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Production, Process Design and Quality Characterization of Catfish Visceral Oil.

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PRODUCTION, PROCESS DESIGN AND QUALITY CHARACTERIZATION OF CATFISH VISCERAL OIL

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To my parents

My parents taught me many things in my life with valuable lessons that I have held close to my heart. With those lessons I have gone many places, met many people, and done many things. But of all the things I have done, the thing that I am most proud of is that I am the son of Mr. Subramaniam and Mrs. Latchumie.

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ABSTRACT

Between 45-55% of viscera, head, skin, intestine and slurries are generally generated as byproducts or waste from catfish filleting process. Our preliminary study showed that the crude fat content of the whole catfish viscera was 30-35% (wet basis) and the total ω-3 fatty acids of oils recovered from the whole and/or portioned viscera ranged from 4.3-20.9 mg/g (dry basis). Catfish viscera may serve as an excellent source of health-promoting oil. Yield of purified (deodorized) catfish visceral oil was 65.7% and contained considerable amounts of DHA (1.21 mg/g of oil) and omega-3 fatty acid (4.6 mg/g of oil). A rapid microwave-assisted method for fatty acid analysis was developed. The microwave heating power (%) and time (sec) required for maximal fatty acid recovery were determined. Heating at 100% power for 80 sec yielded the highest recovery of DHA (C22:6 ω-3) and arachidonic acid (C20:4 ω-3). The adsorption of free fatty acid of crude catfish oil was performed with chitosan, activated carbon and/or activated earth as adsorbents. Batch adsorbent experiments were carried out to assess equilibrium parameters. Chitosan was the most effective adsorbent to adsorb free fatty acids. The fixed-bed adsorption study showed that the highest percent of bed length was used when chitosan was used as an adsorbent. Melting points, specific heat capacity, enthalpy, and heat resistance were studied using DSC (Differential Scanning Calorimeter) and TGA (Thermogravimetric analysis). Melting points, enthalpy, and specific heat capacity varied greatly among the catfish oils from different processing stages due to differences in impurities. Catfish visceral oils from different processing steps showed different rates of weight loss with increased temperature. Suitability of Newtonian, Herschel-Bulkley, Bingham plastic, and Casson models to characterize the

flow behavior of catfish oils form different processing steps was investigated. The Casson equation sufficiently described the flow behavior of catfish oils. The research findings from this study will benefit the whole catfish industries and will set a good model for fish oil recovery from other fish species as well.

CHAPTER 1. INTRODUCTION

1.1 Catfish Production

The channel catfish (*Ictalurus punctatus*) industry in the United States has been recently growing during its 35-year history. The total water surface acreage for the catfish production has increased from about 56,000 acres in 1980 to more than 185, 700 acres in 2001 (Catfish Production, 2001). The catfish processing industry has also grown dramatically to accommodate rapid changes in supply and consumer demand.

Catfish processed in 1980 in the U.S. was approximately 46.5 million pounds (live weight). By 2000, this number increased to about 594 million pounds, a 13-fold increase in 20 years. Producer sales (farm value) for the food-size catfish totaled almost $446 million for 2000, at an average price of 75 cents per pound (Catfish Processing, 2001).

More than 297 million pounds of processed catfish were consumed in 2000 through various markets, with a per capita consumption of more than 1.07 pounds. Total revenue to processors was about $708 millions. Catfish is now the fourth most popular seafood product in the U.S.

1.2 Catfish Product Forms

A traditional product form of catfish is the whole dressed fish. To produce a whole dressed catfish, it must be headed, gutted and skinned with tail left intact (Silva and Dean, 2001). The dressed fish is also further processed into a variety of cuts or form, including fillets, nuggets, fingers, steaks, breaded fillets and nuggets, marinated fillets, and smoked fillets and dressed fish.

1.3 Catfish Processing Waste

Silva and Dean (2001) illustrated a hypothetical catfish product mix (Figure 1.1). The yields and properties of product mix of various catfish product forms were calculated based on an original weight of 100, 000 pounds of live catfish

Volumes given in Figure 1 were based on 100,000 pounds of live fish. The yield of the whole-dressed fish is 62%. As further processing occurs, 47.5% of the a whole dressed fish was used for filleting process and 2.5% for steak cuts. Over 70 percent of the whole dressed fish was further processed into fillets, steaks and nuggets. Of this amount, 4.5 percent is converted in to steaks and the remaining 66.5% as fillets.

Thus, from an input of 100,000 pounds of live catfish weight, a total of 5,301 pounds was converted into salable product and the rest became a processing waste (Silva and Dean, 2001). The yield of catfish when processed as whole fillets is around 45 %, generating about 55 % waste. The Department of Food Science at LSU is actively involving in research on catfish processing waste. The preliminary studies show that the fat content of catfish viscera is 30-35% (dry basis). It clearly indicates that multimillion-kilogram of catfish fat is being wasted and currently has no market value.

1.4 Catfish Consumption

Per capita consumption of catfish products has significantly increased over the last decade from 0.41 pounds in 1985 to 1.12 pounds in 2000 (Top 10 Seafood and Fish Products, 2000), partly because of intensive marketing efforts within the industry. Rapid changes in consumption patterns and an apparent desire for new and exciting food products have been equally important.

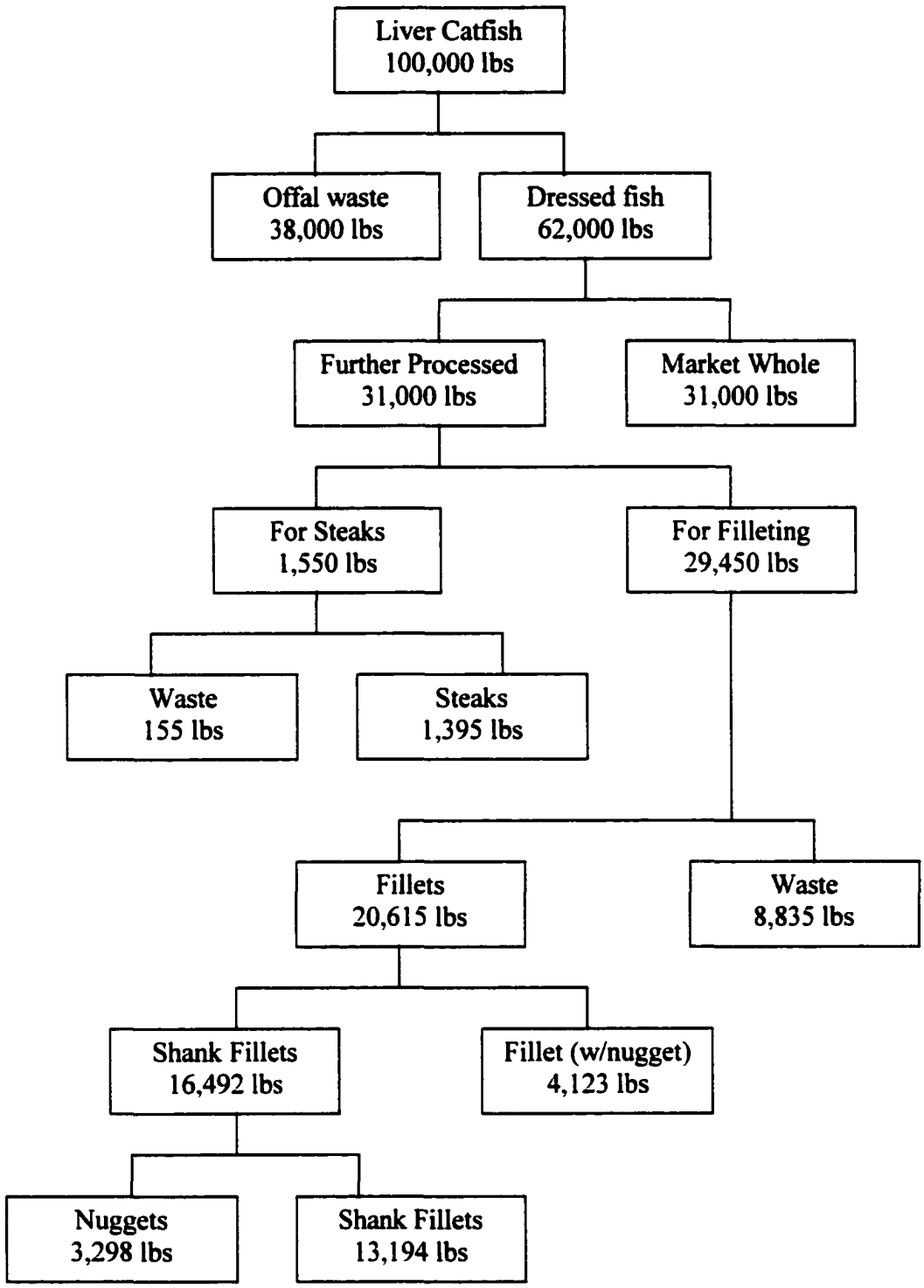

Figure 1.1 A hypothetical product mix for catfish processing (Silva and Dean, 2001)

This is evidenced by the introduction of more than 10,000 new food products each year. The catfish industry has recognized these trends and has introduced value-added products to keep abreast of consumers' demand. Some of these products (commercial or researched) are gumbo, sausage, corn dog, nuggets, fingers and others. Food service/institutional sales are rising, so product development for this industry is critical for growth.

1.5 Total Utilization of Seafood Processing Waste

The concept of "total resource utilization," "by-product recovery," and "value-added products" has been increasingly recognized. Significant changes have occurred in terms of fishery harvest, based on fishing regions as well as in catch of particular species. Fishery and seafood scientists now realize that the sea is no longer an infinite supply of an expanding resource of wild stock. The maximal sustained yield has been met with various fish species and exceeded with some in different regions of the world oceans. No longer can we think of materials from processing operations as "waste."

Waste management and utilization are an important issue facing food scientists/technologists and seafood industries from both regulatory and economic standpoints. Up to 25-50% of the catfish raw materials are utilized for primary products (fresh/frozen fillets, steaks, surimi, etc), leaving about 50-75% processing byproducts, which are normally considered as "wastes." The processing waste have been utilized for low- value products or discarded without pretreatments.

1.6 Utilization of Catfish Processing Waste

The lipid of catfish contains an abundant amount of monounsaturated fatty acids, such as palmitic, stearic, palmitoleic, oleic, and linoleic acids and they comprise more

than 50% of the total fatty acids. Saturated fatty acids made up about 30%, while polyunsaturated fatty acids account for 20 % of catfish oil (Fisher, 1983; Yang, 1984; Silva, 1984; Woodruff, 1987; Nettleton, 1990; Freeman, 1990; Kim et al., 1996). This indicates that catfish and its waste may have plenty of unsaturated fat and can be used as a rich resource for producing edible oil. However, it has been found that dietary changes can cause alterations in the fatty acid profile of fish fillets (Watanabe, 1982; Fair et al., 1993). The fatty acid content of channel catfish can be increased by supplementation of fish oil in prepared diets (Stickney and Andrews, 1971 and 1972; Worthington and Lovell, 1973; Gatlin and Stickney, 1982). Chanmugam et al. (1986) reported that cultured channel catfish fed with a practical feed formulation had lower levels of n-3 fatty acids compared to wild channel catfish. This was due to the relatively low level of n-3 fatty acids in the formulated diet of cultured catfish.

The processing wastes, especially catfish viscera contain high quantity of crude fat, which can be used as a raw material for producing omeg-3 enriched fish oil. Fish oils and other animal- or plant-derived bioactive compounds are currently a center of attention in the nutraceutical and functional foods arena. Fish oil containing long-chain polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), have received tremendous research attention. Omega-3 fatty acids have been claimed to reduce the risk of various diseases especially cardiovascular diseases. The majority of the American population does not consume an adequate level of omega-3 PUFAs through their natural diets. Fish oil, containing high concentration of EPA and DHA taken as a dietary supplement, is an attractive alternative to many health-conscious consumers. The market for omega-3 long chain PUFA

nutraceutical products is currently more established in U.S. With awareness of health benefits of fish oil, development of omega-3 enriched fish oil for nutraceutical products has been increased.

1.7 Fish Oil Composition

The nature of fatty acids and chemical distribution of fish oil and marine oils are generally characterized by a large group of saturated and unsaturated fatty acids, which are commonly associated with mixed triglycercides. In addition, triglycercides of body/muscle oils from fish and marine animals usually include minor amounts of fatty acids as substituents of phospholipids and other lipids. In comparison to body oils, liver oils and oils from particular parts (e.g., viscera) of fish and marine animals often contain large amounts of fatty acids associated with phospholipids, glyceryl ethers and wax, esters depending on the source of oils and lipids (Lovern, 1962).

The complex nature of fish oil depends upon a number factors. One of the major factors is the type of fat in fish diet. Kelly et al. (1958) has experimentally shown that the type of fat in fish diets can influence the proportional distribution of fatty acids in fish oils. They fed fish oil to freshwater fish and found that the fatty acids of fresh-water fish changed to resemble the dietary fish oil. Similar results were observed by Brenner et al. (1963); Mead et al. (1960); Brockerhoff et al. (1964). Researchers have studied the fatty acid of marine plankton and fish fatty acids because marine plankton is a major food source for fish (Kelly et al., 1959). A group of polyunsaturated fatty acids were isolated from marine plankton. They found that these fatty acids were found in fish and marine animals whose main diets are marine plankton (Farkas and Herodek, 1964).

Environmental factors, such as geographic locations of catch, seasons of the year, and water temperatures, also affect proportions of fatty acids of fish oils. Lewis (1962) investigated the influence of season, water temperatures, and pressure on fatty aid composition. He found two changes in the fatty acids, i.e., an increase in degree of unsaturation and reduction in chain length with a reduction in environmental temperature. Farkas and Herodek (1964) found that the amount of C20-C22 polyunsaturated fatty acids in fresh water planktonic crustacea increased with decreased temperatures.

1.7.1 Fish Oil Quality

The condition of the fish at the time of processing affects the oil physically, chemically and nutritionally. Fish of poor quality yields a malodorous oil with high contents of free fatty acids (FFA) and sulfur. These undesirable properties affect both the economic value and the applications of the oil. Some sulfur compounds have an inactivating effect on the nickel catalyst used for hydrogenation (called "poisoning of the catalyst"), thus the catalyst has to be replaced frequently.

To produce an oil with desirable prosperities, the fish should be as fresh as possible, and the oil should be cooled before delivery to the storage tank and should be pumped in near the bottom of tank (not right at the bottom) and removed from top. The sludge and water should be regularly drained from the bottom of tank to prevent an increase in FFA during storage (FAO, 1986).

1.7.2 Fish Oil

The world total production of fats and oils is in the range of 60-70 million metric tons per year and 20% production of oil comes from soybean, while marine oils come

from whale, fish liver and fish body oils which account for 1.97 % (U.S. Department of Agriculture, 1986). According to preliminary data, the U.S. production of edible fats and oils was about 7.6 million MT in 1988, with soybean oil accounting for about 67% of the total production of all fats and oils. The U.S. production of marine oils totaled 135 thousand metric tons in 1987 and was composed of 134 thousands metric tons of menhaden oil (99%) and 1.5 thousand metric tons of oil from miscellaneous fish (U.S. Department of Commerce, 1988).

Over one million MT of fish oils are used annually for preparing food. Furthermore, fish oils are usually competitive with other fats and oils available on the world market and have a wide range of functionality, especially the partially hydrogenated form which is used for the baking industry (Bimbo et al., 1987).

When the fish oil is winterized, a liquid fraction is obtained, which can be blended with another soft oil, such as soybean oil and used as a salad oil. It can also be used for single-use shallow frying. Hydrogenated fish oil can be used for deep-fat frying, either alone or as the major component of a blend with palm oil. Partially hydrogenated fish oil can be used for margarine and shortening production. It is also used in the production of emulsifiers for food applications

In addition, fish oil can be used in a variety of products and processes, including soaps, fatty acids, fatty chemicals, leather tanning, protective coatings, lubricants and greases, pneumatic tool lubricants, rubber compounds, caulking compounds, glazing compounds, gasket manufacture, core oils, tin-plating oils, rust proofing agents, refractory compounds, cutting oils, plasticizers, printing inks, linoleum, press-wood fiber boards, oiled fabrics, ceramics, ore flotation, fermentation substrates, illuminating oils,

mushroom culture, fire retardant, polyurethane foams and animal feeds. There is clearly a high demand for fish oil in both U.S. and other parts of the world. Therefore, it may be economical by possible to produce catfish oil because it can be used not only for food product development but also by other industries.

1.7.3 History of Fish Oil Developments in the United States

In early 1640, the first marine-origin oil, whale oil, was produced in the United States. However, whale oil is not a fish oil but a marine mammal oil. In 1811, menhaden oil was manufactured in the United States in Rhode Island as the first American fish oil. According to the literature, the menhaden fish were boiled in pots or kettles, then the fish were transferred to casks, and the oil and water pressed out by the pressing stone. The extracted oil was skimmed off the top and placed into a barrel (Stansby, 1978).

In 1860, a modern plant was established in Rhode Island, and it was comprised of steam cookers and mechanical screw presses. During the period 1873-1911, the U.S. production of fish oil remained stable at about 2 million gallons per year and was produced from between 110,000 and 115,000 metric tons of fish. In 1912, the production was increased to 6.6 million gallons from 323, 000 metric tons of fish and remained at that level until mid 1940s (Fitzgibbon, 1969). In 1940, the fish oil industry began to use centrifugation to separate oil from water and the recovered fish oil was used as a oil material in soap, paints, and linoleum. Due to introduction of centrifugation, there was a rapid increase in production of menhaden oil in the United States which was referred to as "Industrial Revolution" of the menhaden Industry in United States (Smith, 1940). In 1953, over 17 million gallons of oil was produced, and peaked in 1956 with

22.5 million gallons of oil (Bimbo, 1990). During 1950s, the United States began to study on other fish species oil, such as anchovy. It was reported that 931, 000 gallons of anchovy and 920,000 gallons of tuna oil were manufactured in California in 1975. In 1976 the production of all American fish oil was 204 million pounds with the value of at about 40 million dollars. Of this total production, 186 million pounds or nearly 90% was menhaden oil. Of the oil from other species, most of it was anchovy and tuna oils in about equal quantities (Stansby, 1978).

The U .S. National Marine Fisheries Service has reported that the total marine oil production in U.S. was 129,000 MT in 1985 and was composed of 98 % (126,000 MT) of menhaden oil and 3, 000 MT of tuna, mackerel, anchovy and other fish oils (U.S. National Marine Fisheries Service, 1985).

