. Journal of Dairy Science 89. 383-401.

Wagenmakers A.J. 1998. Muscle amino acid metabolism at rest and during exercise: role in human physiology and metabolism. Exercise and Sport Sciences Reviews 26. 287-314.

Walstra P. 1983. In: P.F. Fox, Editor, Developments in Dairy Chemistry 2—Lipids, Applied Science Publishers, London. 119-158.

Wanasundara N. U. and Shahidi F. 1998. Lipase-assisted concentration of n-3 polyunsaturated fatty acids in acylglycerols from marine oils. Journal of American Oil Chemists' Society 75 (8). 945-951.

WHO: World Health Organization (2004). Available at:
http://www.who.int/cardiovascular_diseases/en/cvd_atlas_14_deathHD.pdf.

Wiedermann L.H. 1981. Degummimg, refming and bleaching of soybean oil. Journal of the American Oil Chemists' Society 58. 159-166.

Wilson R.P. 2002. Amino acids and proteins. In: Halver JE,Hardy RW (eds) Fish nutrition, 3rd edn. Academic press, Elsevier Science, pp 144-175.

Wright I. 2004. Salmon by-products. Aqua Feeds: Formulation and beyond, 1 (1): 10-12. http://www.feedware.com/aqua/magazine/v1i1/salmonby.html

Yadwad V.B., Ward O.P., and Noronha L.C. 1991. Application of lipase to concentrate the docosahexaenoic acid (DHA) fraction of fish oil. Biotechnology and Bioengineering 38. 956-959.

Yanez-Fernandez J., Ramos-Ramirez E. G. and Salazar-Montoya J. A. 2008. Rheological characterization of dispersions and emulsions used in the preparation of microcapsules obtained by interfacial polymerization containing Lactobacillus sp. European Food Research and Technology 226. 957-966.

Yang R.T. 2003. Basic considerations for sorbent design, Adsorbents Fundamentals and Applications, John Wiley & Sons Inc., Hoboken, New Jersey, USA, pp. 12-15.

Yang B., Zhou R., Yang J., Wang Y., and Wang W. 2008. Insight into the enzymatic degumming process of soybean oil. Journal of the American Oil Chemists' Society 85 (5). 421-425.

Yang L.Y., Kuksis A., and Myher J.J. 1989. Lumenal hydrolysis of menhaden and rapeseed Oil and their fatty acid methyl and ethyl esters in the rats. Biochemistry and Cell Biology 67. 192-204.

Yehuda S., Rabinovitz S., and Mostofsky D.I. 1999. Essential fatty acids are mediators of brain biochemistry and cognitive functions. Journal of Neuroscience Research 56. 565-570.

Young F.V.K. 1978. Processing of oils and fats, Chemical Industry 692, p. 703.

Young F.V.K. 1986. The chemical and physical properties of crude fish oils for refiners and hydrogenators, Fish Oil Bulletin No. 18, Fish oil International Association of Fish Meal Manufacture, Herefordshire, UK, pp. 1-19.

Young F.V.K., Poot C., Biernoth E., Krog N., Davidson N.G.J. and Gunstone F.D. 1994. Processing of oils and fats. In The Lipid Handbook. 2nd Ed., (F.D. Gunstone, J.L. Harwood and F.B. Padley, eds.) pp. 249-318, Chapman & Hall, New York.

Yusuf S., Reddy S., Ounpuu S., and Anand S. 2001. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. Circulation 104. 2746-2753.

Zhang Z. K., Li G. Y. and Shi B. 2006. Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes. Journal of the Society of Leather Technologists and Chemists 90. 23-28.

Zhao L. Yalkowsky S.H. 1999. A combined group contribution and molecular geometry approach for predicting melting points of aliphatic compounds. Industrial and Engineering Chemistry Research 38. 3581-3584.

APPENDIX 1: PURIFYING FISH OILS BY CONVENTIONAL METHODS

Methods

The PV and FFA of the oil samples were measured by the methods described in 2.2.1. The moisture content was measured according to the method in 2.2.2.