1.7.4 Essential Fatty Acids

Experimental work in the 1930's on animals and humans demonstrated that certain long chain polyunsaturated fatty acids, especially linoleic and arachidonic, are essential for growth and good skin and hair quality. Linoleic and linolenic acids are termed "essential" because they cannot be synthesized by the body and must be supplied in the diet. Arachidonic acid can, however, be synthesized by the body from dietary linoleic acid. Arachidonic acid is considered an essential fatty acid because it is an essential component of membranes and a precursor of a group of hormone-like compounds called eicosanoids including prostaglandins, thromboxanes, and prostacyclins which are important in the regulation of widely diverse physiological processes. Linolenic acid is also a precursor of a special group of prostaglandins. The dietary fatty acids that can function as essential fatty acids must have a particular

chemical structure, namely, double bonds in the *cis* configuration at specific positions (carbons 9 and 12 or 9, 12, and 15 from the carboxyl carbon atom or carbons 6 and 9 or 3, 6 and 9 from the methyl end of the molecule) on the carbon chain. The requirement for these essential fatty acids has been demonstrated clearly in infants. While the minimum requirement has not been determined for adults, there is no doubt that they are essential nutrients.

1.7.5 Fish Oil and Heart Diseases

Fish oil has been used since ancient time. For example, the effective use of fish oils for treating night blindness is mentioned in the Bible before anything was known about vitamins (Rosenberg, 1942). The first suspicion of a role of fish oil in heart diseases was that the consumption of considerable fish provided a beneficial effect on atherosclerosis or other cardiac diseases occurred in Norway.

Cardiovascular diseases, which include heart attack and stroke, are the leading causes of death in the United States. The most predominant form of cardiovascular disease is coronary heart disease or CHD (commonly referred to as "heart attack") which is the single leading cause of death in America. Atherosclerosis, the gradual blocking of arteries with deposits of lipids, smooth muscle cells, and connective tissue, contributes to most deaths from cardiovascular disease.

Researchers now recognize that total serum cholesterol is distributed largely between two general classes of lipoprotein carriers, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). The largest portion of total cholesterol is in the LDL fraction, and elevated levels of LDL cholesterol are associated with increased coronary heart disease risk. On the other hand, high levels of HDL cholesterol have been

associated with protection against coronary heart disease. One factor that has been related to increased levels of HDL cholesterol is regular exercise. However, it is uncertain whether diet or exercise related modifications of LDL or HDL levels might affect development of coronary heart disease. Long-term studies are currently in progress to address these questions.

The levels of total cholesterol and the LDL and HDL fractions in the blood are influenced by a combination of factors, including age, sex, genetics, diet, and physical activity. Diet and exercise are factors which individuals can modify and thus have been a basis for recommendations to reduce risk factors for chronic diseases such as coronary heart disease. The three major categories of dietary fatty acids (saturated, monounsaturated, and polyunsaturated) appear to influence total, LDL, and HDL cholesterol in different ways. In general, diets high in saturated fatty acids increase total as well as LDL and HDL cholesterol levels compared to diets low in saturated fatty acids. The specific saturated fatty acids palmitic (the principal saturated fatty acid in the U.S. diet), myristic and lauric acids are considered to be cholesterol raising, whereas stearic acid and medium-chain saturated fatty acids (6 to 10 carbon atoms) have been considered to be neutral with respect to effects on blood lipids and lipoproteins.

Monounsaturated (found in, e.g., olive, canola) and polyunsaturated (found in, e.g., sunflower, corn, soybean) fatty acids are cholesterol lowering fatty acids when they replace significant levels of saturated fatty acids in the diet. Clinical and epidemiological studies indicate that polyunsaturated fatty acids lower LDL and total cholesterol. Some studies have found that diets high in monounsaturated fatty acids compared with polyunsaturated fatty acids decrease LDL cholesterol while maintaining

HDL cholesterol levels (Mattson and Grundy, 1985; Mata et al., 1992). Other work has suggested that the effect of consuming polyunsaturated fat compared with monounsaturated fat is similar and results in a decrease in both LDL and HDL cholesterol (Gardner and Kraemer, 1995).

In the late 1940s, it became known that high serum cholesterol levels could cause heart attacks and that such high levels of serum cholesterol could be decreased by the presence of polyunsaturated fat or oil in the diet. At first fish oils were considered useless because they contained almost equal proportions of saturated and polyunsaturated fatty acids. In this earlier period it was believed that only certain vegetable oils were suitable. By mid 1950s, research had shown that fish oils was far superior to any vegetable oils in their ability to lower serum cholesterol levels (Peifer, 1967). Until the 1970s, it was generally believed that fish oils in the diet was effective in alleviating problems in connection with heat disease but the beneficial effect of the long - chain polyunsaturated on heart diseases was never mentioned. The first series of investigations that eventually led to work on omega-3 fatty acids in fish oil began in 1970. The researchers at the Aalborg Hospital, Aalborg, Denmark, became interested on Eskimos food. They examined the Eskimo food and compared it with typical Danes. They observed that the omega-3 fatty acids in marine oil might act similar to aspirin and related drugs (Bang and Dyerberg ,1972; Dyerberg et al., 1975).

Another situation analogous to Eskimo diet was noted in Japan. Japanese fisherman families have less incidences of coronary diseases. This situation has been investigated by various laboratories in Japan. Chiba University School of Medicine, Chiba, Japan conducted a study and found a relationship between eicosapentaenoic acid

(EPA) and coronary disease. They compared the effects of consumption of highly purified EPA and docosahexaenoic acid (DHA) and the effect of EPA in patients with thrombotic diseases (Hirai et al., 1987). Other researches carried out at the University of Oregon in Portland, Oregon in the United States, examined the effect of omega-3 fatty acids on coronary disease (Connor and Connor, 1972). In England a relatively large number of omega-3 investigations were conducted, especially the effect of omega-3 capsules (MAXEPA) (Durie, 1984).

In addition, other countries have carried out a number of investigations on omega-3 and heart diseases. Researches are being conducted world wide on various aspects of the effects of omega-3 fatty acids and fish oils on coronary disease. Examples of these are: Nestel et al. (1987), in Australia; Hornstra (1984) in the Netherlands; Budowski, (1988) in Israel; Gibney and Connolly (1988) in the Irish Republic; Tilvis et al. (1987) in Finland; and Sametz and Juan (1985) in Austria; Weber et al. (1986) in Germany; Ackman (1982) in Canada.

1.7.6 Fish Oil and Cancer

Cancer is the second leading cause of death in the United States, exceeded only by heart disease. The American Cancer Society (1998) predicted that about 564,800 Americans would die of cancer in 1998. The three most common sites of fatal cancer in men are lung, prostate, and colo-rectal whereas lung, breast, and colo-rectal for woman. In men and women, cancers at these top three sites account for about half of all cancer fatalities.

Cancer is diseases are characterized by uncontrolled growth and spread of abnormal cells. If the spread is uncontrolled, it can result in death. Cancer is caused by

both external factors (e.g., chemicals, radiation, and viruses) and internal factors (e.g., immune conditions and inherited mutations). Causal factors may act together or sequentially to initiate or promote carcinogenesis. Frequently the time period between exposures or mutations and appearance of cancer is 10 years or longer. Risk factors contributing to cancer development include cigarette smoking, certain dietary patterns, exposure to sunlight, exposure to radioactive materials or specific chemicals, and family history. All cancers caused by cigarette smoking and heavy use of alcohol are likely preventable. Many cancers related to dietary factors or to sunlight exposure are also felt to be preventable. Unlike heart disease in which blood cholesterol levels serve as an indictor of risk, there are no similar types of markers to indicate that a cancer may be developing. Early detection of cancer, for instance through regular screening examinations, greatly increases the chances of successful treatment.

Laboratory animal studies on diet and cancer have dealt largely with the response of chemically induced or transplanted tumors to increased calories from excessive fat in the diet. Some of these studies have suggested that dietary calories and the type of fat consumed may be related to cancer incidence, particularly with breast and colon cancer. Other animal studies have indicated that moderate caloric restriction may result in lower cancer incidence and, for a given level of unsaturated fat in the diet, animals may develop a higher incidence of cancer. Some studies suggest that a high level of dietary fat may act as a promoter of carcinogenesis rather than as an initiator of tumors. A promoter is a compound that by itself is not carcinogenic but enhances the ability of a carcinogen to induce cancer. The existence of a direct relationship between caloric content, fat unsaturation, and carcinogenesis is, however, still unclear.

In addition to interest in effects of the total amount of fat in the diet, there is also interest in whether individual types of fatty acids can affect cancer risk. Specific saturated, monounsaturated, or polyunsaturated fatty acids are found not to affect cancer risk (ILSI North America, 1997). Although animal studies have suggested that polyunsaturated fatty acids may increase tumor growth, no relationship has been found between polyunsaturated fatty acids and cancer in humans (Zock and Katan, 1998). Similarly, studies in animals have found that omega-3 fatty acids (e.g., from fish oils) suppress cancer formation, but there is no currently direct evidence for protective effects in humans. Oleic acid and saturated fatty acids have not been found to have any specific effects on carcinogenesis. Available scientific evidence does not support a relationship between *trans* fatty acids and risk of cancer at any of the major cancer sites. Recent studies have shown that conjugated linoleic acid, found primarily in lipids from ruminant animals, appears to be unique among fatty acids because low levels in the diet produce significant cancer protection. This effect seems to be independent of other dietary fatty acids.

Diets rich in antioxidant vitamins (in particular, vitamins A and C) may help reduce the risk of some cancers. Vitamins A and C are abundant in many fruits and vegetables. Vitamin E is an antioxidant found in vegetable oil products. There is less epidemiological evidence regarding vitamin E; however, laboratory and animal data support its anticarcinogenic activity.

1.7.7 Effects of Fish Oil on Other Diseases

A number of researches have been carried out on the effects of omega-3 fatty acids on heart diseases. However, there are evidences that omega-3 fatty acids can be

used to control or cure some diseases other than the heart. For examples, dietary fish oil or omega-3 fatty acids can greatly diminish the arthritis (Kremer and Jubiz, 1987); reduce the severity of glomerular nephritis (Thais and Stahl, 1987; Robinson et al., 1987); favorably affect lupus erythematosis (Kelley et al., 1985; Accinni and Dixon, 1979); a multiple sclerosis (Bates et al, 1989); strokes (Hirai et al., 1987; Land, 1982; Budowski, 1988); breast cancer (Karmeli, 1987; Carter et al., 1987; Cohen, 1987; Stampfer et al., 1987; Cave and Jurkowski, 1987) and effect on colon cancer (Reddy, 1987); certain skin disease (Rhodes, 1984; Kromann and Green 1980); and blood pressure within the brain and retina (Neuringer et al., 1988).

Besides the health benefits, the fish oils can be used in food production. Hardened fish oil is used almost entirely in margarine and shortenings production. Because fish oils have a widely varied chain length, margarines prepared from them have an excellent plastic consistency (FAO, 1986).

1.8 Production of Fish Oil

1.8.1 Raw Materials

The Food and Agricultural Organization of the United Nations (FAO, 1986) categorizes the raw materials which are used for reduction to meal and oil as follows:

1. Fish caught specifically for reduction to meal and oil such as menhaden, anchovy and sardines;
2. Incidental or by-catch from another fishery, for example shrimp by catch which could range from 5 to 15 million metric tons annually;
3. Fish offal or waste from the edible fisheries such as cutting from filleting operations fish cannery waste, roe fishing waste, and more recently surimi processing.

In general, fish are processed by the wet reduction method, in which the principal operations are cooking, pressing, separation of the oil and water emulsion with recovery of oil, and drying of the residual protein material. The wet rendering process is used in the majority of the factories that produce fish oil worldwide. This process is universal. Factories all over the world both on land and ships employing it with slight differences in equipment types, but the major steps of cooking, pressing and separating, are always present (Bimbo, 1990).

1.8.2 Commercial Processing of Fish Oil

Commercial processing of fish oil involves many steps (Bimbo, 1990). After the fish are caught and transported to the factory, they are cooked with steam to denature the protein and to release bound water and fish oil (Bimbo, 1989). After the fish and liquid (fish oil and water) are separated, the fish are pressed to remove most of the liquid. The pressed fish is called "press cake"; the liquid is called "press liquor." The press liquor contains particles of fish; therefore, the liquid is centrifuged and the removed particles are returned to the press cake (Bimbo, 1989 and 1990). The press liquor is heated and centrifuged to separate the fish oil from the stickwater (water and small suspended fish particles). At this stage, the oil is crude commercial fish oil. However, before the fish oil can be consumed, it must be undergone further refining and processing to produce a more pure and stable product. The steps used in the oil processing include degumming, neutralization, washing, drying, bleaching, filtration, deodorizations, and stabilization. Degumming and neutralization are often done together and involve addition of sodium hydroxide solution to the heated oil. Free fatty acids and other components such as the

phospholipids, trace metals, pigments, and oil-insoluble materials are removed (Bimbo, 1987; Young, 1985a; 1985b).

1.8.3 Rendering

Rendering is referred to as the extraction of fat or oil mainly from animal tissues heat. Almost all the animal fats are recovered by rendering, whereas vegetable fats are obtained by crushing, solvent extraction or by both. In general, rendering can be wet or dry rendering. Wet rendering is carried out in the presence of large amounts of water. The fat cell walls are hydrolyzed by steam under pressure until they are partially liquefied and the released fat floats onto the surface of the water. Separated fat is formerly removed by skimming, but centrifugal methods are widely used.

In dry rendering, the tissues are dehydrated until brittle, and the fat cells break and release the fat. Both methods need a pressing either in hydraulic or continuous screw presses to complete the recovery. Generally, wet rendering is used for edible products where color, flavor, and keeping qualities are of prime importance. Dry rendering is preferred for inedible products where flavor and odor are secondary and the production of large amounts of high quality residue is important (Dormitzer, 1956; Downing, 1959).

In general, the wet rendering method is used to produce fish oil. The apparatus used in wet rendering is a vertical cylindrical steel autoclave or digester with a cone bottom designed for a steam pressure of 40-60 psi and a corresponding high temperature. The vessel is filled with fatty material and a small amount of water. The usual digestion time is 4-6 hours. Under the high temperature employed, the fatty materials in the digester disintegrate to some extent. There is a very efficient separation of the fat, which

rises fat to top of the vessel, leaving a layer of solids and stick water in the bottom. The fat is drawn off and purified from water and solid material by settling or occasionally by centrifuge (Norris, 1982).

The optimum extraction was believed to take place at 100°C and atmospheric pressure; however, recent experiments have shown that the fat cells are broken down before the temperature reaches 50°C. Therefore, theoretically it should be possible to separate liberated oil from solid materials at 50°C. Furthermore, the coagulation of the fish protein is completed at about 75°C and that the process is vary rapid. This investigation leads to the conclusion that there is very little, if anything, to be gained by heating the material beyond 75°C or by using a long heating time (FAO, 1986).

One of the advantages of wet rendering is that an efficient recovery of fat is obtained with relatively simple equipment and that it is adaptable to a wide range of raw materials. In addition, there is little tendency for proteins and other substances to dissolve or disperse in the fat in the presence of water. On the other hand, wet rendering is less rapid and less efficient than dry tendering. Furthermore, some hydrolysis of fat occurs during steam rendering and the free fatty acids produced depends on the rendering time and temperature; storage temperature; and duration of storage of the fatty stock before it is processed (Norris, 1982).

There are several modifications of wet rendering operations which produce a better oil product. Addition of antioxidants before wet rendering greatly enhances the stability of the fat produced (Dugan, 1954). Another modification is known as the Kingan process. In this process, the raw material is finely ground, pumped through an

appropriate heat exchanger, and reground in a hammer mill. The fat is then separated from the protein and waste by a special type of centrifuge.

1.8.4 Pressing

The purpose of pressing is to squeeze out as much liquid as possible from the solid phase. This is important to improve the oil yield. Both continuous single-and twin-screw presses are used in the fishmeal industry today. The screw in the single-screw press is designed with a taper and exerts an increasing pressure on the fish pulp by reducing the volume as it progresses through the press. Some problems occur when the fish are soft and slip through the press while being squeezed.

The twin-screw press consists of a press chamber with two hollow interlocked cylinders. The cylinder contains heavily supported strainer plates, and the two screws have tapered shafts that are pitched opposite to the shaft taper. The screws operate in opposite directions and appear more capable of handling soft fish (Bimbo, 1990).

1.8.5 Centrifugation

In the early days of fish oil production, the oil was separated from the press liquor (sludge, water) by a gravity method. If pressed liquor is left for some time in a tank, it will settle out in three layers: sludge at the bottom, water in between, and oil at the top. This gravity separation method is extremely slow. Many modern industries including the fish meal and oil industry are using a centrifugation method as a standard operation to separate solids from liquid. There are several advantages of using a centrifuge instead of a press to separate solids from liquid in cooked fish. These include simplicity and quickness of the process.

Table 1.1 Crude fish oil quality assessment test and significance

Test	Significance
Moisture (%)	For contractual and yield purposes
Insoluble Impurities (%)	For contractual and yield purposes
Free Fatty Acids (%)	Contractual, yield & general quality purposes
Peroxide Value (Milliequivs active oxygen/Kg)	A measure of primary oxidation
Anisidine Value	A measure of secondary oxidation
Iodine Value	For fish oil type identification and as guide line for hydrogenation
Color	Quality Indicator
Iron (μg/g)	Autoxidation catalyst
Copper (μg/g)	Autoxidation catalyst
Phosphorus (μg/g)	Refining treatment/catalyst poison
Sulfur (μg/g)	Refining treatment/catalyst poison
Soap (μg/g)	Presence of soap indicates adulteration with alkali-refined oil
Laboratory Refining Laboratory Hydrogenation Laboratory Bleachability	Quality Assurance tests

Source: Young, 1985b

Perhaps the most important advantage of centrifugation is the ability to process soft and very fluid material where the press cannot be used. The hygiene and simpler procedures for washing and cleaning operations are another positive side of the centrifugation.

However, centrifugation tends to produce more fine emulsions, causing problems in the subsequent separation of oil, water and sludge in the liquid phase.

An important prerequisite for efficient separation is high temperature, implying that the press liquor should be reheated to 90°- 95°C before entering the centrifuge. This applies to sludge removal as well as to separation of oil and water (Bimbo, 1990).

1.8.6 Crude Oil

The oils obtained directly from rendering contain varying but relatively small amounts of naturally occurring non-glyceride materials that are removed through series of processing steps. For example, crude oils contain some free fatty acids, water, and protein that must be removed. Not all of the non-glyceride materials are, however, undesirable elements. Tocopherols, for example, perform the important function of protecting the oils from oxidation and provide vitamin E. Processing is carried out in such a way as to control retention of these substances. It is therefore necessary to determine certain key analytical values (Table 1.1) in order to modify processing conditions so as to obtain a product of satisfactory quality (Young, 1985a).

1.9 Processing of Fish Oil

The general objective of processing fats and oils is the removal of impurities that cause the original product to have an unattractive color or taste or that cause harmful metabolic effect. Crude fats and oils intended for edible purposes are, therefore, further processed to remove these substances while retaining desirable features. Furthermore, recent emphasis is to preserve some of natural components such as omega-3 fatty acid and tocopherols. Therefore, the process should be designed to preserve such components while reducing off-flavor, cholesterol and other impurities (Takao, 1986).

1.9.1 Degumming

Degumming is a treatment designed to remove the impurities such as phospholipids, free fatty acids and trace metals with the least possible damage to the natural oil. The impurities are complex molecules and are generally classified as "gum." The presence of gum in the oil determines the final oil color, flavor, foaming and smoking stability, and they must be removed. Occasionally, the gum settles out in a storage tank and will cause high refining losses. The gum can be removed from oil with water or acids such as phosphoric acid or citric acid (Carr, 1976; Cowan, 1976; Norris, 1982).

The gum in oil is broadly divided into hydratable and non-hydratable types. The hydratable type is treated with water or steam and converted to a hydrated gum which is insoluble in oil and can be separated by centrifuge (Carr, 1976). For water hydration, the crude oil is heated to 70°C and 1-3 % of water by weight is mixed into the oil. The mixture is agitated slowly for about 30 min and then centrifuged to remove the gum. The non-hydrated gum is usually removed by treating the oil under vacuum with acids such as phosphoric and citric acids. In acid degumming, the oil is heated to 70 to 85°C and about 0.1 % (weight basis) phosphoric acid 0.3 % (weight basis) of citric acid is added and intensively mixed for about 30 sec. After an additional 15-min agitation by a stirrer, 1.0 % (weight basis) demineralized water is added to the acid-in-water emulsion and agitated for another 15 min and oil is removed by centrifuge (Nilsson et al., 1989).

Degumming is not ordinarily carried out in fish oils processing, because they are very low in phosphatides. In some refineries, however, an acid pretreatment, designed to hydrate gums and remove phosphorous and other trace metals, is applied to oil as it

enters the alkali refining plant. The pretreatment of fish oils with phosphoric acid prior to caustic refining is a standard practice in Europe (Brekke, 1980). Degumming has shown to effectively reduce lead, copper, arsenic, and zinc in menhanden oil (Elson and Ackman,1978 and Elson et al.,1981).

Dijkstra and Opstal (1987 and 1989) developed a novel degumming process that can be used with undegummed as well as water-degummed oils. The process lowers the iron and phosphorous content so that the oil can be physically refined and bleached to produce a stable oil similar to alkali refined oils. In this process, phosphoric or citric acid is dispersed into the oil, and after sufficient contact time, caustic soda is added and the mixture is pumped to a centrifuge where the gums are removed with little oil loss. The oil stream is then pumped to a second centrifuge, where the remaining gums are removed, and after normal drying, the oil is either alkaline or physically refined.

1.9.2 Neutralization

The term "refining" refers to as any purifying treatments designed to remove nonglyceride impurities such as free fatty acids in the oil. Crude fats and oils produced by rendering, or solvent extraction contain variable amounts of nonglyceride impurities. But, not all impurities in crude oils are undesirable. For examples, tocopherols are considered as nonglyceride impurities in fish oil; however, they perform the important function of protecting the oil from oxidation. For this reason, they may be classed as highly desirable constituents of most oil and fat products. Most of the other impurities are objectionable and they cause the oil to foam or smoke, or are precipitated when the oil is heated in subsequent processing operations. The objective of refining is to remove the undesirable impurities from the oil with the least possible damage to either

glycerides or tocopherols or other desirable impurities, and with the least possible loss of oil. In general, the refining process can be done in two ways: alkali refining (Neutralization) and physical refining.

In the case of alkali refining, an alkali solution is added to crude oil. Causing chemical and physical changes. The alkali combines with free fatty acids present in the oil to form soaps (Norris, 1982). The alkali most commonly employed for refining oils is caustic soda, which is much more effective in its decolorizing action than weaker alkalis. Caustic soda, however, saponifies some of the neutral triglycerides, causing a higher refining loss. The amount of neutral oil lost in alkali refining depends primarily on the amount and the of impurities that are present (Norris, 1982).

Another method to remove free fatty acids and volatile components is physical refining. Since free fatty acids are more volatile than glycerides, it is also possible to remove them from the oil by high-temperature steam distillation (Sullivan, 1976). This process is called "physical refining." However, the fish oils are not normally physically refined because they are too unstable. The highly unsaturated triglycerides would tend to polymerize during the distillation and produce a rapid flavor reversion after refining (Bimbo, 1990).

Fernandez (1986) explained a cation strong-acid microporous resins process for the refining of fish oil for human consumption. Conventional refining processes require high temperature treatment that damages omega-3 fatty acids in fish oil. On the other hand, resins, do not require high temperature, are of consistent quality, and can be regenerated. Fernandez (1986) demonstrated said that cation strong-acid macro porous

resins in columns produced superior fish oil to anything currently available to the food industry.

1.9.3 Bleaching

Bleaching is designed to improve color, flavor and oxidation stability of the oil by removal of compounds responsible for color and off-flavor. Many compounds in crude oil responsible for the color are broken down at high temperatures and the volatile products are removed under deodorization conditions. Bleaching involves the adsorption of color in the oil by activated clay (Bimbo, 1990). The bleaching step is also important to remove soap, trace metals and sulfur compounds. During bleaching, peroxide are broken down to aldehydes and ketones and these secondary oxidation products are adsorbed onto the activated earth surface such that the filtered oil after bleaching should have a low peroxide value (PV) compared with the oil before bleaching.

Adsorption is commonly used for bleaching and iis done by transfering fluid phase to the surface of a solid adsorbent. Usually the small particles of adsorbent are held in a fixed bed, and the fluid is passed continuously through the bed until the solid is nearly saturated and the more desired separation can no longer be achieved. The flow is then switched to a second bed until the saturated adsorbent can be replaced or regenerated (McCabe et al., 1993).

The two types of commercial bleaching clays used in processing edible oils may be characterized as "Natural Bleaching Earth" and " Activated Bleaching Earth" (Richardson, 1978). Natural bleaching earth, otherwise known as Fuller's earth is basically a hydrated aluminum silicate. In recent years, there has been considerable

interest in acid activated clays for bleaching of oil and fats. The raw materials used for the manufacture of this type of bleaching clay consist mostly part of bentonites or montmorillonite, which have little or no decolorzing power in the raw state. In general, the clays are treated with sulfuric or hydrochloric acid. The acid treatment extends the surface of the clay and causes important changes in its chemical or physicochemical nature. Acid-activated clays retain more oil per unit weight of clay than do natural earths. The apparent densities of natural earth, activated earth, and activated carbon are approximately 50, 30, and 25 lb/ft3 , respectively.

In general, however, the range of clay used in the United States is 0.15-3% (Norris, 1982). Certain types of color are extremely difficult to remove. The greatest usefulness of activated clays is in the treatment of off-grade oils. For example, the green color due to chlorophyll in some soybean oils is much more responsive to a slightly acid earth than one of ordinary type because the pigment is unstable under acid conditions (Norris, 1982).

Carbon is also a superior adsorbent for traces of soap in refined oils. It is particularly effective in removing the red, blue, and green pigments of coconut and palm kernel oils and the better grades of animal fats. Unlike bleaching earths, carbon imparts no foreign flavor or odor to the oil treated. Because of its very porous nature, carbon retains much greater amounts of oil than do any of the clays. The choice of an adsorbent generally depends on cost, activity, and oil retention. The amount of adsorbent required for any given bleaching operation will vary greatly with the activity and the nature of the adsorbent, the types of oil, the color of the unbleached oil, and the color desired in the bleached oil.

1.9.4 Fractionation and Winterization

Fractionation or winterization operation in the processing of edible oils is basically the separation of oils into two or more fractions with different melting points (Kreulen, 1976). Cooling was previously employed to isolate solid glycerides from natural fats. Either liquid oils or melted fats were directly cooled and allowed to form crystals, and crystalline mass was separated from the remaining liquid. This process is known as dry fractionation (Thomas, 1985). Dry fractionation has been employed to separate solid and liquid triglycerides from numerous fat sources. This process is most effective when the crystals to be isolated are large and easily separated by filtration or centrifugation.

Winterization is a specialized form of dry fractionation in which small quantities of solid fats are separated from a large amount of liquid oil. Dry fraction is applicable to oils having appreciable quantities of both solid and liquid constituents. The objective of both processes is to remove trisaturated and disaturated glycerides.

The winterization process is known to be an old practice that removes small quantities of solid fat fractions that normally cloud when oil is held at refrigerated temperature. In this process, the oils are cooled and kept at low temperature for sometime. During the winter period, cottonseed oil was kept in large storage tanks and the deposition of stearine was noticed. The liquid oil was separated and it became known as " winter" oil and was bottled for use as salad oil (Weiss, 1967). These oils remain clear when refrigerated. For this reason, winterization became economically the most important of all fractional crystallization procedures (Weiss, 1983).

Today triglyceride oils are winterized for the following reasons: to remove waxes and other nontriglyceride constituents, to remove naturally occurring high-melting triglycerides, and to remove high-melting triglycerides formed during partial hydrogenation and interesetrification. The major problem with winterized oil production is the formation of crystals suitable for separation from the liquid oil. The process is conducted in refrigerated rooms or well-insulated refrigerated tanks in rooms at ambient temperature. The tanks are equipped with cooling coils containing refrigerant and good temperature controls. In order to prevent shock chilling of the oil near the coil, the temperature difference between the refrigerant and oil should be from 10^0C-25^0C. Another major problem is filtration, the winterized oil is usually filtered out in ordinary plate and frame filter presses. It is a slow process, and the entire winterization process from start to finish takes up to six days.

Solvent winterization of triglycerides is a relatively recent development. In this process, the oil viscosity is reduced by means of a solvent such as hexane. Fish oils are cooled in the presence of a solvent yielding high -melting crystals that are separated by filtration. In this process, acetone or some other ketone or hydrocarbon solvent is used (Kokubu et al., 1984).

1.9.5 Hydrogenation

A long time ago, the direct addition of hydrogens at double bonds of the unsaturated fatty acids chains was discovered for vegetable oil. Since then it has been developed into the largest single chemical process in fat and oil industries. This is known as "hydrogenation" and this process reduces the number of double bonds and changes the physical characteristics of the fatty acid chain. Due to reduction in the

number of double bonds, oxidation is decreased and flavor stability is increased. The reaction is not a simple saturation of double bond with hydrogen, but is an extremely complex series of reactions that result in a myriad of products. By controlling of the reaction conditions of pressure, temperature, agitation, catalyst type, and concentration, the desired product, such as margarine, shortening , coatings fats, frying fats, may be obtained (Allen, 1978; Sathivel, 1994).

Vegetable oils and their hydrogenation products have received much attention. A considerable number of studies have been conducted on the subject of fish oil hydrogenation (Ackman et al., 1971; Sebedio et al., 1981; Sebedio and Ackman, 1983a; Sebedio and Ackman, 1983b; Bittner, et al., 1982). Hydrogenation has had the greatest effect on the interchangeability of oils because the process results in the production of a very large number of alternatives to naturally occuring hard fats. Over 1 million tons of fish oil per year, which in its extracted state contains fatty acids with up to six double bonds in chains, have been made available to the edible-oil industry. By virtue of their wide triglyceride composition resulting from fatty acid chain lengths from C14 to C24 in significant quantities, hardened fish oils possess useful crystal-stabilizing properties and assist in the creaming performance of bakery products (Young, 1985a). The degree of hydrogenation of the oil is directly related to its iodine value. Most hydrogenation is done in batch reactors. The feed stock must be refined, bleached, free of soap, and dry because free fatty acids, soap, and water can all act as poisons that reduce catalyst activity and selectivity. The hydrogen must be dry and as pure as possible (Young, 1985b).

1.9.6 Deodorization

Deodorization is the last major processing step in the refining of edible oils. Due to current harvesting, processing practices, high concentration of polyunsaturated fatty acids and other contaminants, crude fish oil are easily subjected to deterioration. This severe deterioration changes the flavor quality of fish oil. Off-odors and flavors in fish oil arise from metabolite contaminants, from fish oil protein spoilage, or from oxidation of the fish oil itself (Stansby, 1971 and 1973). For example, Hsieh et al. (1989) studied the volatile components of crude winterized menhaden oil by dynamic head space gas analysis and they found that many odor components are derived from lipid oxidation, including short chain saturated and unsaturated aldehydes, ketones, and carboxylic acids.

Undesirable ingredients produced by previous refining, bleaching, hydrogenation or even storage conditions may effect the flavor quality of fish oil. Therefore, undesirable odors and volatile components should be removed during refining and deodorization to obtain food grade oil with good cooking quality. Deodorization has been considered as a unit process that finally establishes the oil flavor and odor characteristics that are most readily recognized by the consumer (Zehnder, 1976; Gavin, 1977 and 1978). Steam deodorization is possible because of the great differences in volatility between the triglycerides and the substances that give oils and fat their natural flavors and odors. It is essentially a process of steam distillation where the volatile compounds are stripped from the nonvolatile oil (Mattil, 1964).

CHAPTER 2. FATTY ACID COMPOSITION OF CATFISH VISCERA, VISCERAL PARTS, FILLET, AND NUGGETS

2.1 Introduction

The catfish industry is the largest aquaculture industry in North America. Total water surface acreage for catfish production has increased from 50,000 acres in 1980 to more than 185,700 acres in 2001 (Catfish Production, 2001). Over 590 million pounds of processed catfish were produced in 2000 by the industry (Catfish Processing, 2001) in the U.S. with little "added value." Most catfish processed in the U.S. is sold as fresh or frozen fillets and whole-dressed fish. Yield from dressed-out catfish from traditional processing is only 45%, while offal (including catfish frames, viscera, skin, and trimmings) derived from the filleting process amounts to 55% of the total live weight, which often end up in landfills or rendering plants.

To the best of our knowledge, very little or no interest has been paid to adding value to catfish viscera, a processing waste. A whole viscera, which includes liver, gallbladder, digestive track (intestine and stomach) and visceral storage fat, weighs about 10% by weight of the whole catfish. Multimillion pounds of catfish oil from processing waste could be recovered and converted into edible oil, but is being wasted instead. Omega-3 fatty acids play a major role in human health (Kromhout et al., 1985; Clandinin, et al., 1994; Neuringer et al., 1994). Fat content and fatty acid (FA) composition of foods are of concern to health – conscious consumers as well as food scientists. Natural fish oils have been claimed to help maintain heart and vascular health in humans (Haglund et al., 1998).

Some beneficial effects of omega-3 fatty acids on certain diseases, functions, and malfunctions were found including heart diseases (O'Keefe and Harris, 2000); lupus

erythematosis (Kelley et al., 1985; Accinni and Dixon, 1979); multiple sclerosis (Bates et al, 1989); strokes (Budowski, 1988); skin disease (Rhodes, 1984; Kromann and Green 1980); lower blood pressure involving the brain and retina (Neuringer et al., 1988); visual and congnitive development (Neuringer et al., 1994); cardio-vascular functions (Heemskerk et al., 1996; McCarthy 1996a; Simopoulos, 1997). Fish oil may be useful in treating or reducing the risk of psoriasis and other skin disorders (Ziboth, 1996; McCarthy, 1997), migarine (McCarty, 1996b), gastrointestinal carcinomas (Eastwood, 1995), and smoking or nicotine (McCarty, 1996c), and may influence the brain growth during early infancy (Xiang et al., 2000).

Catfish oil recovered from processed wastes may provide a good source of health-promoting fatty acids. This study was conducted (1) to characterize the fatty acid (FA) profile of oil recovered from catfish viscera and various parts of viscera, i.e., liver, digestive track, gallbladder, and visceral storage fat, and (2) to compare the profile with that of fillets and nuggets (abdominal portion).

2.2 Materials and Methods

2.2.1 Sample Preparation

An average weight of 450g of 24 fresh catfish was collected from a local seafood store in Baton Rouge, LA. All the catfish were headed, gutted and skinned and the following parts of catfish were separated: viscera (WV), gallbladder (GB), liver (L), digestive track (DT), visceral storage fat (VSF), fillet (F) and nugget (N), and were individually weighed and ground with a commercial blender for 10 minutes and stored at –20°C until analyzed.

2.2.2 Fat, Protein and Moisture Analysis

The fat content of samples was analyzed according to the procedure 985.15 (AOAC, 1990). An automated solvent extractor equipped with a microwave moisture analyzer (CEM Corp., Matthews, NC) was used for this analysis. Approximately 4 g of ground samples was used. Methyl chloride was used as a solvent for fat extraction. Percent protein (Kjeldahl Nx 6.25) was determined by the procedure as outlined by AOAC (1990).

2.2.3 Fat Extraction

The fat extraction was done for each of the seven individual samples (WV, GB, L, DT, VSF, F, and N). About 5g of each ground sample was placed in a screw cap test tube, then 5ml of distilled water, 20 ml of chloroform and 20 ml methanol (1:4:4 by volume) were added to the tube and the mixture was homogenized on a vortex in a screw-cup test tube for 10 minutes. The homogenized mixture was filtered through Whatman No.1 filter paper. The filtrate was placed into a separatory funnel. The bottom layer of the solution was collected. Anhydrous sodium sulfate (5.7g) was added to the collected solution to remove the water. Residual solvent was removed from the solution by the Meyer-N-Evaporator (an analytical evaporator) under nitrogen atmosphere. The evaporation was continued until the solution was free from chloroform. The extracted sample was kept at -20°C until used. Fat extraction was repeated 9 times (3 batches; 3 extractions for each batch) of the seven individual samples.

2.2.4 Esterification of Fatty Acid

The fatty acids methyl esters (FAMEs) were prepared following the procedure

969.33 (AOAC, 1990). Each extracted catfish oil was separately placed into a 50-ml flat bottom-boiling flask containing approximately 4 ml of methanolic sodium hydroxide (2g of NaOH dissolved in 100 ml methanol) and 10 boiling chips were added into the flask. The condenser and reflux units were attached to the flask and refluxing took place with immediate addition of 7 ml of boron trifluoride through the condenser for 10 minutes. Refluxing was allowed for another 2 minutes. The esterified fatty acids were extracted from the mixture by adding 5 ml of heptane and refluxing for 1 minute. The esterified solution was allowed to cool to room temperature. A saturated solution of sodium chloride was added and the flask gently rotated. Saturated sodium chloride solution was added until the heptane solution reached the neck of the flask. The heptane solution containing FAMEs was recovered, dehydrated with 1.5g anhydrous sodium sulfate, and stored under nitrogen in teflon-capped vials at –20°C until analyzed.