Conventional methods:

1. Degumming: 100 g crude catfish oil was removed from frozen storage, placed in a 600-mL beaker, and heated to 70 °C in a temperature-controlled water bath; 3 mL 3% aqueous citric acid solution was added to the oil, and the mixture was thoroughly mixed at 70 °C for 1 min. The oil was then cooled to room temperature and centrifuged at 2,560×g for 10 min to remove precipitated gum.
2. Neutralization: Sodium hydroxide (12.6 g of 9.5% NaOH solution) was added to the fish oils (100 g) and the mixture was heated to 65°C for 30 min with constant stirring with a magnetic stirrer. The sample was then cooled to room temperature and kept undisturbed for 6 h. After centrifuging at 2,560×g for 10 min, the oil was decanted from the precipitated soap. Demineralized water (50 mL) was added to wash out any remaining soap; this was repeated three times. Water and impurities were removed by centrifuging at 2,560×g for 10 min.
3. Bleaching: Fish oils were heated in a water bath and bleached with 4% (w/w) activated earth at 70 °C for 10 min with constant stirring using a magnetic stirrer. The activated earth with absorbed impurities was removed from the oil by centrifuging at 2,560g for 30 min.
4. Deodorization: The fish oils were was deodorized using a laboratory distillation unit. The distillation unit consisted of a 500-mL round bottom boiling flask with three outlets. One

outlet was connected to a vacuum pump, another outlet was connected to a glass distillation column, and the remaining outlet was sealed with a thermometer. The flask was placed on a heating system. The oil (100 mL) was added to the flask and heated to 100 °C for 30 min under vacuum (5 mmHg). The temperature was manually controlled. The volatile products were condensed in a cooling system installed on the vacuum line and the distillate was collected.

Results

Menhaden and salmon oils were purified by the four main conventional methods (degumming, neutralization, bleaching, and deodorization) and the results on removal of peroxides and free fatty acids are shown in tables 7.1 and 7.2. Degumming was effective removing peroxides but not free fatty acids from the MO and SO and had the highest oil loss. Neutralization was most effective decreasing PV and FFA; however the yield was comparatively lower. Bleaching had similar effects with degumming however the yield was higher than degumming method. Deodorization method didn't cause obvious changes on PV and FFA contents.

Table 7.1 – PV, FFA, and Yield of the menhaden oil purified by conventional methods

	PV (meq/kg)	FFA (%)	Yield (%)
MO	24.22±1.28^{a}	2.79±0.02^{b}	
MD	17.82±0.74^{b}	2.68±0.05b^{b}	38.80%
MN	8.62±0.14^{d}	0.14±0.00^{c}	47.10%
MB	12.07±2.62^{c}	2.62±0.11b^{b}	76.30%
ME	20.72±0.71^{a}	2.05±0.11^{a}	99.90%

Values are means ± SD of triplicate determinations. ^{abcd}Means with the same letters in each column are not significantly different ($P > 0.05$). MO = unrefined menhaden oil; MD = menhaden oil purified by degumming process; MN = menhaden oil purified by neutralization process; MB = menhaden oil purified by bleaching process; ME = menhaden oil purified by deodorization process.

Table 7.2 - PV, FFA, and Yield of the salmon oil purified by conventional methods

	PV (meq/kg)	FFA (%)	Yield (%)
SO	35.46±1.54[a]	2.43±0.12[b]	
SD	28.57±1.14[b]	2.47±0.02[b]	36.45%
SN	10.58±0.37[d]	0.12±0.03[c]	52.89%
SB	18.97±0.28[c]	2.22±0.09[b]	69.87%
SE	31.40±2.71[a]	1.81±0.25[a]	98.56%

Values are means ± SD of triplicate determinations. [abcd]Means with the same letters in each column are not significantly different ($P > 0.05$). SO = unrefined salmon oil; SD = salmon oil purified by degumming process; SN = salmon oil purified by neutralization process; Bleaching = salmon oil purified by bleaching process; SE = salmon oil purified by deodorization process.