2.2.5 Fatty Acids Analysis

The FAMEs were quantified by the Hewlett Packard 5890 Series II Gas Chromatograph equipped with a 7673A autosampler and interfaced to a 5970 mass selective detector (Agilent Technologies, Palo Alto, CA). The GC was equipped with an EZ-Flash fast temperature programmable column (Thermedics Detection, Inc., Chelmsford, MA). The column phase was RTX-2330 (90%-biscyanopropyl/10% phenylcyanopropyl polysiloxane) with the dimension: 5 meter long, 0.25 mm inside diameter with a 0.2-μm phase thickness. One μL was injected using the inlet in a split mode. The head pressure was set at 2 psi and the split vent flow was 7 mL/m. The injector temperature was 260°C. Column flow rate at 2 psi was 0.68 mL/m. The column temperature was ramped from 50° to 260°C at a 20°C/second and was held at 260°C for

90 seconds. Run length was 5 minutes. The transfer line temperature was 280°C. The MSD was operated with the selected ion monitoring mode. Fatty acids were identified with retention times obtained from the fatty acids methyl esters standards (Sigma Company, St. Louis, MO). Three experimental replications (batches) were conducted, each with 3 extractions and 3 GC-injections per extraction. FA content was reported as mg/g dry-sample weight.

2.2.6 Statistical Analysis

All data were analyzed using SAS (version 8.1, 2001). Analysis of variance (ANOVA) was performed to determine differences in fatty acid profiles of samples. Tukey's Studentized Range test was performed for post-hoc multiple comparisons. Group differences, expressed in terms of differences in mean vectors of the fatty acids, were determined using multivariate analysis of variance (MANOVA). Principal component analysis was used to group the samples with similar fatty acids. Descriptive discriminant analysis (Huberty, 1994) was performed to identify fatty acids that largely underlie group differences among FA profiles of whole and various parts of viscera.

2.3 Results and Discussion

2.3.1 Proximate Composition

Whole viscera, which includes liver, digestive track (intestine and stomach), gallbladder and visceral storage fat, weighs about 265 g, which is approximately 14% by weight of a live catfish. The average weight of catfish liver, gallbladder, digestive track and visceral storage fat was 65g, 8g, 90g and 80g, respectively. Weight of catfish liver was relatively greater than that of marine fishes (e.g., notothenioid fishes from Admiralty Bay) reported by Kamler et al. (2001). The fillet and nugget made up an

average of 45% to 55 % of the whole catfish weight. Fat, protein, and moisture contents of the various parts of catfish are shown in Table 1.1. The carbohydrate and fiber contents of viscera were less than 5% except that of gallbladder. Fat content (% wet basis) of WV, L, DT, GB, VSF, F, and N was 33.6, 8.8, 5.8, 0.3, 90.7, 9, and 14.7, respectively. Catfish store fat in the visceral cavity as visceral storage fat. The fat content of catfish viscera from our study was greater than that reported by Belal and Assem (1995).

Table 2.1 The fat, protein, and moisture contents of whole viscera, visceral parts, fillet and nugget

Catfish Parts	Fat[a] %	Protein[a] %	Moisture %
Whole viscera	33.6	14.7	50.1
Digestive track	5.8	13.4	79.5
Liver	8.8	11.4	74.9
Gallbladder	0.3	2.6	88.9
Visceral storage fat	90.7	1.3	8
Fillet	9	14.4	74.4
Nugget	14.7	13.5	71.2

[a] Wet weight basis

Average protein percent of fillet and nugget (Table 2.1) agreed with values from other studies of Liebowitze (1981); Belal and Assem (1995); Brooks (1982). Average moisture content of nugget and fillet was similar to that reported by Belal and Assem (1995). However, the moisture content was higher than that reported by Liebowitze

(1981), and Brooks (1982). Researches have shown that an inverse relationship existed between the total lipid content and moisture content and it was a common relationship to all the species of fish (Love, 1970).

2.3.2 Fatty Acids Profile

Fatty acid composition of WV, DT, L, GB, VSF, F, and N is shown in Table 2.2 The major fatty acids present in processing waste were palmitic (C16:0), stearic (C18: 0), oleic (C18:1), and linoleic (C18:2). The catfish oil from processing waste was characterized by a high level of unsaturated fatty acids. Oleic acid was dominant among unsaturated fatty acids, whereas palmitic acid was dominant among saturated fatty acids. The unsaturated fatty acid of the WV was greater than that of portioned visceral parts, almost equal to that of fillet, but less than that of nugget. Among all fatty acids, oleic acid (C18:1 ω-9) was present in the largest quality in WV as well as DT, liver and storage fat. Gallbladder contained 9.2 mg/g of DHA (dry basis), whereas fillet had the greatest amount of DHA (10.7 mg/g of dry basis). The greatest amount of myristic acid (9.5 mg/g of dry tissue) and steric acid (32.9 mg/g of dry tissue) were found in whole viscera when compared to L, GB, DT, and VSF.

Unsaturated fatty acids accounted for 307.6, 261.3, 259.3, 102.1, 94.7, 79.4, and 28 (mg/g of dried tissue) for N, WV, F, VSF, DT, GB, and L, respectively. A significant difference was found in the level of unsaturated fatty acids, and ω-3 and ω-6 fatty acid for all processing waste parts. The total ω-3 fatty acids (combined C18:3 ω-3 and C22:6 ω-3) in WV, DT, L, GB, VSF were 11.7, 20.9,4.3, 9.4, and 6.1 (mg/g of dry tissue), respectively, whereas 15.3 and 18.6 (mg/g of dry tissue) for fillet and nugget, respectively.

Table 2.2 Fatty Acids profiles of various parts of catfish viscera, fillet, and nuggets (mg/g dry basis)[a]

Fatty acids	Whole viscera	Digestive track	Liver	Gallbladder	Storage fat	Fillet	Nugget
C14:0	9.5	1.4	0.3	0.31	5.2	6.8	10.4
C16:0	76.2	43.2	7.2	5.3	33.9	70.4	83.6
C16:1	10.9	3.7	1.1	1.3	5.1	14	10.8
C18:0	32.9	10.9	6.7	13.9	13.1	29.7	35.6
C18:1	145.7	62	12.2	3.1	52.7	149.5	175.7
C18:2	73.1	1.5	2.8	0.4	29.5	65.6	81.2
C18:3	7.47	17.3	0.3	0.24	4.3	6	8.3
C20:0	1.91	0.62	0.2	0.5	0.9	1.5	1.9
C20:1	11.9	1.9	1	0.9	4.6	7.9	10.9
C20:2	3.5	1.3	0.2	2.1	2.2	2.3	3.6
C20:4	4.5	3	6.4	2.8	1.9	4.7	6.4
C22:6	4.2	3.6	4	9.2	1.8	9.3	10.7
Saturated	121	56.2	14.4	25	53	108.4	131.5
Unsaturated	261.3	94.7	28	79.4	102.1	259.3	307.6

[a] mean values of 27 measurements (3 batches, each with 3 extractions and 3 injections per extraction). Standard deviation of each fatty acids is less than 5%.

Table 2.3 Comparison of Fatty Acid profiles of Whole Viscera of Catfish with some fish fillets from USDA data

Fatty Acids (g/100 of raw tissue)	Wild Catfish[a]	Farmed Catfish[a]	Farmed Salmon[a]	Bluefin Tuna[a]	Whole Viscera
C14:0	0.06	0.09	0.49	0.14	0.42
C16:0	0.44	1.23	1.3	0.81	3.35
C18:0	1.5	0.35	0.28	0.31	1.44
Monounsaturated	0.84	5.59	3.87	1.6	7.4
C16:1	0.18	0.28	0.67	0.16	0.48
C18:1	0.59	3.17	1.78	0.92	6.4
C20:1	0.02	0.07	1.19	0.28	0.55
Polyunsaturated	0.87	1.57	3.93	1.43	4.1
C18:2	0.1	0.88	0.59	0.05	3.21
C18:3	0.07	0.1	0.09	0	0.33
C20:4	0.15	0.09	1.15	0.04	0.2
C22:6	0.23	0.21	1.29	0.89	0.18
Omega-3	0.3	0.31	1.38	0.89	0.51

[a] Reference: USDA (2001)

Table 2.4. Canonical structure's indicating discriminating fatty acids

Fatty acids	CAN1	CAN2	CAN3
C14:0	0.410342	0.045013	-0.090079
C16:0	0.088155	0.046879	0.241991
C16:1	0.097705	0.050886	0.105486
C16:2	0.129619	0.179777	0.128813
C18:0	0.149494	0.139335	0.335687
C18:1	0.237364	0.162011	0.199828
C18:2	0.382133	-0.003049	-0.049986
C20:0	0.351113	0.205034	0.313439
C20:1	0.315523	0.304401	0.096263
C20:2	0.169628	-0.115654	0.151203
C20:4	-0.017687	0.763122	-0.268654
C22:6	0.007587	0.425394	0.213213
Saturated	0.123607	0.088773	0.242402
Unsaturated	0.219265	0.203546	0.340944
Cumulative variance explained (%)	93.0	97	99.8

The ω-3/ω-6 ratio was lower in all the catfish parts. This ratio is typically lower in cultured fish lipids, which ranges from 0.5 to 3.8 (Hearn et al., 1987). Fatty acids of fish are derived from two sources, namely, biosynthesis (Voss et al., 1991 and Aveldano et al., 1993) and diet (Chanmugam et al., 1986; Cai and Curtis, 1989; Morris et al., 1995; Kamler et al., 2001). Catfish whole viscera, fillet, and nugget were characterized as having greater quantities of 18:2 ω-6; however, there were small quantities in the digestive track, gallbladder, and liver.

The predominance of 18:2 ω-6 in catfish has been attributed to the fishmeal, especially from soy products. Fish can also accumulate ω-3 fatty acids in liver lipids when the diet contains either linolenic acid (18:3 ω-3) or docosahexanenoic acid (DHA, 22:6 ω-3) (Satoh et al., 1989). The mean level of 18:3 ω-3 was 0.3 mg/g of tissue in liver, although it is low but was similar to other fish such as Japanese surgeonfishes (Naso lituratus, Acanthurus lineatus) (Montgomery, 1999). Diet has a major effect on the fatty acid composition of liver lipids, especially ω-3 fatty acids (Satoh et al., 1989).

In Table 2.3, we compared selected fatty acids in WV with the values from USDA fatty acids data of selected fish. Polyunsaturated fatty acids of whole catfish viscera (4.10 g) were similar to that of the muscle of farmed raised salmon (3.93 g) but greater than Bluefin tuna (1.43 g) and farmed catfish (1.57g). Total omega-3 fatty acids (C18:3 and C22:6) in whole viscera accounted for 12.4 % of the polyunsaturated fatty acids, whereas 35% and 62 % in muscle tuna and salmon, respectively.

Among FAs (C14:0, C16:0, C16: 1, C18:0, C 18: 1, C18:2, C18:3, C20:0, C20:1, C20:2, C20:4, and, C22:6) found in catfish viscera, C18:1, C16:0, and C18:2 were predominant and found greatest quantities in WV. Principal component analysis

indicated clear distinction among FA profiles of whole from viscera and various parts of viscera. Discriminant analysis (Table 2.4) identified C14:0/C18:3/C20:0 (the first dimension with 93% variance explained) and C20:4/C22:6 (the second dimension with 97% cumulative variance explained) as discriminating fatty acids in catfish oils.

2.4 Conclusion

This study showed that about 34% crude fat (wet basis) could be recovered from the whole catfish viscera, which is currently considered as processing waste and has no market value. The recovered catfish visceral oil was characterized by a high level of polyunsaturated fatty acids which are similarly found in catfish fillet. The total ω-3 fatty acids of oil recovered from the whole and portioned viscera ranged from 4.3-20.9 mg/ g (dry weight basis). The whole catfish viscera may serve as a good source of edible oil that contains health-promoting fatty acids. Value addition to catfish processing wastes would economically impact the entire catfish industry.

CHAPTER 3. CATFISH OIL PRODUCTION FROM VISCERA

3.1 Introduction

There is a sizeable and growing world market demand for high-quality fish oil, and production can be quite profitable if suitable raw material is available. The fish industry would be wise to handle the by-products from gutting, filleting, and other fish-processing operation with care because they are good raw materials for fish meal and oil production. The by-products of catfish processing consists of heads, frames, skin, and viscera. Catfish may be classified as a fatty fish since the fat content of visceral is 30% to 35%. Catfish waste would provide a larger quantity of raw material for production of the fish oil. Viscera can be used for recovery of fish oil. The average weight of viscera is about 265g, which is about 14% by of weight of a live catfish.

Fish oils comprise a complex of fatty acids moieties mostly straight chain with an even number of carbon atoms. The fatty acids, usually present as their glycercides, are either saturated or mono or polyunsaturated. Unlike vegetable oils and fats from terrestrial animals which contain mainly fatty acids having a maximum of eighteen carbons and two or three double bonds, fish and marine mammal oils contain substantial amounts of fatty acids having twenty or twenty-two carbons and four, five or six double bonds. Omega-3 fatty acids include C18:3 and C22:6, which are unique to fish oil.

During the last two decades, interest in dietary effects of marine omega-3 fatty acids, (C20:5 n-3 and C22:6) has increased because it has become widely accepted that these fatty acids may prevent coronary heart diseases (Simpoulos et al., 2000; Haglund et al., 1998; Herrmann et al., 1985). Supplement intake of fish oil decrease arthritis (Kremer et al., 1995). Furthermore, marine omega-3 fatty acids appear to be necessary

for the development of the brain and visual function of the infant (Xiang et al., 2000). Long-chain polyunsaturated fatty acids, such as arachidonic and docosahexanenoic (DHA), are important for early growth and development. Omega –3 fatty acids may also be helpful in the treatment of dementia and efficacious treatment for psychiatric disorders in pregnancy and in breastfeeding (Freeman, 2000).

Oil production begins with crude oil recovery and extraction. In general, crude oil contains insoluble and soluble materials. Separation of insoluble materials can be achieved through gravity methods. Fish oil processing steps include degumming, neutralization, bleaching, and deodorization. Both insoluble and soluble material is removed through a degumming step (List et al., 1993; Young, 1978; Dijkstra and Opstal. 1989). Neutralization of crude oil with caustic soda is to remove free fatty acids. Bleaching removes soap, trace metals, sulphurous compounds, and part of the more stable pigments and pigment-breakdown products, aldehydes, and ketones (Young, 1978; Richardson, 1978; Goebel, 1976). The purpose of deodorization is to remove residual free fatty acids, aldehydes and ketones, which are responsible for unacceptable oil odor and flavor (Young, 1978; Gavin, 1977, and 1978; Zehnder, 1975).

Many marine fishes have been studied for fish oil production but little attention has been paid to produce fish catfish oil from fish processing waste. A major question is whether it is possible to produce edible catfish fish oil from fish processing waste, especially from viscera. Therefore, the objectives of this study were to produce oil from catfish viscera and to determine the effect of processing steps on the composition of fatty acids and the quality of the catfish oil.

3.2 Materials and Methods

3.2.1 Samples and Sample Preparation

Catfish viscera was obtained from the local fish processing plant in Baton Rouge, LA. The viscera was frozen at - 20°C until used. The slightly thawed viscera was finely ground in Hobart Chopper Bowl (84181D) at 3,450 rpm at for 10 minutes. Water was added (water: ground viscera, 5:1 V/W) and the mixture was heated at 70°C for 15 minutes. The purpose of the heating step was to coagulate the protein of the catfish viscera, so that liquids and solids can be mechanically separated. During mild heating, fat cells are ruptured, releasing the oil into the liquid phase. The denatured proteins were separated from the liquid phase (oil and soluble particles) by filtration through cheesecloth and the cooked viscera were pressed manually to remove most of liquid liquid. The crude oil was separated from the water phase and other fish particles by centrifugation at 5,000 rpm for 30 minutes. Separated crude oil was stored at – 20°C until used. Three experimental batches were conducted. Crude menhaden oil was supplied by the Omegaprotein Inc, Reedville, VA. Both catfish and menhaden crude oils were purified as explained below.

3.2.2 Degumming

Crude oil was removed from the storage at –20°C and 100g of the crude oil was taken for the degumming process and placed in a 600-ml beaker and heated to 70°C in a water bath. Three ml of citric acid (3%) was added to the oil, thoroughly mixed,and the sample was heated at 70°C for one minute, then centrifuged at 5,000 rpm for 10 minutes to remove impurities.

3.2.3 Neutralization

Sodium hydroxide solution (14 Baume; 13g NaOH/100mL; 0.25 normal NaOH) was added to 100 g of degummed oil as explained in the AOCS method 9b-52Ca (1989) and heated to 65°C for 30 minutes. The sample was cooled to room temperature and kept for six hours. The precipitated soap was removed by centrifugation at 5000 rpm for 10 minutes. Fifty ml of demineralized water was added to the oil and to wash out remaining soapl. This was repeated for three times. Remaining water was removed by centrifugation at 5,000 rpm for 10 minutes.

3.2.4 Bleaching

Neutralized oil was heated to 70°C in a water bath and was bleached with 4% w/w activated earth (the American Oil Chemistry Society, Champaign, IL) at 70°C for 10 minutes. The activated earth with impurities was removed from the oil by centrifugation at 5,000 rpm for 30 minutes.

3.2.5 Deodorization

The laboratory distillation unit was used for deodorization. The unit consisted of a round bottom boiling flask with three outlets. One outlet was attached to a vacuum pump, another outlet was attached to a glass distillation column and the last outlet was sealed with a thermometer inserted. The flask was placed on a heating system. Bleached oil was added into the flask and heated to 100°C for 30 minutes under vacuum pressure at 65 cmHg. The temperature was maintained manually. The volatile products were condensed with a circulated liquid cooling system around the distillation unit and distillate was collected.

3.2.6 Esterification of Fatty Acid

Esterification of fatty acid was followed by the procedure 969.33 (AOAC, 1990). About 1g of crude catfish oil and oil from each processing step was separately placed into a 50-ml vial with a taflon cap and a stirring magnet. Approximately 4 ml of methanolic sodium hydroxide (2g of NaOH dissolved in 100 ml methanol), 7 ml of boron trifluoride and 5 ml of heptane were added to the vial. The heptane solution containing FAMEs was recovered from the upper layer (neck) of the vial, dehydrated with 1.5g anhydrous sodium sulfate, and stored under nitrogen in teflon-capped vials at – 20°C until analyzed.