APPENDIX 2: SPRAY DRYING PROCESS AND CALCULATIONS

The emulsions were dried using a pilot plant scale spray dryer (FT80 Tall Form Spray Dryer Armfield Inc., Ringwood, UK) under co-current drying conditions. A schematic representation of the pilot scale FT80 tall form spray dryer is shown in Fig. 7.1. The FT80 spray dryer includes inlet and exhaust air fans, an electrical air heating chamber, a tall dryer chamber, and a cyclone separator. Ambient air was blown into the air heating chamber by the inlet fan where the ambient air was heated by an electric resistance heater to 150°C. The heated air (inlet air) was blown into the top of the drying chamber. The temperature of emulsions was measured at the beginning of the procedure, and then the emulsion was separately fed through the hygienic progressing cavity pump to a spray nozzle where it was atomized and sprayed into the dryer chamber. The emulsion droplets were dried in the drying chamber yielding dried powder and dust. The dried powder, dust, and air were pulled to the bottom of the drying chamber and then to the cyclone separator by the exhaust fan. The powder and dust were separated in the cyclone separator. The powder separated by the cyclone separator was collected in the powder collector and the exhaust air was released though filter bag to the atmosphere. The filter bag captured the dust.

The powder samples and dust were analyzed for moisture content according to the AOAC official method 930.15 (AOAC, 1999). The powder production rate was estimated and compared with the actual powder production rate. The actual powder production rate was the mass of the powder recovered from the powder collector divided by the time of production. The estimated production rate was the sum of the actual production rate and the average rate at which powder was retained within the spray dryer by such mechanisms as sticking to the walls of the spray dryer. The mass flow rate for water entering and leaving the spray dryer and the energy required to dry the emulsion in the production of powder were determined.

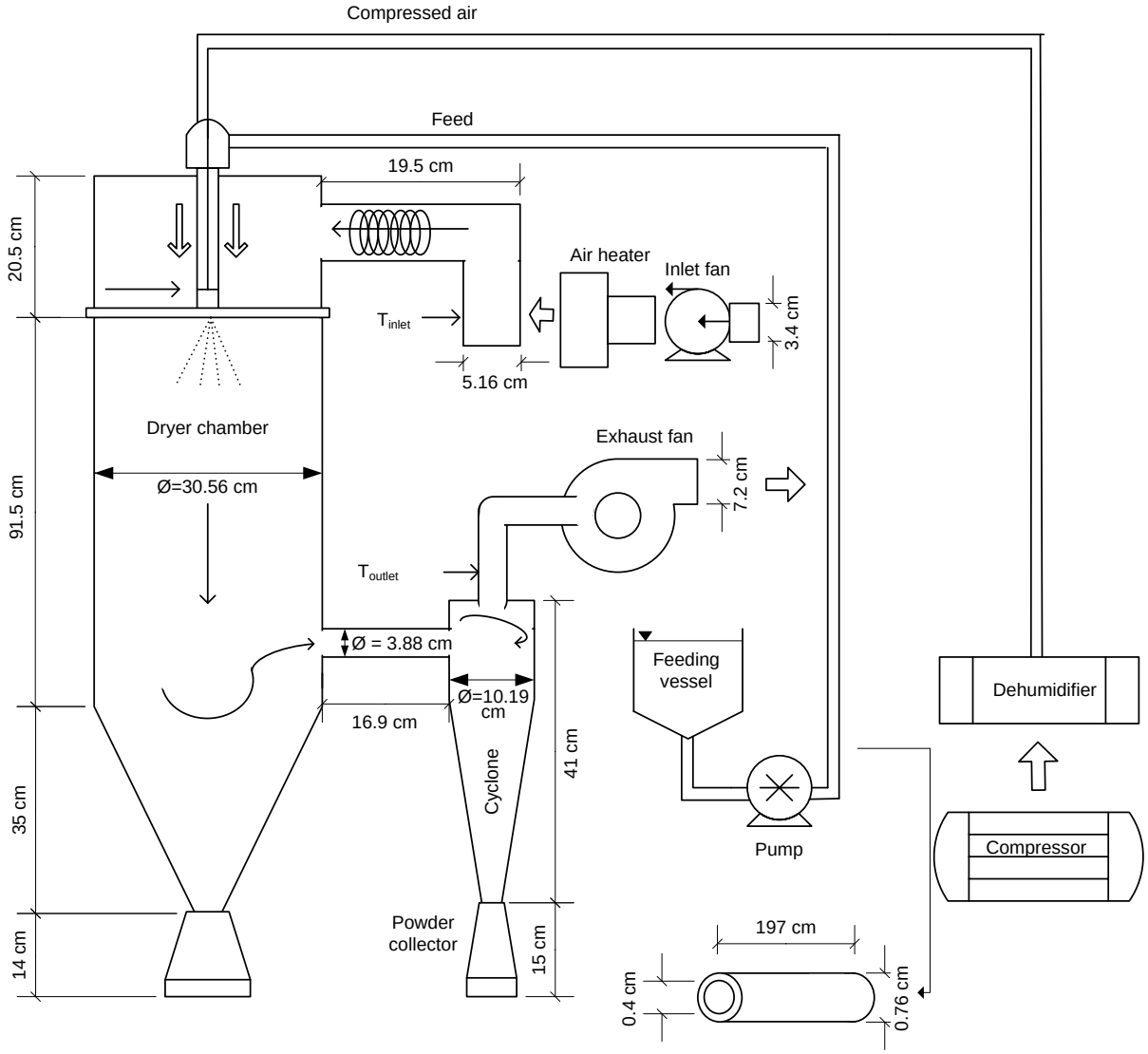


Figure 7.1 - Schematic representation of the pilot scale FT80 Tall Form Spray Dryer-Armfield Limited®.

Table 7.3 - Parameters for the spray dryer and spray drying process when drying MOEE

	Inlet ambient air	Outlet air
Temperature (°C)	26.23±0.38	80.53±0.78
Velocity (km/h)	70.73±0.92	19.19±0.14
Relative humidity (%)	39.67±0.32	5.40±0.03
Internal pipe diameter (m)	0.034	0.072
Partial pressure exerted by water vapor (kPa)	1.41±0.01	2.56±0.01
Saturation pressure of water vapor (kPa)	3.57	47.39
Absolute humidity $\times 10^3$ (kg water/kg dry air)	8.81±0.07	16.11±0.08
Specific volume of inlet or outlet air (m^3/kg dry air)	0.86±0.00	1.03±0.00
Mass flow rate (kg dry air/h)	74.80±0.93	76.14±0.39
Specific heat of dry air at 26.23°C (kJ/kg.K)	1.0126	
Specific heat of water vapor at 26.23°C (kJ/kg.K)	1.88	

Table - 7.4 Parameters for the spray dryer and spray drying process when drying SOEE

	Inlet ambient air	Outlet air
Temperature (°C)	26.26±0.21	80.70±0.70
Velocity (km/h)	70.64±0.53	19.21±0.17
Relative humidity (%)	39.53±0.31	5.35±0.12
Internal pipe diameter (m)	0.034	0.072
Partial pressure exerted by water vapor (kPa)	1.41±0.00	2.54±0.06
Saturation pressure of water vapor (kPa)	3.57	47.39
Absolute humidity $\times 10^3$ (kg water/kg dry air)	8.75±0.02	15.97±0.37
Specific volume of inlet or outlet air (m^3/kg dry air)	0.86±0.00	1.03±0.00
Mass flow rate (kg dry air/h)	74.70±0.59	76.19±0.56
Specific heat of dry air at 26.34°C (kJ/kg.K)	1.0126	
Specific heat of water vapor at 26.34°C (kJ/kg.K)	1.88	

Estimation of Production Rate

The material balance expressed as average flow rates of dry solids entering and leaving the spray dryer system (Fig. 7.2) is described by Eq. (1).

$$m_e = mP + m_d \quad (1)$$

The production rate was estimated by the Eq. (2)

$$mP = m_e - m_d \quad (2)$$

where m_e is the average emulsion flow rate (kg dry solids/h); m_d is the average dust flow rate (kg dry solids/h); mP is the estimated powder production rate which included both the average actual production flow rate (m_p) for the powder collected through powder collector vessel and product retained in the spray dryer. It was assumed that the physical properties of product retained in the spray dryer were the same as the powder product collected in cyclone collector vessel.