3.2.7 Fatty Acids Analysis

The FAMEs were quantified by the Hewlett Packard 5890 Series II Gas Chromatograph equipped with a 7673A autosampler and interfaced to a 5970 mass selective detector (Agilent Technologies, Palo Alto, CA). The GC was equipped with an EZ-Flash fast temperature programmable column (Thermedics Detection, Inc., Chelmsford, MA). The column phase was RTX-2330 (90%-biscyanopropyl/10% phenylcyanopropyl polysiloxane) with the dimensions: 5 meter long, 0.25 mm inside diameter with a 0.2-μm phase thickness. One μL FAMEs was injected using the inlet in a split mode. The head pressure was set at 2 psi and the split vent flow was 7 mL/m. The injector temperature was 260°C. The column flow rate at 2 psi was 0.68 mL/m. The column temperature was ramped from 50° to 260°C at 20°C/second and was held at 260° C for 90 seconds. Run length was 5 minutes. The transfer line temperature was 280°C. The MSD was operated with the selected ion monitoring mode. Fatty acids were identified with retention times obtained from using the fatty acids methyl esters

standards (Sigma Company, St. Louis, MO). Three experimental replications (batches) were conducted, each with 3 extractions and 3 GC-injections per extraction. FA content was reported as mg/g dry-sample weight.

3.2.8 Free Fatty Acids Analysis

Free fatty acid (FFA) content was determined in duplicate for each batch by a titration method according to the AOAC official method 940.28 (1990). FFA was expressed as mg of oleic acid/ g of oil.

3.2.9 Water Activity

Water activity of oil was measured in using the of water activity meter (AW sprint, Novasina, Switzerland) at 25°C.

3.2.10 CIE-L*a*b* Color Measurement

Colorimetric measurements of catfish oil from different processing stages were determined in compared with the commercial refined menhaden oil using a Minolta Spectrophotometer CM 3500d (Minolta Instrument Systems, Ramsey, NJ). The instrument was calibrated with zero and white calibrations to compensate for the effects of stray light and to eliminate variations in measure values due to changes in ambient temperature and the internal temperature of spectrophotometer. The spectrophotometer was set to obtain color values based on 10° standard observation and D65 illuminants. Results were expressed as L*, a*, and b* values. Psychometric color terms involving hue angle $\left[\tan^{-1}(b^*/a^*)\right]$ and chroma (C*) $\left[b^{2*}/a^{*2^{1/2}}\right]$ were calculated. Color difference (ΔE) was calculated by $\left[\Delta a^{*2}+\Delta b^{*2}+\Delta C^{*2}\right]^{1/2}$ using the refined menhaden oil as reference. L* values measure lightness (0 = black and 100 = white); + a* value represents redness and - a* value represents greenness; + b* value represents yellow and

- b* value represents blue. The hue angle represents an actual color, and chroma evaluates purity or intensity of the color.

3.2.11 Mineral Analysis

Minerals of catfish oil from different processing steps were determined in by the acid digestion method involving CEM innovators in microwave technology. Half of the gram oil sample was separately placed in the vessel and 6 ml of HNO_3 was added to the vessel and sealed. The sealed vessel was placed into the turntable. The heating program was run until the digestion process had completed. Then the sample digested was cooled for 5 minutes and transferred to a flask with a filtration step. Absorption spectrophotometer was used to analyze Ca, Fe, Mg, and P.

3.2.12 Statistical Analysis

All data were analyzed using SAS (version 8.1, 2001). Analysis of variance (ANOVA) was performed to determine differences in fatty acid profiles of samples. Tukey's Studentized Range test was performed for post-hoc multiple comparisons. Group differences, expressed in terms of differences in mean vectors of the fatty acids, were determined using multivariate analysis of variance (MANOVA).

3.3 Results and Discussion

3.3.1 Material Balance

Total yield of crude catfish oil from viscera was calculated on the basis of weight of catfish viscera and fat content of viscera. An average of 3150g catfish viscera was required to produce 815g of crude oil. Our chemical analysis showed that viscera contain 33% of fat. According to the calculation, 78% of the fat was recovered as oil from the viscera and this value can be increased by optimizing the cooking temperature

and time. The amount of catfish oil and menhaden oil produced from each processing steps are given Table 3.1. 1000g of crude oil was required to produce 657g deodorized catfish oil; on the other hand, 1000g of crude menhaden oil was required to produce 763g of deodorized oil.

Table 3.1 Oil losses during the processing steps (g)

Fish Oil	Crude	Degummed	Neutralized	Bleached	Deodorized
Catfish	1000	810	713	683	657
Menhaden	1000	890	807	783	763

The major weight losses of oil was observed between crude oil to degummed oil, and was 19% and 11% for catfish oil and menhaden oil, respectively.

3.3.2 Composition of Fatty Acids

The fatty acid compositions of catfish oil and menhaden oil from each processing stage, respectively, are given in Tables 3.2 and 3.3. The 12 fatty acids found in catfish oil included (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3,C 20:0, C20:1, C20:2, C20:4, and C22:6. Saturated fatty acids (mg/g of oil) from crude, degummed, neutralized, bleached, and deodorized oils accounted for 46.6, 42.1, 41.7, 40.4, and 33.6, respectively (Table 3.2). Steric acid was the predominant fatty acid accounting for about 50% of all saturated fatty acids. Total unsaturated fatty acid present in catfish oil was almost two fold higher than total saturated fatty acid; It amounted to 87.1, 81.1, 79.4, 75.8, and 68.6 crude oil, degummed oil, neutralized oil, bleached oil, and deodorized oil, respectively. Among unsaturated fatty acids, oleic acid was a predominant fatty acid accounting for almost 30% of all unsaturated fatty acids.

Table 3.2. Fatty acids profiles of catfish oil from each processing step (mg/g of oil)

Fatty Acids	Crude	Degummed	Neutralized	Bleached	Deodorized
C14:0	4.9± 0.71[a]	3.85 ± 0.60[b]	3.82 ± 0.57[b]	3.81 ± 0.40[b]	3.53 ± 0.38[b]
C16:0	16.72 ± 4.24[a]	15.20 ± 1.56[ab]	15.14 ± 1.02[ab]	13.50 ± 2.48[ab]	13.20 ± 1.28[b]
C16:1	14.91 ± 2.45[a]	13.79 ± 2.14[a]	13.42 ± 2.08[ab]	13.41 ± 2.47[ab]	10.80 ± 1.64[b]
C18:0	23.90± 3.03[a]	22.24± 2.38[a]	22.00± 1.31[a]	21.87 ± 1.91[a]	16.10 ± 2.24[b]
C18:1	26.32 ± 7.24[a]	25.53 ± 6.11[a]	24.95 ± 3.93[a]	22.82 ± 4.64[a]	22.10 ± 1.34[a]
C18:2	25.02 ± 3.04[a]	22.65 ± 3.55[ab]	22.02 ± 2.70[ab]	20.83 ± 3.65[b]	20.746 ± 2.13[b]
C18:3	4.50 ± 0.73[a]	4.26 ± 0.41[a]	4.14 ± 0.43[a]	4.120 ± 0.35[a]	3.37 ± 0.63[b]
C20:0	1.05 ± 0.39[a]	0.83 ± 0.13[a]	0.82 ± 0.28[a]	0.78 ± 0.36[a]	0.77 ± 0.08[a]
C20:1	9.42 ± 1.68[a]	8.25 ± 0.61[a]	8.24 ± 0.82[a]	8.19 ± 0.71[a]	6.40 ± 0.78[b]
C20:2	4.12 ± 0.73[a]	3.82 ± 0.36[a]	3.81 ± 0.64[a]	3.72 ± 0.41[a]	2.83 ± 0.26[b]
C20:4	1.83 ± 0.27[a]	1.63 ± 0.29[a]	1.58 ± 0.12[a]	1.58 ± 0.11[a]	1.10 ± 0.17[b]
C22:6	1.24 ± 0.12[a]	1.24 ± 0.30[a]	1.24 ± 0.25[a]	1.23 ± 0.45[a]	1.21 ± 0.18[a]
Sat	46.56 ± 6.99[a]	42.14 ± 1.93[ab]	41.65 ± 2.28[ab]	40.04 ± 2.76[b]	33.63 ± 2.10[c]
Unsat	87.1± 12.13[a]	81.16± 9.99[a]	79.39 ± 6.48[ab]	75.81 ± 9.08[b]	68.60 ± 3.32[b]
Omega-3	5.70 ± 0.68[a]	5.50 ± 0.40[a]	5.40 ± 0.55[ab]	5.35 ± 0.68[ab]	4.60 ± 0.66[b]

For each fatty acid, mean values (an average of 27 measures) with the same letters are not significantly different (P>0.05).

Table 3.3 Fatty acids profiles of menhaden oil from each processing step(mg/g of Oil)

Fatty Acids	Crude	Degummed	Neutralized	Bleached	Deodorized
C14:0	18.40 ± 1.70^{a}	18.34 ± 3.67^{a}	17.33 ± 1.87^{a}	16.67 ± 5.19^{a}	16.15 ± 1.45^{a}
C16:0	17.18 ± 0.50^{a}	17.01 ± 0.95^{a}	16.04 ± 1.17^{ab}	15.40 ± 1.92^{ab}	14.42 ± 2.69^{b}
C16:1	16.65 ± 2.18^{a}	16.50 ± 3.28^{a}	14.46 ± 5.43^{a}	14.43 ± 4.53^{a}	14.22 ± 4.60^{a}
C18:0	11.97 ± 1.14^{a}	11.82 ± 1.64^{a}	11.69 ± 0.72^{a}	11.58 ± 0.66^{a}	11.53 ± 3.96^{a}
C18:1	22.08 ± 4.38^{a}	20.11 ± 4.70^{a}	18.74 ± 1.93^{a}	18.65 ± 3.82^{a}	18.35 ± 1.96^{a}
C18:2	5.59 ± 0.15^{a}	5.23 ± 0.60^{ab}	5.11 ± 0.26^{ab}	5.01 ± 0.35^{b}	4.96 ± 0.52^{b}
C18:3	5.81 ± 0.16^{a}	5.63 ± 0.20^{a}	5.45 ± 0.38^{a}	5.34 ± 0.52^{a}	5.15 ± 0.90^{a}
C20:0	1.33 ± 0.14^{a}	1.19 ± 0.05^{ab}	1.17 ± 0.09^{b}	1.16 ± 0.12^{b}	1.13 ± 0.12^{b}
C20:1	6.72 ± 0.68^{a}	6.14 ± 0.53^{a}	6.13 ± 0.59^{a}	6.12 ± 0.50^{a}	6.11 ± 0.51^{a}
C20:2	1.69 ± 0.22^{a}	1.11 ± 0.34^{b}	1.04 ± 0.30^{b}	0.98 ± 0.32^{b}	0.92 ± 0.24^{b}
C20:4	4.81 ± 0.44^{a}	4.68 ± 0.28^{a}	4.66 ± 0.44^{a}	4.63 ± 0.59^{a}	3.03 ± 0.31^{b}
C22:6	19.85 ± 1.62^{a}	19.23 ± 2.31^{a}	18.98 ± 0.86^{a}	18.85 ± 1.18^{a}	18.72 ± 1.25^{a}
Sat	48.88 ± 2.58^{a}	48.26 ± 4.64^{a}	46.23 ± 3.27^{a}	44.81 ± 7.24^{a}	43.24 ± 5.32^{a}
Unsat	83.19 ± 5.93^{a}	78.62 ± 9.53^{ab}	74.57 ± 7.07^{ab}	74.00 ± 9.35^{ab}	71.48 ± 5.83^{b}
Omega-3	25.70 ± 1.68^{a}	24.87 ± 2.34^{a}	24.43 ± 1.04^{a}	24.18 ± 1.48^{a}	23.87 ± 1.22^{a}

For each fatty acid, mean values (an average of 27 measures) with the same letters are not significantly different ($P>0.05$).

The deodorized catfish oil contained 1.21 mg of DHA/g of oil, the polyunsaturated fatty acid considered to be of major importance in terms of human health.

Most of the difference can be explained by the content of DHA, with catfish oil having up to 1.21–1.24 mg/g of oil, whereas processed menhaden oil contained 18.7–19.9 mg/g of oil. Combined omega-3 fatty acids (C18:3 and C22:6) in the deodorized catfish oil accounted for 4.5% of total fatty acids whereas in purified menhaden oil they constituted about the 20.8% of total fatty acids. On a quantitative basis, the purified menhaden oil had approximately five times the level of combined omega-3 (C18:3 and C22.6) than that of the deodorized catfish oil. The value of DHA in deodorized menhaden was at least 15.5 times higher than that of deodorized catfish oil; however, the amount of DHA present in our deodorized menhaden oil was greater than that given by (Young, 1986), who reported for DHA in menhaden oil was between 0.1 to 8.8%.

Table 3.4 shows reported data (USDA, 2001) for the amount of saturated, unsaturated, and omega-3 of sardine, menhaden, herring, cod liver oils compared with our experimentally extracted and purified (deodorized) catfish oil.

Table 3.4 Comparison of catfish oil with major marine fish oil

Fatty acids	Sardine [a]	Menhaden [a]	Herring [a]	Cod liver [a]	Purified[b] Catfish
Saturated	31.3	33.3	22.8	24.6	32.9
Unsaturated	68.7	66.7	77.2	75.4	67.7
DHA	11.1	9.8	4.5	11.9	1.1
Omega-s (C22:6 + C18:3)	13.3	12.4	5.3	13.0	4.5

[a] Reference: USDA (2001); [b] our result

The amount of total saturated fatty acid presented in sardine oil, menhaden oil, was 31.3%, and 33.3%, respectively; both of which were similar to that of our purified catfish oil (32.9%). However, total saturated fatty acid of herring oil (22.8%) and cod liver oil (24.6%) was lower than that of purified catfish oil. The total unsaturated fatty acids of purified catfish oil (67.7%) was somewhat similar to that reported for sardine (68.7%) and menhaden (66.7%) but lower than herring (77.2%) and cod liver oil (75.4%).

Omega-3 (C22:6 and C18:3) fatty acids in purified catfish oil (4.5%) was lower that reported for other fish oil (Table 3.4). There was at least two times greater quantity of omega-3 reported in sardine (13.3%), menhaden oil (12.4%), and cod liver oil (13%) than that of purified catfish oil. Investigations have shown that fatty acids composition of fish lipid was highly dependent on a number of factors, especially fish diets (Ackman and Sipos, 1965; Chanmugam et al., 1986; Cai and Curtis, 1989; Morris et al., 1995); environment (Farkas and Herodek, 1964); seasons (Aro et al, 2000). The predominance of 18:2 ω-6 in catfish oil is attributed to the fishmeal, especially from soy products. On the other hand, marine plankton, a major food source for marine fishes contains high quantity of polyunsaturated fatty acids. Ackman and Sipos (1965) examined a number fish oil fatty acids and they noted that the fatty acids in marine fish were found in phytoplankton.

Loss of each fatty acid during the processing steps was calculated based on quantity of each fatty acids originally present in crude catfish oil (Tables 3.5 and 3.6). It can be clearly seen there were losses in fatty acids from crude oil to final deodorized oil.

Table 3.5. Loss of fatty acids (%) of catfish oil during processing

Fatty Acids	Degummed	Neutralization	Bleaching	Deodorization
C14:0	21.43	22.04	22.24	27.96
C16:0	9.09	9.45	19.26	21.05
C16:1	7.51	9.99	10.06	27.57
C18:0	6.95	7.95	8.49	32.64
C18:1	3.00	5.21	13.30	16.03
C18:2	9.47	11.99	16.75	17.08
C18:3	5.33	8.00	8.44	25.11
C20:0	20.95	21.90	25.71	26.67
C20:1	12.42	12.53	13.06	32.10
C20:2	7.28	7.52	9.71	31.31
C20:4	10.93	13.66	13.66	39.89
C22:6	0.00	0.00	0.81	2.42
Saturated	9.49	10.55	14.00	27.77
Unsaturated	6.82	8.85	12.96	21.24
Omega-3	3.51	5.26	6.14	19.30

Table 3.6. Loss of Fatty acids (%) of menhaden oil during processing

Fatty Acids	Degummed	Neutralization	Bleaching	Deodorization
C14:0	0.33	5.82	9.40	12.23
C16:0	0.99	6.64	10.36	16.07
C16:1	0.90	13.15	13.33	14.59
C18:0	1.25	2.34	3.26	3.68
C18:1	8.92	15.13	15.53	16.89
C18:2	6.44	8.59	10.38	11.27
C18:3	3.10	6.20	8.09	11.36
C20:0	10.53	12.03	12.78	15.04
C20:1	8.63	8.78	8.93	9.08
C20:2	34.32	38.46	42.01	45.56
C20:4	2.70	3.12	3.74	37.01
C22:6	3.12	4.38	5.04	5.69
Saturated	1.27	5.42	8.33	11.54
Unsaturated	5.49	10.36	11.05	14.08
Omega-3	3.23	4.94	5.91	7.12

In the case of catfish oil (Table 3.5), the loss of omega-3 fatty acids between crude oil to deodorized oil accounted for 19.3%, whereas saturated and unsaturated fatty acids losses between crude oil to deodorized oil were 27.77% and 21.24%, respectively. Highest amounts of saturated, unsaturated, and omega-3 fatty acids were lost as much as 13.8%, 8.23% and 13.2%, respectively, between bleached oil to deodorized oil. In the case of menhaden oil (Table 3.6) losses of saturated, unsaturated, omega-3 fatty acids between crude oil to deodorized oil accounted for 11.54%, 14.08 % and 7.12%, respectively. The overall losses of fatty acids were higher in catfish oil than menhaden oil.

Scott and Latshaw (1991) reported that processing caused losses in fatty acid composition of fish oil. Maza et al. (1992) reported that the deodorization parameters such as temperature and time have a significant impact on the quality of finished oil. According to Norris (1982), there were complex chemical and physical phenomena involved during the deodorization. Hydration of tryglycerides, vaporization of oil composition, and entrainment of neutral oil droplets in distillate may have changed the fatty acid composition. Furthermore, the chemical and physical effects were responsible for the yield decrease in substantial during deodorization (Spinelli et al., 1987; Takao et al., 1985; Rivers, 1986). Furthermore, Spinelli et al. (1987) ; Takao et al. (1985); Rivers (1986) described that the current method of purification of oils with distillation destroyed the polyunsaturated fatty acids.

3.3.3 Free Fatty Acid (FFA)

Free fatty acids were gradually removed throughout the oil purification process (Table 3.7). Crude oil contained the highest amount of free fatty acids (4.53 mg of oleic acid/g

of oil), whereas the final deodorized oil containing the lowest acids (3.25 mg of oleic acid/g of oil).

The decreases in free fatty acid may have been due to vaporization ability of FFA during deodorization process. It was reported that considerable amount of FFA was vaporized during distillation (Norris, 1982).