Estimation of Evaporation Rate

The moisture balance expressed as water entering and leaving the spray dryer system is described by Eq. (3).

$$m_{aa}AH_{aa}+m_ew_e = m_{ao}AH_{ao}+m_dw_d+mPw_p \quad (Eq.3)$$

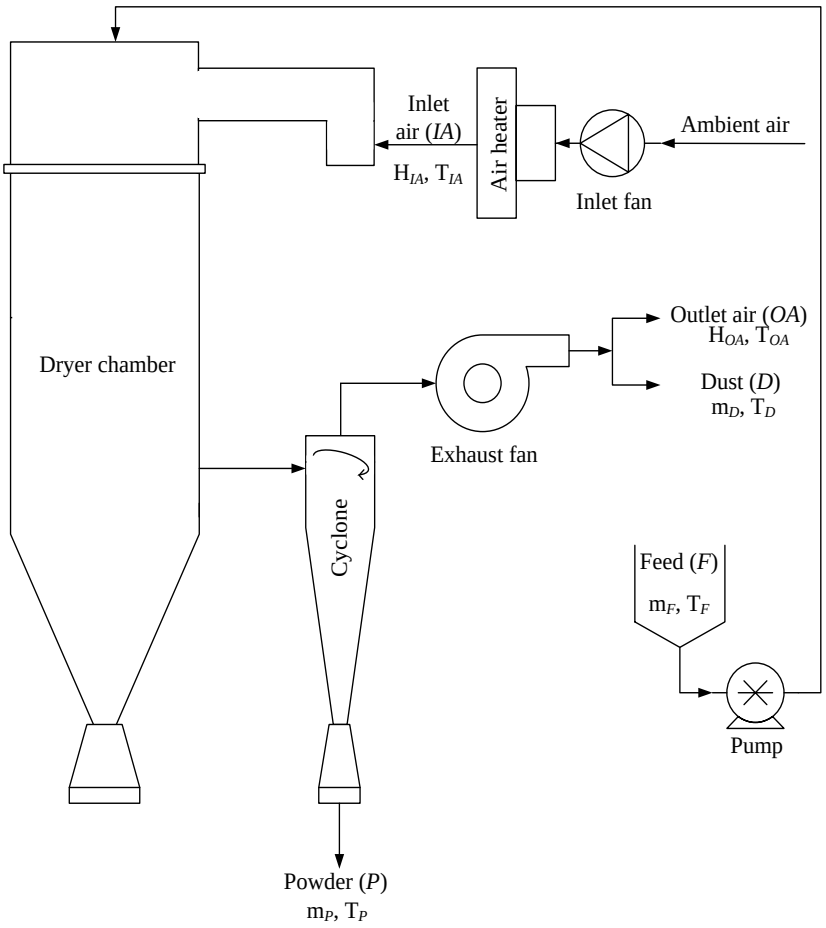


Figure 7.2 - Material balance of a spray drying system.

where m_{aa} is the dry air mass flow rate at the inlet (ambient air) (kg dry air/h); m_{ao} is the dry air mass flow rate of outlet air (kg dry air/h); m_e is the mass flow rate of the emulsion (kg dry solids/h); m_d is the mass flow rate of dust (kg dry solids/h); mP included both the product flow rate (m_p) for the powder collected through cyclone vessel and product retained in the spray dryer; AH_{aa} is the absolute humidity of inlet ambient air (kg water/kg dry air); AH_{ao} is the absolute humidity of outlet air (kg water/kg dry air); w_e is the moisture content (dry basis) of emulsion (kg water/kg dry solids); w_d is the moisture content (dry basis) of dust (kg water/kg dry solids); w_p is the moisture content (dry basis) of product (kg water/kg dry solids). It has been assumed that the powder retained in the spray dryer has essentially the same moisture content as the

collected powder and that the encapsulation effectively removes that moisture from the air stream.