Table 3.7 Free fatty acids, water activity and some minerals catfish oil at different processing steps

Properties	Crude	Degummed	Neutralized	Bleached	Deodorized
Free fatty acid (mg/g of oleic acid	4.53	4.28	4.25	3.80	3.25
Water activity	0.838	0.756	0.696	0.651	0.555
Magnesium (ppm)	4.99	4.64	-	-	-
Calcium(ppm)	10.7	-	-	-	-
Iron (ppm)	0.64	-	-	-	-
Phosphorus (ppm)	107.6	99.2	-	-	-

- (trace amount)

Furthermore, removal of FFA from the oil is inversely proportional to the system pressure and directly proportional to the vapor pressure of the FFA. Gavin (1978) found that a lower system pressure at a fixed temperature showed a greater reduction of the FFA. Vapor pressure of FFA was directly proportional to the temperature, and therefore the increased temperature tentatively increased FFA reduction. Young (1986) reported that under appropriate processing conditions, the FFA could be reduced up to 50% during deodorization.

3.3.4 Water activity

The water activity gradually decreased from crude oil to deodorized oil. Highest water activity was found in crude oil (0.838) and it was reduced to 0.555 in deodorized oil (Table 3.7). This clearly indicated that some free and bound water was removed during oil processing.

3.3.5 Minerals

Table 3.7 shows the mineral contents of catfish oil at different processing steps. Mg, Ca, Fe, and P were major minerals found in catfish oil and other minerals were not reported since there were only trace amounts. During the processing, all these major minerals reduced to a trace level. Crude catfish oil contained high amount of phosphors (107.6 ppm) and it was reduced 99.2 ppm after the degumming process. Ca, Mg, and Fe were removed after the degumming process, while Mg and P were removed after the neutralization process. As explained before, during the degumming process, most of the impurities were removed after due to filtration and repeated washing.

3.3.6 Color

Color characteristics of catfish oil from different processing steps compared with that of the refined menhaden oil are presented in Table 3 8. Refined menhaden oil and bleached catfish were lighter than (higher L*) than crude, degummed, neutralized and deodorized oils. There was no specific pattern observed for color lightness changed during processing steps. Bleaching did increase color lightness of catfish oil. All the oils had negative a* values indicating the slight green color. Oil from all steps of catfish oil production had a positive b* (yellowish) value. The lowest b* value was observed for bleached catfish oil. Total color difference (ΔE) of all the catfish oils were > 1.0;

therefore they may be perceptibly different from the refined menhaden oil consumers point of view. Hue angle values of all oils were higher than 90°. Oil with hue angle between 90-180 were more greenish yellow.

Table 3.8 Color characteristics of catfish oil from different processing steps corresponded with refined menhaden oil.

	L*	a*	b*	C*	h	ΔE
Crude	3.09	-0.809	1.79	1.96	114.37	6.20
Degummed	2.99	-0.84	2.04	2.2	112.34	6.41
Neutralized	1.29	-0.48	1.32	1.40	109.88	7.65
Bleached	5.15	-1.21	0.12	1.22	174.28	3.59
Deodorized	2.99	-1.25	1.29	1.79	134.18	6.04
Refined menhaden oil [a]	8.45	-1.20	-1.32	1.78	227.34	0

[a] used as a standard for ΔE calculation

Commercially refined menhaden oil had highest hue angle (227. 34) and its color was visually observed as light greenish blue.

Color lightness (L*) of catfish oil slightly decreased between the crude oil to neutralized oil. It was visually observed that when citric acid solution was added during the degumming process, and when caustic soda was added during neutralization process, the oil turned into a cloudy mixture and color became dull. During bleaching operation, the bleaching earth adsorbed pigments, water, minerals, and soap. As the result,

bleached oil had a higher lightness (L*). However, the L* value of deodorized oil was lowered than that of bleached oil. It may be due to heat decomposition..

3.4 Conclusions

This has demonstrated a processing that can be used to extract and purifying oil recovered from catfish viscera the yield of deodorized catfish oil was 65.7% compared with 76.3% of deodorized menhaden oil. Major yield loss took place during the degumming process. The combined omeg-3 fatty acids content of deodorized catfish oil was about 5 times less that that of menhaden oil. The percent loss at 19.3, 27.77 and 21.24% was observed for omega-3, saturated and unsaturated fatty acids after the deodorization process. Free fatty acid, water activity, and some minerals were decreased during processing. Bleaching removed pigment, thus resulting in oil with greater lightness and less yellowness.

CHAPTER 4. MICROWAVE -ASSISTED FATTY ACID ANALYSIS OF OIL RECOVERED FROM CATFISH PROCESSING WASTE CONTAINING HIGH MOISTURE CONTENT

4.1 Introduction

The analysis of lipids and fatty acid compositions of foods and ingredients is an important element in food chemistry. Nutritional studies have shown the potential benefits of long-chain polyunsaturated fatty acids (PUFAs). PUFAs, e.g., eicosapentaenoic acid (20:5ω3, EPA), arachidonic acid (20:4ω6), and docosahexaenoic acid (22:6ω3), are precursors of prostaglandins and leukotrienes. PUFAs are important to human health and essential to the development of the fetus and infants. Dietary intake of PUFAs reduces incidence of coronary diseases and some cancers (Innis, 1991; Burns and Spector, 1994). Fatty acid composition of foods, especially PUFAs, is of interest to consumers as well as food scientists because of the nutritional/health benefit. A number of extensive research programs have been oriented toward development of enriched PUFAs-containing products. This type of research requires a routine fatty acid analysis, which is time consuming and labor intensive. Thus, a faster, yet simple and reliable method is needed.

Gas chromatography (GC) analysis is commonly used to determine fatty acid profiles of lipids in biological materials, and normally requires a methyl esterification of fatty acids (FAMEs) (Bligh and Dyer, 1959). The FAMEs preparation requires fat extraction from biological materials with organic solvents, followed by esterification of the fat to form fatty acid methyl esters (FAMEs). Organic solvents commonly used for fat extraction include chloroform, dichloromethane, hexane, toluene, benzene and methanol or a mixture of them. After extraction, the solvent is evaporated from the

mixture by mild heating under nitrogen gas. The traditional fat extraction method requires a large volume of solvents. It is a multistep procedure, not suitable for handling a large number of samples, and it may lead to introduction of contaminants and losses of esters. Therefore, it may not be practical in laboratories where a number of tests are required in a short period of time.

Several methods of fatty acid methyl ester preparation have been developed to reduce the number of steps to one or two. For example, Ichihara et al. (1996) rapidly transesterified a lipid extract with methanolic HCl and resolved major fatty acids using high temperature gas liquid chromatography (GLC). Shimasaki et al. (1977) chemically removed water from brain and plasma samples with 2,2'-dimethoxypropane, and then transesterified the lipids to produce FAMEs. Lepage and Roy (1984, 1986 and 1988) reported a one-step procedure for fatty acid methyl ester preparation with acetyl chloride applied to several classes of lipids and sources. A similar approach was reported by Sukhija and Palmquist (1988), but was applied to freeze-dried materials. Ohta et al. (1990) directly applied plasma samples to thin layer chromatography (TLC) plates to separate lipids and then transesterified the silica-adsorbed fatty acids with boron-trifluoride in methanol. Except for the study by Ichihara et al. (1996), the aforementioned studies have in common the fact that samples were not solvent-extracted before lipids were transesterified. Lipid extraction procedures, for example those reported by Floch et al. (1957) and Bligh and Dyer (1959), ideally separated all lipids from bulk of the material, which were then portioned into a hydrophobic phase for separation and subsequent purification.

The use of microwave irradiation has been suggested as an efficient technique for the extraction of lipophilic substances from biological tissue (Pare et al., 1997). Microwave ovens are credited for rapid heating rates and high efficiency, because of their high penetration power (Burfoot, et al., 1990). Yoshida et al. (1995) reported the effects of microwave heating on acyl lipids in whole soybeans in relation to moisture.

The objective of this study was to develop a rapid microwave–assisted method for fatty acids analysis for materials containing high moisture content. The microwave heating power (%) and time (sec) required for maximal fatty acids recovery was determined. Catfish liver with approximately 75% moisture content was used for demonstration.

4.2 Materials and Methods

4.2.1 Microwave –Assisted FAMEs Preparation

Approximately 5g of finely ground and homogenous catfish liver was placed in a 50-ml sample vial. The vials were placed in a microwave oven (Model R-508AK, Sharp Carousel, 1000 Watts and 2450MHz) and heated as explained in Table 4.1. A 4x4 full factorial deign was employed. The concentration of fatty acids were dependent variables and the microwave power (100, 80, 60, 40 %) and heating time (80, 60,40,20 sec) were independent variables.

After microwave treatment, the vials were sealed with teflon coated caps and cooled to room temperature. After cooling, each vial was weighed. The moisture loss of liver was calculated as [(initial wt of the sample + vial)-(wt of the sample + vial after microwave heating)]x 100/ initial weight of sample. 6 ml methanolic sodium hydroxide (2% NaOH in methanol), 7 ml BF_3 and 5 ml heptane were added to each vial. The

reaction mixture was stirred with a teflon-coated magnetic stirrer bar in a 70°C controlled water bath for 30 minutes. The heptane containing FAMEs was collected from the upper layer and was dehydrated with anhydrous sodium sulfate, and stored under nitrogen in a teflon-capped 20 ml vial at –20°C until further analyzed (experimental replication); each using a different batch of catfish liver. All the treatments were repeated three times.

Table 4.1 Microwave treatment

	Time (sec)			
Power (%)	80	60	40	20
100	A	B	C	D
80	E	F	G	H
60	I	J	K	L
40	M	N	O	P

4.2.2. Fat Extraction by a Traditional Method

About 5g the finely ground and homogenous catfish liver sample was placed in a screw cap test tube, then 5ml of distilled water, 20 ml of chloroform and 20 ml Methanol (1:4:4 by volume) were added to the tube and the mixture was thoroughly mixed on a vortex in a screw-cap test tube for 10 minutes. The homogenous mixture was filtered through Whatman No.1 filter paper. The filtrate was placed into a separatory funnel. The bottom layer of the solution was collected. Anhydrous sodium sulfate (5.7g) was added to collected solution to remove water. The residual solvent was removed from the

solution by the Meyer-N-Evaporator (an analytical evaporator) under a nitrogen atmosphere. The evaporation was continued until the solution was free from chloroform. The extracted oil samples were kept at -20°C until analyzed. Fat extraction was repeated for 3 times, each using a different batch of catfish liver.

4.2.3 FAMEs Preparation by a Traditional Method

The fatty acids methyl esters (FAMEs) were prepared following the procedure 969.33 (AOAC, 1990). The extracted catfish oil was separately placed into a 50-ml flat bottom-boiling flask containing approximately 4 ml of methanolic sodium hydroxide (2g of NaOH dissolved in 100 ml methanol) and 10 boiling chips were added into the flask. The condenser and reflux units were attached to the flask and refluxing took place with immediate addition of 7 ml of boron trifluoride through the condenser for 10 minutes. Refluxing was allowed for another 2 minutes. The esterified fatty acids were extracted from the mixture by adding 5 ml of heptane and refluxing for 1 minute. The esterified solution was allowed to cool to room temperature. A saturated solution of sodium chloride was added and the flask gently rotated. Saturated sodium chloride solution was added until the heptane solution reached the neck of the flask. The heptane solution containing FAMEs was recovered, dehydrated with 1.5g anhydrous sodium sulfate, and stored under nitrogen in teflon-capped vials at –20°C until analyzed.

4.2.4 Fatty Acids Analysis

The FAMEs obtained from microwave heated and traditionally treated samples were quantified by the Hewlett Packard 5890 Series II Gas Chromatograph equipped with a 7673A autosampler and interfaced to a 5970 mass selective detector (Agilent Technologies, Palo Alto, CA). The GC was equipped with an EZ-Flash fast temperature

programmable column (Thermedics Detection, Inc., Chelmsford, MA). The column phase was RTX-2330 (90%-biscyanopropyl/10% phenylcyanopropyl polysiloxane) with the dimensions: 5 meter long, 0.25 mm inside diameter with a 0.2-μm phase thickness. One μL of FAMEs was injected using the inlet in a split mode. The head pressure was set at 2 psi and the split vent flow was 7 mL/m. The injector temperature was 260°C. Column flow rate at 2 psi was 0.68 mL/m. The column temperature was ramped from 50° to 260°C at a 20°C/second and was held at 260°C for 90 seconds. Run length was 5 minutes. The transfer line temperature was 280°C. The MSD was operated in the selected ion monitoring mode. Fatty acids were identified with retention times obtained from the fatty acids methyl esters standards (Sigma Company, ST. Louis, MO). Three experimental replications (batches) were conducted for both microwave-assisted and traditional method, each with 3 extractions and 3 GC-injections per extraction. FA content was reported as mg FA/g remaining moisture of 1g microwaved sample. The unit used in this study was to reflect the effect of microwave on moisture and fatty acid profile of the samples.

4.2.5 Statistical Analysis

All data were analyzed using SAS (version 8.1, 2001). Analysis of variance (ANOVA) was performed to determine differences in fatty acid profiles of microwave treated and traditional (control) samples. Tukey's Studentized Range test was performed for post-hoc multiple comparisons. Group differences, expressed in terms of differences in mean vectors of the fatty acids, were determined using multivariate analysis of variance (MANOVA). Principal component analysis was used to group the samples based on similarity (correlation) in the fatty acid profile. Descriptive discriminant

analysis (Huberty, 1994) was performed to identify fatty acids that largely underlie group differences among FA profiles of traditional (control) and microwave-treated samples.

4.3 Results and Discussion

4.3.1 Moisture Loss

Figure 4.1 shows the moisture loss of the catfish liver sample for each microwave heat treatment. The moisture loss increased with an increase of magnitude of power and time. Pronounced effects were observed when heating time was increased to 80 second.

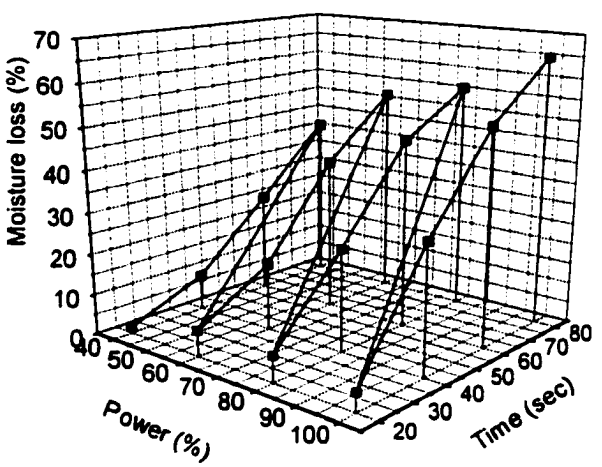

Figure 4.1 Effects of microwave treatments on moisture of catfish liver

Initial moisture content of liver tissue was 75%. Microwave-heating at 60-100% power for 80 sec removed over 50% water from the samples. Microwave energy was

one of the rapid heating sources. Water in a sample facilitates increased fat extraction, during microwave heat treatment. The moisture content in the samples to be fat extracted is a critical factor as water is very efficient at absorbing microwave radiation. Fat molecules are surrounded by biological materials, particularly protein molecules. Rapid removal of water in the sample may disrupt the cell structure and supports removal of lipids from their association with cell membranes and proteins. By denaturing the protein molecules, the fat molecules can be liberated to the surface of the biological materials. When a biological material is microwave–heated, moisture in the tissue creates localized superheating, causing a rapid release of moisture, expulsion of fat from the fat cells, and release of oil to the surface

4.3.2 Fatty Acid Composition

The values obtained for fatty acid composition of catfish liver oil from the microwave treated and control (traditional method) samples are shown in Table 4.2. Significant differences in the content of recovered fatty acids were observed in the microwave treated samples and traditionally treated sample. Results showed that the fatty acids content recovered by the microwave-assisted method was generally greater than that of the traditional method. Significant differences were observed for almost all fatty acids present in catfish liver samples due to various microwave treatments. We found that C20:0 and C20:4 were not present in any microwave treated samples, except in 100% power for 80 seconds. PUFAs showed the drastic differences among the treatments, with important variants in the magnitude of C22:6 ω-3. Results showed that there were significant differences in saturated and unsaturated fatty acids for both methods.

Table 4.2 Effects of microwave heating on fatty acids composition of catfish oils

Power (%)	Time (sec)	C14:0	C16:0	C16:1	C18:0
100	80	$0.07 \pm 0.03^{a*}$	$0.23 \pm 0.10^{a*}$	$0.45 \pm 0.17^{a*}$	$1.77 \pm 0.71^{a*}$
100	60	$0.04 \pm 0.01^{bcd*}$	$0.07 \pm 0.02^{bcd*}$	$0.25 \pm 0.04^{bcd*}$	$0.91 \pm 0.17^{cd*}$
100	40	0.02 ± 0.01^{ef}	0.03 ± 0.01^{def}	0.12 ± 0.03^{fe}	0.44 ± 0.10^{efgh}
100	20	0.02 ± 0.01	0.02 ± 0.01	0.08 ± 0.07	0.26 ± 0.26
80	80	$0.06 \pm 0.02^{ab*}$	$0.10 \pm 0.02^{b*}$	$0.35 \pm 0.09^{ab*}$	$1.35 \pm 0.42^{b*}$
80	60	$0.05 \pm 0.01^{bc*}$	$0.07 \pm 0.01^{bcde*}$	$0.28 \pm 0.03^{bc*}$	$0.98 \pm 0.17^{bc*}$
80	40	0.03 ± 0.01^{ed}	0.03 ± 0.01def	0.15 ± 0.07^{cd}	0.41 ± 0.09^{efgh}
80	20	0.03 ± 0.02^{def}	0.02 ± 0.01ef	0.11 ± 0.06^{ef}	0.31 ± 0.14^{fgh}
60	80	$0.04 \pm 0.01^{bcd*}$	$0.08 \pm 0.02^{bc*}$	$0.24 \pm 0.0^{cd*}$	$0.72 \pm 0.17^{cde*}$
60	60	$0.03 \pm 0.00^{cde*}$	$0.04 \pm 0.01^{cdef*}$	$0.17 \pm 0.03^{ed*}$	0.53 ± 0.07^{defgh}
60	40	0.02 ± 0.00	0.02 ± 0.00	0.10 ± 0.03^{ef}	0.33 ± 0.08^{efgh}
60	20	0.02 ± 0.00^{ef}	0.02 ± 0.00^{f}	0.10 ± 0.01	0.30 ± 0.02
40	80	0.03 ± 0.00^{de}	0.04 ± 0.01^{cdef}	0.16 ± 0.02^{ed}	0.60 ± 0.06^{cdefg}
40	60	0.03 ± 0.01^{def}	0.03 ± 0.01^{def}	0.12 ± 0.06^{ef}	0.60 ± 0.15^{cdefg}
40	40	$0.03 \pm 0.01^{cde*}$	$0.02 \pm 0.00^{ef*}$	$0.17 \pm 0.05^{de*}$	0.68 ± 0.17^{cdef}
40	20	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.01	$0.18 \pm 0.02^{*}$
Control		0.02 ± 0.01	0.48 ± 0.17	0.07 ± 0.02	0.45 ± 0.18

For each fatty acid, means (excluding that of control) with the same letter are not significantly different ($P>0.05$). Paired comparison between microwave treated and control samples was significant at $P<0.05$ as indicated by*.