The evaporation rate (E_{va}) was estimated from the moisture removed by the dry air as shown by Eq. (4).

$$E_{va} = m_{ao}AH_{ao} - m_{aa}AH_{aa} \quad \text{(Eq.4)}$$

Also, the evaporation rate (E_{vp}) was estimated based on the moisture content of emulsion, powder collected through cyclone vessel and dust using Eq. (5).

$$E_{vp} = m_e w_e - m_d w_d - mPw_p \quad \text{(Eq.5)}$$

The dry air mass flow rate of inlet ambient air and dry mass flow rate of outlet air were estimated as described by the AlChE Equipment Testing Procedure (2003) using Eq. (6).

$$m = \frac{V}{V'} \quad \text{(Eq.6)}$$

where m is the dry air mass flow rate (kg dry air/h); V is the volumetric flow rate of inlet or outlet air (m^3/h); V' is the specific volume of inlet or outlet dry air (m^3/kg dry air).

The volumetric flow rate of inlet ambient air and outlet air was calculated as described by Eq. (7)

$$V = v \times A \quad \text{(Eq.7)}$$

where v is the average velocity of the inlet or outlet air (m/s) and A is the cross sectional area of the inlet or outlet air pipe (m^2).

The specific volumes of inlet or outlet dry air were calculated using Eq. (8) as described by Singh and Heldman (2001).

$$V' = (0.082T + 22.4)\left(\frac{1}{29} + \frac{AH}{18}\right) \quad \text{(Eq.8)}$$

where T is the temperature of inlet ambient or outlet air (°C); AH is the absolute humidity of inlet ambient or outlet air (kg water/kg dry air).

The absolute humidity of the inlet ambient and the outlet air were calculated as Eq. (9) as described by AlChE Equipment Testing Procedure (2003)

$$AH = 0.622x\frac{p_w}{101.325 - p_w} \quad \text{(Eq.9)}$$

where AH is the absolute humidity of the inlet ambient or outlet air (kg water/kg dry air); and p_w is the partial pressure exerted by water vapor (kPa).

The partial pressure exerted by water vapor is estimated with Eq. (10) as described by Singh and Heldman (2001)

$$p_w = p_v \text{ x RH} \quad \text{(Eq.10)}$$

where p_w is the partial pressure exerted by water vapor (kPa); p_v is the saturation pressure of water vapor (kPa); RH is the relative humidity (%).

Estimation of Energy Required Drying the Emulsions

The estimation of the energy required to dry the emulsions was obtained with Eq. (11) as described by Singh and Heldman (2001).

$$Q = m_{aa}c_p\Delta T = m_{aa}(c_{aa} + c_v\, AH_{aa})(T_{ad} - T_{aa}) \quad \text{(Eq.11)}$$

where m_{aa} is dry air mass flow rate of inlet ambient air (kg dry air/h); c_p is specific heat of inlet ambient air (kJ/[kg K]); c_{aa} is specific heat of inlet ambient dry air (kJ/[kg K]); c_v is the specific heat of water vapor (kJ/[kg K]); AH_{aa} is the absolute humidity of inlet ambient air (kg water/kg dry air); ΔT is the temperature difference between inlet ambient air and heated air (K); T_{ad} is the temperature of the inlet drying air (K); and T_{aa} is the temperature of inlet ambient air (K).