(table continued)

Power (%)	Time (sec)	C18:1	C18:2	C18:3	C20:0
100	80	$6.13 \pm 2.51^{a*}$	$1.00 \pm 0.66^{a*}$	0.05 ± 0.02	0.01 ± 0.00
100	60	$3.71 \pm 0.77^{bc*}$	$0.82 \pm 0.15^{bcd*}$	0.02 ± 0.01	0.00 ± 0.00
100	40	$2.04 \pm 0.40^{cde*}$	0.41 ± 0.16^{efg}	0.01 ± 0.00	0.00 ± 0.00
100	20	1.32 ± 0.03	0.24 ± 0.03	0.01 ± 0.03	0.00 ± 0.00
80	80	$4.86 \pm 2.08^{ab*}$	$1.20 \pm 0.43^{b*}$	0.04 ± 0.01	0.00 ± 0.00
80	60	$3.70 \pm 1.17^{bc*}$	$0.99 \pm 0.13^{bc*}$	0.03 ± 0.01	0.00 ± 0.00
80	40	1.71 ± 0.15^{de}	$0.47 \pm 0.24^{defg*}$	0.02 ± 0.01	0.00 ± 0.00
80	20	1.71 ± 0.66^{de}	0.28 ± 0.12	0.01 ± 0.01	0.00 ± 0.00
60	80	$2.63 \pm 0.66^{cd*}$	$0.76 \pm 0.20^{cde*}$	0.03 ± 0.01	0.00 ± 0.00
60	60	$2.43 \pm 0.31^{cde*}$	$0.59 \pm 0.13^{cdef*}$	0.02 ± 0.01	0.00 ± 0.00
60	40	1.72 ± 0.35^{de}	0.33 ± 0.06^{fg}	0.01 ± 0.00	0.00 ± 0.00
60	20	1.66 ± 0.16	0.29 ± 0.03^{fg}	0.01 ± 0.00	0.00 ± 0.00
40	80	$2.59 \pm 0.19^{cd*}$	$0.57 \pm 0.12^{def*}$	0.02 ± 0.00	0.00 ± 0.00
40	60	$2.54 \pm 0.74^{cd*}$	$0.46 \pm 0.11^{defg*}$	0.01 ± 0.00	0.00 ± 0.00
40	40	$2.90 \pm 0.70^{cd*}$	$0.54 \pm 0.15^{defg*}$	0.02 ± 0.01	0.00 ± 0.00
40	20	0.83 ± 0.14	0.15 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
Control		0.81 ± 0.19	0.19 ± 0.08	0.02 ± 0.01	0.02 ± 0.01

For each fatty acid, means (excluding that of control) with the same letter are not significantly different ($P>0.05$). Paired comparison between microwave treated and control samples was significant at $P<0.05$ as indicated by*.

(table continued)

Power (%)	Time (sec)	C20:1	C20:2	C20:4	C22:6	Sat	Unsat
100	80	0.22 ± 0.08	0.17 ± 0.09	0.01 ± 0.00	0.96 ± 0.35	2.08 ± 0.81	9.68 ± 3.68
100	60	0.10 ± 0.02	0.09 ± 0.04	0.00 ± 0.00	0.54 ± 0.11	1.03 ± 0.18	5.53 ± 1.04
100	40	0.04 ± 0.01	0.04 ± 0.01	0.00 ± 0.00	0.26 ± 0.10	0.49 ± 0.11	2.93 ± 0.69
100	20	0.02 ± 0.02	0.02 ± 0.02	0.00 ± 0.00	0.18 ± 0.19	0.29 ± 0.28	1.87 ± 1.69
80	80	0.15 ± 0.04	0.15 ± 0.04	0.00 ± 0.00	0.83 ± 0.19	1.48 ± 0.44	7.58 ± 2.75
80	60	0.11 ± 0.01	0.11 ± 0.02	0.00 ± 0.00	0.67 ± 0.09	1.10 ± 0.17	5.90 ± 1.34
80	40	0.40 ± 0.02	0.06 ± 0.03	0.00 ± 0.00	0.31 ± 0.14	0.47 ± 0.11	2.76 ± 0.58
80	20	0.02 ± 0.01	0.03 ± 0.02	0.00 ± 0.00	0.21 ± 0.09	0.36 ± 0.16	2.38 ± 1.04
60	80	0.08 ± 0.02	0.10 ± 0.03	0.00 ± 0.00	0.52 ± 0.12	0.84 ± 0.18	4.35 ± 1.04
60	60	0.05 ± 0.01	0.05 ± 0.02	0.00 ± 0.00	0.38 ± 0.08	0.60 ± 0.08	3.69 ± 0.36
60	40	.03 ± 0.01	0.03 ± 0.01	0.00 ± 0.00	0.24 ± 0.07	0.38 ± 0.09	2.46 ± 0.53
60	20	0.02 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.23 ± 0.02	0.35 ± 0.02	2.35 ± 0.22
40	80	0.06 ± 0.01	0.05 ± 0.01	0.00 ± 0.00	0.34 ± 0.06	0.66 ± 0.06	3.78 ± 0.34
40	60	0.05 ± 0.01	0.04 ± 0.01	0.00 ± 0.00	0.33 ± 0.10	0.66 ± 0.16	3.56 ± 0.95
40	40	0.06 ± 0.02	0.05 ± 0.01	0.00 ± 0.00	0.40 ± 0.13	0.74 ± 0.18	4.14 ± 1.06
40	20	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.09 ± 0.01	0.20 ± 0.02	1.15 ± 0.16
Control		0.06 ± 0.02	0.05± 0.02	0.43 ± 0.16	0.27 ± 0.06	0.96 ± 0.36	1.90 ± 0.52

For each fatty acid, means (excluding that of control) with the same letter are not significantly different (P>0.05). Paired comparison between microwave treated and control samples was significant at P<0.05 as indicated by*.

Recovery of C16:0, C20:4, and C20:0, and of all microwave heated samples were lower than that of the traditional method; however, greater recovery was observed for C20:1, C18:0, C16:1, and C22:6 at 80-100% power for 60-80 sec.

4.3.3 Multivariate Statistical Analysis

To provide a method for differentiating the treatment microwave heat effects on fatty acid composition, a multivariate statistical analysis was carried out. Summary of the MANOVA is given in Table 4.3. The model includes 14 variables. GLM analysis indicated that differences were found in each fatty acid content among 16 microwave heat treatments. Considering all 14 variables simultaneously, there were differences among the fatty acids profile of all 16 microwave–heated samples. This was substantiated by the statistics value for Wilk's lambda, Pillai's Trace, Hotelling –Lawley and Roy's Greatest Root.

Table 4.3 MANOVA for fatty acid analysis

Statistics	Value	F-Value	Num DF	Den DF	Pr>F
Wilks' Lambda	0.001	7.5	192	1397.5	<0.0001
Pillai's Trace	3.5	3.9	192	1836	<0.0001
Hotelling -Lawley	23.65	17.3	192	891.37	<0.0001
Roy's Greatest	14.18	135.6	16	153	<0.0001

4.3.4 Principal Component Analysis

The principal component scores are plotted between PC1 versus PC2 in Figure 4.2. The PCA plot and scores (Table 4.4) shows three clearly differentiated samples:

one corresponding to control (Q), and the others including treatments 100 % power for 80 sec (A) and 80% power for 80 sec (E). The PCA plots also indicated that samples microwave-heated for less than 80 sec at the power of less than 80% are more alike, although some fatty acids are significantly different as indicated by the ANOVA results.

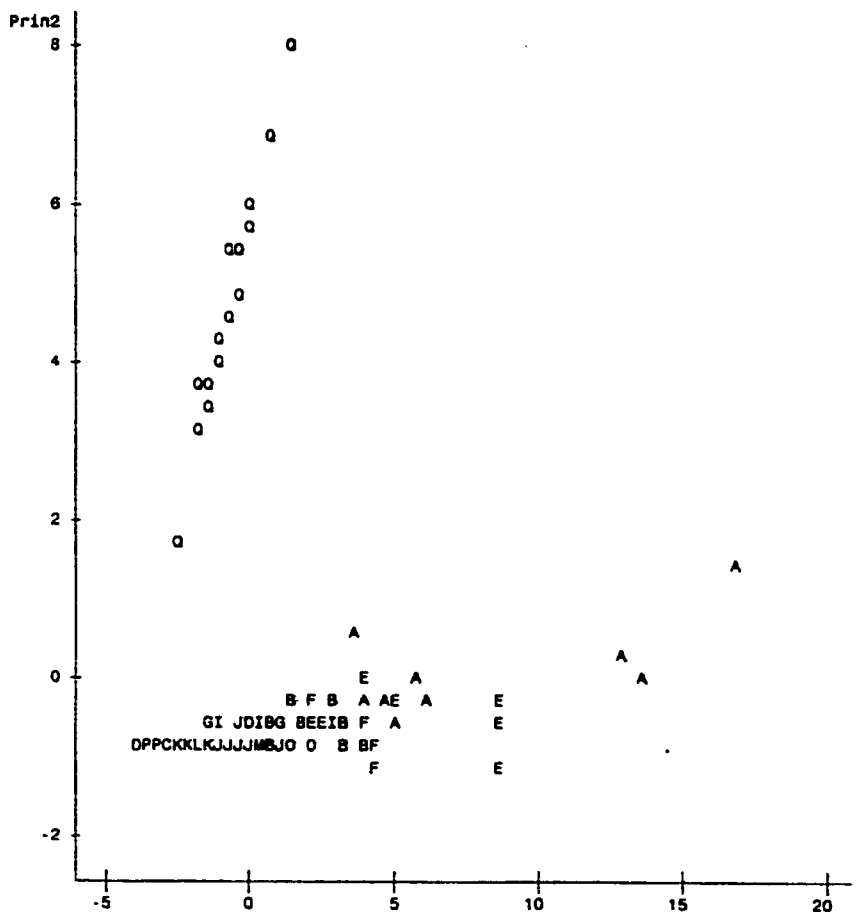

Figure 4.2. Plots (PC1 and PC2) of principal component scores for all 16 microwave-treated and the control samples.

A=100 % power for 80 sec; B=100 % power for 60 sec; C=100 % power for 40 sec; D=100 % power for 20 sec; E= 80 % power for 80 sec; F= 80 % power for 60 sec G= 80 % power for 40 sec; H= 80 % power for 20 sec; I= 60 % power for 80 sec; J= 60 % power for60 sec; K= 60 % power for 40 sec; L= 60 % power for 20 sec; M= 40 % power for 80 sec; N= 40 % power for 60 sec; O = 40 % power for 40 sec;P= 40 % power for 20 sec; Q = control

4.3.5 Discriminant Analysis

Discrimination analysis (Table 4.4) with emphasis on canonical correlation was employed to determine which fatty acids variables contribute the most to differences among 16 microwave-heated and the control samples.

Table 4.4 Discrimination analysis of fatty acids

Fatty Acids	Can1[a]	Can2	Can3	Prin1[b]	Prin2	Prin3
C14:0	-0.1587	0.50436	0.44194	0.30555	-0.0564	-0.0526
C16:0	0.59508	0.29106	0.10977	0.04741	0.54039	-0.0294
C16:1	-0.2017	0.60024	0.49293	0.30418	-0.908	-0.244
C18:0	-0.1109	0.63855	0.35262	0.31057	0.00707	0.27157
C18:1	-0.2191	0.49812	0.2637	0.28993	-0.1489	0.58446
C18:2	-0.2159	0.6245	0.38288	0.29941	-0.1092	-0.1259
C18:3	0.01012	0.46165	0.51764	0.28969	0.14169	-0.3335
C20:0	0.44617	0.14491	0.12546	0.02918	0.54175	0.12666
C20:1	-0.0386	0.75215	0.37019	0.30447	0.08815	0.01028
C20:2	-0.0776	0.52394	0.55039	0.3	0.02586	-0.3978
C20:4	0.67146	0.04302	0.15984	-0.0326	0.54294	0.05107
C22:6	-0.1336	0.60722	0.63316	0.30727	-0.033	-0.2518
Sat	0.05891	0.61531	0.33345	0.29164	0.19211	-0.221
Unsat	-0.183	0.57391	0.39506	0.306117	-0.87461	0.324323
Cumulative variance explained (%)	59.97	88.37	95.53	72.55	96.17	97.50

[a] Can = Canonical Correlation; [b] Prin= Principal component

Fatty acids (C20:4/C16:0/C20:0) of the first canonical variables appear to be the attributes that mostly contribute to the differences. Canonical correlations for C20:4/C16:0/C20:0 were 0.671464, 0.595077, and 0.446174, respectively, and 59% of total variance explained. The second canonical variables included C20:1, C18:0, C18:2, C16:1, and C22:6 explaining a cumulative 88% variance.

4.4 Conclusions

This research investigates the feasibility of preparing FAMEs from microwave-heated samples without solvent extraction. Heating at 100% power for 80 sec yielded the highest recovery of DHA (C22:6 ω-3) and arachidonic (C20:4 ω-3). Fatty acid profiles of microwave-heated samples were different. C20:4, C16:0, and C20:0 are discriminating fatty acids. Recovery of C20:4, C16:0, and C20:0 of all microwave-heated samples were lower than that of the control, while higher recovery was observed for C20:1, C18:2, C16:1, and C22;6 at 80-100% power for 60-80 sec or 60% power for 80 sec. In addition to speed and ease of use, the advantages of microwave-assisted technique were no solvent consumption and low energy consumption. The total process took a few minutes compared with several hours if done using conventional fat extraction methods. This technique also generates less chemical wastes.

CHAPTER 5. REMOVAL OF FREE FATTY ACIDS FROM CATFISH VISCERAL OIL BY BATCH ADSORPTION

5.1 Introduction

Non-triglyceride materials, such as free fatty acids and pigments, are present in crude oil and they must be removed to produce an oil of acceptable quality. Conventional edible oil refining process includes degumming, neutralization, bleaching and deodorization, and is designed to remove impurities. Free fatty acids (FFA) are precipitated as soaps and removed during the neutralization process (Mounts and Khym, 1980). Pigments from oil are adsorbed by bleaching clays (Brekke, 1980). Adsorption techniques can be used to remove impurities in oils. Adsorption can be defined as a separation process often used to remove solutes from liquid solution when the quantity of solute is small (Geankoplis, 1993; Suzuki, 1990; Kennedy and Cabral, 1993).

Many studies have shown that an adsorption process used for edible oil purification not only removes pigments but also removes free fatty acids (Taylor and Ungermann, 1984; Proctor and Palaniappan, 1990; Palaniappan and Proctor, 1991; Toro-Vazquez and Rocha-Uribe, 1993; Farook and Ravendren, 2000; Toro-Vazquez and Mendez-Montealvo, 1995; Clark and Proctor, 1993; Proctor and Toro-Vazquez, 1996).

Due to their physical and chemical characteristics' chitosan and activated carbon may be used as adsorbents to effectively remove FFA from catfish visceral oil. Chitosan is a copolymer of linked β, (1→ 4), 2-amino-2-deoxy-D-glucose and 2-acetamidodeoxy-D-glucan. Chitosan bears hydroxyl and amino groups, which are excellent functional groups for use as an adsorbent for vast variety of complex compounds (Wan-Ngah and

Liang, 1999; Piron et al., 1997; 1998; Peniche-Covas et al., 1992; Quignard et al., 2000; Juang and Ju, 1998).

Activated carbon is a well known versatile product used in many applications. It is used to remove impurities from gaseous and liquid streams for purification and separation in several industrial processes (Ahmedna et al., 1997; Bernardo et al., 1997; Migo et al., 1993; Castro et al., 2000). The carbons have nonpolar surfaces that are used to adsorb nonpolar molecules, especially hydrocarbons. They are manufactured from nut shells and rice hulls, among other agricultural wastes (Dai and Antal, 1999). The ability of activated carbon and activated earth to adsorb impurities from the oil has been reported (Riberio et al., 2001; Ferreira-Dias et al., 2000; Toro-Vazquez and Rocha-Uribe, 1993)

In the case of chitosan, there have been no studies showing investigating kinetics for adsorption of free fatty acids. The objective of this study was to evaluate the feasibility of using chitosan, activated carbon and activated earth for removal of free fatty acids from crude catfish oil. Variables studied were contact time, composition of fatty acids, free fatty acids, and water activity of catfish crude oil.

5.1.1 Theory

Equation 1 was used to calculate the initial adsorption kinetic coefficient as described by Kadirvelu et al. 2000:

$$c = \left(\frac{dC}{dt}\right)_{t \to 0} \frac{V}{mC_o} \qquad (1)$$

where c is the initial adsorption kinetic coefficient ($mlmg^{-1}min^{-1}$), t is the time (min), C is the concentration of FFA (mgg^{-1}) at time t; Co is the initial concentration of FFA (mgg^{-1}), m is the adsorbent weight (g); and V is a crude oil volume (ml).

The external film mass transfer coefficient k_sA (mls^{-1}) was calculated from equation 2 (Kadirvelu et al. 2000):

$$-In\left(\frac{C}{C_o}\right) = ksA\frac{t}{V} \quad (2)$$

Intraparticular diffusion coefficient k_w ($mgml^{-1}s^{-0.5}$) was calculated from equation 3 (Morris and Weber, 1962; Kadirvelu et al., 2000):

$$\frac{m}{V}q = k_w t^{1/2} \quad (3)$$

where q (mgg^{-1}) is the adsorption at time t.

Partition coefficient kd (mlg^{-1}) is calculated from equation 4 (Piron et al., 1997):

$$kd = \frac{V(Co - C)}{Cm} \quad (4)$$

Where V is the volume of liquid, m is the mass of adsorbent.