Table - 7.5 Production rate, evaporation rate, and energy required for spray drying MOEE and SOEE

	MOEE	**SOEE**
Estimated Production rate x 10^{-3} (Kg dry solids/h)	79.68±0.54	85.17±0.93
Real Production rate x 10^{-3} (Kg dry solids/h)	76.82±0.02	81.61±0.02
Evaporation rate (kg water/h)^{1}	0.57±0.01	0.56±0.02

(table 7.3 continued)

Evaporation rate (kg water/h)[2]	0.58±0.00	0.58±0.00
Energy required to increase ambient temperature to inlet temperature (kJ/kg)	9528.32±118.19	9511.07±74.88

[1]Calculated based on the moisture uptake by the dry air (kg water/h). [2]Calculated based on the moisture content of the emulsion, powder collected through collector vessel, and dust (kg water/h). MOEE= Emulsion containing enzyme treated menhaden oil, SOEE= Emulsion containing enzyme treated salmon oil.

APPENDIX 3: AUTHOR'S PUBLICATIONS

Yin H., Mis K.S., Huang J., Sathivel S., and Bechtel P.J. 2011. Effects of oil extraction methods on physical and chemical properties of red salmon oils (*Oncorhynchus nerka*). Journal of American Oil Chemists' Society 88. 1641-1648.

Yin, H., Wan Y., Pu J., Bechtel P. J., and Sathivel S. 2011. Functional properties of protein fractions of channel catfish (*Ictalurus punctatus*) and their effects in an emulsion system. Journal of Food Science 76 (3). E283-E290.

Yin, H., and Sathivel S. 2010. Physical properties and oxidation rates of unrefined menhaden oil (*Brevoortia patronus*). Journal of Food Science 75. E163-E168.

Yin, H., Pu J., Wan Y., Xiang B., Bechtel P. J., and Sathivel S. 2010. Rheological and functional properties of catfish skin protein hydrolysates. Journal of Food Science 75. E11-E17.

Sathivel, S., **Yin H**., Bechtel P. J., and King J. M. 2009. Physical and nutritional properties of catfish roe spray dried protein powder and its application in an emulsion system. Journal of Food Engineering 95. 76-81.

Sathivel, S., **Yin H**., Prinyawiwatkul W., and King J. M. 2009. Comparison of chemical and physical properties of catfish oils prepared from different extracting processes. Journal of Food Science 74. E70-E76.

Sathivel, S., **Yin H**., Prinyawiwatkul W., King J.M. and Xu Z. 2008. Economical methods to extract and purify catfish oil for Louisiana catfish processors. Louisiana Agriculture Magazine, summer, 13.

VITA

Huaixia Yin was born in Weifang, Shandong Province, People's Republic of China. She attended Shandong Agricultural University, China, and graduated with a Bachelor of Science degree in food science and engineering in June 2004. In June 2007, she earned her Master of Science degree in Food Science from South China University of Technology, China. She came to Louisiana State University in food science in August 2007. Currently, she is a candidate for the degree of Doctor of Philosophy in food science in the College of Agriculture. She will receive her doctoral degree in fall 2011.

During her graduate student career, she has been actively involved in multiple research projects. Huaixia has to date: 1) published seven manuscripts in refereed journals, 2) given one presentation at the 2011 Institute of Food Technologists (IFT) annual meeting in New Orleans, LA; 3) presented one technical paper at the 2011 American Oil Chemists' Society meeting in Cincinnati, OH., 4) given two presentations at the 2010 Institute of Food Technologists (IFT) annual meeting in Chicago, IL, and 5) received three Louisiana Gulf Coast Section of IFT awards to attend the 2008, 2009, and 2010 IFT Annual Meetings. She was selected four times as a 'finalist' for four of her papers for the 2009 IFT Aquatic Food Product Division Graduate Student Paper Competition, and also as a finalist in the 2008 IFT Aquatic Food Product Division Graduate Student Paper Competition. She also received a first place award at the 2009 Pacific Fishery Technology Annual Meeting in Portland, Oregon for presenting a research article on fish oil extraction. Huaixia was a recipient of the 2010-2011 IFT Graduate Fellowship and also the recipient of the 2011 American Oil Chemists' Society (AOCS) Honored Student Award. Huaixia is a very active member of the Department's Food Science Club, for which she has served as the club treasurer.