5.2 Materials and Methods

5.2.1 Crude Oil and Adsorbents

Catfish viscera was obtained in three batches (experimental replications) from the local fish processing plant in Baton Rouge, LA. The viscera was frozen at - 20°C until used. The slightly thawed viscera was finely ground in a Hobart Chopper Bowl (84181D) at 3,450 rpm for 10 minutes. Water was added (water: ground viscera, 5:1 V/W) and the mixture was heated at 70°C for 15 minutes. The purpose of the heating step was to coagulate the protein of the catfish viscera, so that liquids and solids can be mechanically separated. During mild heating, fat cells are ruptured, releasing the oil into the liquid phase. The denatured proteins were separated from the liquid phase (oil and

soluble particles) by filtration through cheesecloth and the cooked viscera were pressed manually to remove most of the liquid. The crude oil was separated from the water phase and other fish particles by centrifugation at 5,000 rpm for 30 minutes. Separated crude oil was stored at – 20°C until used. Three experimental batches were conducted.

The activated earth (American Oil Chemistry Society, Champaign, IL) and crab chitosan with degree of deacetylation 80% (Vanson Inc, Redmond, WA) and CO_2 - activated carbon from pecan shell supplied by USDA were used in this study.

5.2.2 Batch Mode Adsorption Study

Batch adsorption study was conducted in a 50 ml vial sealed with teflon cap. Twenty ml of crude oil was placed into each vial and 0.8g of an adsorbent (activated earth, chitosan, or activated carbon) was added separately. Adsorption reaction was carried out with constant agitation using a magnetic stirrer at 25°C $\pm$ 1°C. Samples were drawn at 1, 2, 3 ,4, and 5-h interval for free fatty acid and fatty acid profile. Experiments were repeated three times.

5.2.3 Free Fatty Acids Analysis

Free fatty acid (FFA) content was determined in replicate by titration according to the AOAC official method 940.28 (1990) and FFA was expressed as mg oleic acid per g of oil.

5.2.4 Esterification of Fatty Acids

About 1g of crude catfish oil drawn at 1, 2, 3, 4, and 5-h interval was separately placed into a 50-ml vial with a teflon cap and a magnetic stirrer. Approximately 4 ml of methanolic sodium hydroxide (2g of NaOH dissolved in 100 ml methanol) 7 ml of boron trifluoride and 5 ml of heptane were added to the vial. The heptane solution containing

free acid methyl esters (FAMEs) was recovered from the upper layer (neck) of the vial, dehydrated with 1.5g anhydrous sodium sulfate, and stored under nitrogen in a teflon-capped vial at –20°C until analyzed.

5.2.5 Fatty Acids Analysis

The FAMEs were quantified by the Hewlett Packard 5890 Series II Gas Chromatograph equipped with a 7673A autosampler and interfaced to a 5970 mass selective detector (Agilent Technologies, Palo Alto, CA). The GC was equipped with an EZ-Flash fast temperature programmable column (Thermedics Detection, Inc., Chelmsford, MA). The column phase was RTX-2330 (90%-biscyanopropyl/10% phenylcyanopropyl polysiloxane) with the dimensions: 5 meter long, 0.25 mm inside diameter with a 0.2-μm phase thickness. One μL of FAMEs sample was injected using the inlet in a split mode. The head pressure was set at 2 psi and the split vent flow was 7 mL/min. The injector temperature was 260°C. The column flow rate at 2 psi was 0.68 mL/min. The column temperature was ramped from 50° to 260°C at 20°C increment/s and was held at 260° C for 90 s. Run length was 5 min. The transfer line temperature was 280°C. The MSD was operated with the selected ion monitoring mode. Fatty acids were identified with retention times obtained using fatty acids methyl esters standards (Sigma Company, St. Louis, MO). Three experimental replications were conducted, each with 3 extractions and 3 GC-injections per extraction. FA content was reported as mg/g dry-sample weight.

5.2.6 Water Activity

Water activity of oil was measured in triplicate by a water activity meter (AW sprint, Novasina, Switzerland) at 25°C.

5.2.7 Statistical Analysis

All data were analyzed using SAS (version 8.1, 2001). Analysis of variance (ANOVA) was performed to determine differences in fatty acid profiles of samples. Tukey's Studentized Range test was performed for post-hoc multiple comparisons. Group differences, expressed in terms of differences in mean vectors of the fatty acids, were determined using multivariate analysis of variance (MANOVA).

5.2.8 Calculation of Kinetic Parameters

A curve fit program in CurveExpert 1.3 (copyright Daniel Hyams), a comprehensive curve fitting system for Windows, was used to calculate the external film mass transfer coefficient (ksA) and the intraparticular diffusion coefficient (kw).

5.3 Results and Discussion

5.3.1 Fatty Acids Loss in the Adsorption Process

Fatty acid composition of crude oil is given in Table 5.1. Of the twelve fatty acids found in crude catfish oil, C16:0, C18:0, C18: 1, C18: 2 were major fatty acids. Saturated fatty acid and unsaturated fatty acids content of crude catfish oil were 46.6 and 87.1 mg/g of oil, respectively.

Changes in fatty acid composition of crude oil was monitored during a five hour adsorption process and the results are given in Tables 5.2, 5.3, and 5.4. This experiment was done to see if there was any loss in fatty acids by adsorbents during the five-hour time interval of the adsorption process. Percent loss of fatty acids of crude catfish oil during five-hour adsorption process with chitosan, activated carbon, and activated earth was calculated on the basis of fatty acid composition of crude oil and the values are given in Tables 5.5, 5.6, and 5.7. Fatty acids loss (%) was observed with increased

adsorption time. However, there were fluctuations which may have been due to changes in overall fatty acids compositions. After 5 hours of adsorption, saturated fatty acids by adsorbed activated earth, activated carbon, and chitosan were 27.66, 27.66, and 26.15, respectively, while 12.58, 9.15, and 10.74 for unsaturated fatty acids, respectively.

Table 5.1. Fatty acids profiles of crude catfish visceral oil (mg/g of oil)

Fatty acids	mg/g of oil
C14:0	4.9±0.71a
C16:0	17.72 ± 4.24[a]
C16:1	14.91 ± 2.45[a]
C18:0	23.90± 3.03[a]
C18:1	26.32 ± 7.24[a]
C18:2	25.02 ± 3.04[a]
C18:3	4.50 ± 0.73[a]
C20:0	1.06 ± 0.39[a]
C20:1	9.42 ± 1.68[a]
C20:2	4.12 ± 0.73[a]
C20:4	1.83 ± 0.27[a]
C22:6	1.24 ± 0.12[a]
Saturated	46.56 ± 6.99[a]
Unsaturated	87.1± 12.13[a]
Omega-3	5.70 ± 0.68[a]

For each fatty acid, mean values with the same letters are not significantly different (P>0.05)

Table 5.2. Remaining fatty acid during 1-5 hr adsorption using chitosan

Fatty Acids (mg/g of oil)	Adsorption time (hr) 1	2	3	4	5
C14:0	4.37 ± 0.72^{ab}	4.30 ± 0.50^{ab}	4.20 ± 0.32^{ab}	3.77 ± 0.68^{ab}	4.34 ± 0.76^{b}
C16:0	17.69 ± 4.48^{a}	15.20 ± 1.69^{a}	16.41 ± 1.66^{a}	15.21 ± 2.63^{a}	14.16 ± 2.35^{a}
C16:1	14.53 ± 2.50^{a}	14.89 ± 2.19^{a}	14.28 ± 1.88^{a}	13.18 ± 1.41^{a}	13.15 ± 0.45^{a}
C18:0	16.73 ± 1.57^{b}	16.52 ± 1.53^{b}	16.36 ± 2.8^{b}	16.00 ± 3.23^{b}	14.62 ± 1.76^{b}
C18:1	25.38 ± 3.94^{a}	25.85 ± 3.36^{a}	25.49 ± 2.45^{a}	24.40 ± 2.32^{a}	24.23 ± 3.51^{a}
C18:2	24.35 ± 2.13^{a}	24.05 ± 2.00^{a}	23.41 ± 2.16^{a}	23.13 ± 4.45^{a}	21.71 ± 3.67^{a}
C18:3	4.14 ± 0.39^{a}	4.08 ± 0.58^{a}	4.00 ± 0.27^{a}	3.90 ± 0.75^{a}	3.89 ± 0.51^{a}
C20:0	1.06 ± 0.17^{a}	1.04 ± 0.16^{a}	1.06 ± 0.19^{a}	1.04 ± 0.18^{a}	1.09 ± 0.04^{a}
C20:1	8.88 ± 0.94^{a}	8.55 ± 0.51^{a}	8.42 ± 0.83^{a}	8.39 ± 3.27^{a}	8.49 ± 0.26^{b}
C20:2	4.08 ± 0.50^{a}	3.80 ± 0.98^{a}	3.65 ± 1.29^{a}	3.63 ± 0.31^{a}	3.59 ± 1.21^{a}
C20:4	1.81 ± 0.49^{a}	1.65 ± 0.43^{a}	1.57 ± 0.55^{a}	1.51 ± 0.34^{a}	1.46 ± 0.30^{b}
C22:6	1.21 ± 0.17^{a}	1.21 ± 0.13^{a}	1.22 ± 0.10^{a}	1.23 ± 0.28^{a}	1.23 ± 0.09^{a}
Sat	39.85 ± 4.91^{b}	37.05 ± 2.22^{b}	38.04 ± 3.44^{b}	36.02 ± 2.52^{b}	34.38 ± 3.74^{b}
Unsat	84.39 ± 7.58^{a}	84.08 ± 5.12^{a}	85.05 ± 5.41^{a}	79.36 ± 7.03^{a}	77.74 ± 4.74^{a}
Omega-3	5.36 ± 0.47^{a}	5.29 ± 0.62^{a}	5.22 ± 0.19^{a}	5.13 ± 0.70^{a}	5.11 ± 0.51^{a}

For each fatty acid, mean values with the same letters are not significantly different (P>0.05)

Table 5.3. Remaining fatty acid during 1-5 hr adsorption using activated carbon

Fatty Acids (mg/g of oil)	Adsorption time (hr) 1	2	3	4	5
C14:0	4.02± 0.60^{b}	3.97 ± 0.46^{b}	3.89 ± 0.36^{b}	3.82 ± 0.59^{b}	3.79 ± 0.33^{b}
C16:0	15.75 ± 1.15^{a}	14.84 ± 2.75^{a}	14.23 ± 2.45^{a}	14.16 ± 1.63^{a}	14.00 ± 0.94^{a}
C16:1	14.04 ± 0.88^{a}	13.77 ± 1.42^{ab}	12.94 ± 0.86^{ab}	12.85 ± 0.72^{b}	12.84 ± 1.23^{b}
C18:0	16.89± 2.20^{a}	15.56± 3.11^{b}	15.35 ± 2.15^{b}	14.71 ± 1.84^{b}	14.62 ± 1.76^{b}
C18:1	25.85 ± 4.85^{a}	25.48 ± 2.91^{a}	25.15 ± 1.24^{a}	24.84 ± 1.84^{a}	24.67 ± 2.60^{a}
C18:2	24.92 ± 2.33^{a}	24.49 ± 2.97^{a}	24.15 ± 2.51^{a}	23.98 ± 2.91^{a}	23.93 ± 2.63^{a}
C18:3	4.04 ± 1.03^{a}	3.93 ± 0.70^{a}	3.90 ± 0.56^{a}	3.87 ± 0.63^{a}	3.85 ± 0.71^{a}
C20:0	0.99 ± 0.08^{a}	0.98 ± 0.18^{a}	0.96 ± 0.19^{a}	0.95 ± 0.07^{a}	0.93± 0.15^{a}
C20:1	8.22 ± 0.83^{a}	8.13 ± 0.55^{ab}	7.99 ± 0.43^{b}	7.89 ± 0.60^{b}	7.68 ± 0.44^{b}
C20:2	3.40 ± 0.37^{a}	3.30 ± 0.54^{ab}	3.20 ± 0.75^{ab}	3.17 ± 0.72^{b}	3.17 ± 0.68^{b}
C20:4	1.82 ± 0.10^{a}	1.80 ± 0.24^{a}	1.77 ± 0.29^{a}	1.77 ± 0.27^{a}	1.77 ± 0.28^{a}
C22:6	1.23 ± 0.12^{a}	1.23 ± 0.19^{a}	1.24 ± 0.08^{a}	1.23 ± 0.22^{a}	1.22 ± 0.19^{a}
Sat	37.65 ± 2.51^{b}	35.36 ± 3.36^{b}	34.43 ± 2.70^{b}	33.64 ± 3.13^{b}	33.68 ± 2.09^{b}
Unsat	83.51± 6.33^{b}	82.14± 4.38^{a}	80.34 ± 4.96^{a}	79.60 ± 4.20^{a}	79.13 ± 5.19^{a}
Omega-3	5.27 ± 1.09^{a}	5.16 ± 0.76^{a}	5.14 ± 0.55^{a}	5.09 ± 0.74^{a}	5.07 ± 0.85^{a}

For each fatty acid, mean values with the same letters are not significantly different (P>0.05)

Table 5.4. Remaining fatty acid during 1-5 hr adsorption using activated earth

Fatty Acids (mg/g of oil)	Adsorption time (h) 1	2	3	4	5
C14:0	4.85 ± 0.33[a]	3.74 ± 0.39[b]	3.61 ± 0.34[b]	3.57 ± 0.39[b]	3.47 ± 0.36[b]
C16:0	16.43 ± 4.70[a]	16.40 ± 0.55[a]	16.21 ± 0.89[a]	16.04 ± 1.06[a]	15.76 ± 0.74[a]
C16:1	12.34 ± 1.39[a]	12.29 ± 1.11[b]	11.82 ± 1.10[b]	11.77 ± 1.21[b]	11.58 ± 1.13[b]
C18:0	15.45 ± 1.19[a]	15.03 ± 1.14[b]	14.96 ± 0.91[b]	14.46 ± 1.01[b]	13.63 ± 1.19[b]
C18:1	25.99 ± 2.75[a]	25.68 ± 2.44[a]	25.12 ± 1.48[a]	25.05 ± 2.78[a]	25.00 ± 2.42[a]
C18:2	24.93 ± 2.59[a]	24.72 ± 2.70[a]	24.61 ± 2.34[a]	24.52 ± 3.65[a]	23.59 ± 3.59[a]
C18:3	3.68 ± 0.19[b]	3.50 ± 0.54[b]	3.49 ± 0.59[b]	3.35 ± 0.40[b]	3.24 ± 0.34[b]
C20:0	0.89 ± 0.12[a]	0.87 ± 0.15[a]	0.84 ± 0.16[a]	0.83 ± 0.16[a]	0.82 ± 0.17[a]
C20:1	6.73 ± 0.77[b]	6.72 ± 0.45[b]	6.71 ± 0.32[b]	6.66 ± 0.65[b]	6.66 ± 0.46[b]
C20:2	3.40 ± 0.39[b]	3.25 ± 0.22[b]	3.23 ± 0.21[b]	3.20 ± 0.37[b]	3.16 ± 0.21[b]
C20:4	1.78 ± 0.20[a]	1.78 ± 0.10[a]	1.77 ± 0.18[a]	1.76 ± 0.22[a]	1.75 ± 0.10[a]
C22:6	1.24 ± 0.13[a]	1.22 ± 0.11[a]	1.22 ± 0.11[a]	1.21 ± 0.18[a]	1.16 ± 0.34[a]
Sat	37.61 ± 4.93[b]	36.05 ± 1.36[b]	36.05 ± 1.36[b]	34.90 ± 1.21[b]	33.68 ± 1.72[b]
Unsat	80.09 ± 4.89[a]	79.16 ± 4.03[ab]	79.16 ± 4.03[ab]	77.53 ± 5.44[b]	76.14 ± 6.25[b]
Omega-3	4.92 ± 0.19[a]	4.73 ± 0.57[ab]	4.73 ± 0.57[b]	4.56 ± 0.49[b]	4.40 ± 0.63[b]

For each fatty acid, mean values with the same letters are not significantly different (P>0.05)

Table 5.5 Fatty acids (%) adsorbed by chitosan

	Adsorption time (hr)				
Fatty acids	1	2	3	4	5
C14:0	10.81	12.24	14.28	23.06	11.42
C16:0	0.16	14.22	7.39	14.16	20.09
C16:1	2.58	0.13	4.22	11.60	11.80
C18:0	30.00	30.87	31.54	33.05	38.82
C18:1	3.57	1.78	3.15	7.29	7.94
C18:2	2.67	3.87	6.43	7.55	13.22
C18:3	8.00	9.33	11.11	13.33	13.55
C20:0	0.00	1.88	0.00	1.88	0.00
C20:1	5.73	9.23	10.61	10.93	9.87
C20:2	0.97	7.76	11.40	11.89	12.86
C20:4	1.09	9.83	14.20	17.48	20.21
C22:6	2.419	2.419	1.61	0.80	0.80
Sat	14.41	20.42	18.29	22.63	26.15
Unsat	3.11	3.46	2.35	8.88	10.74
Omega-3	5.96	7.19	8.42	10	10.35

Table 5.6. Fatty acids (%) adsorbed by activated carbon

	Adsorption time (hr)				
Fatty acids	1	2	3	4	5
C14:0	17.95	18.97	20.61	22.04	22.65
C16:0	11.11	16.25	19.69	20.09	20.93
C16:1	5.84	7.65	13.21	13.82	13.88
C18:0	29.33	34.89	35.77	38.45	38.82
C18:1	1.79	3.191	4.45	5.62	6.26
C18:2	0.40	2.11	3.48	4.16	4.35
C18:3	10.22	12.66	13.33	14	14.44
C20:0	6.60	7.55	9.43	10.38	12.30
C20:1	12.73	13.69	15.18	16.24	18.47
C20:2	17.47	19.90	22.33	23.06	23.05
C20:4	0.55	1.64	3.28	3.28	3.28
C22:6	0.80	0.81	0	0.81	1.61
Sat	19.13	24.05	26.05	27.74	27.66
Unsat	4.12	5.69	7.76	8.61	9.15
Omega-3	7.54	9.47	9.82	10.70	11.05

Table 5.7. Fatty acids (%) adsorbed by activated earth

	Adsorption time (hr)				
	1	2	3	4	5
C14:0	1.02	23.67	26.32	27.14	29.18
C16:0	7.27	7.44	8.52	9.48	11.06
C16:1	17.23	17.57	20.72	21.05	22.33
C18:0	35.35	37.11	37.40	39.49	42.97
C18:1	1.25	2.43	4.55	4.82	5.01
C18:2	0.36	1.19	1.63	1.99	5.71
C18:3	18.22	22.22	22.44	25.55	28.0
C20:0	16.03	17.92	20.75	21.69	22.64
C20:1	28.55	28.66	28.76	29.29	29.29
C20:2	17.47	21.11	21.60	22.33	23.30
C20:4	2.73	2.73	3.27	3.82	4.37
C22:6	0.00	1.61	1.61	2.41	6.45
Sat	19.22	22.57	22.57	25.04	27.66
Unsat	8.05	9.11	9.11	10.98	12.58
Omega-3	13.68	17.01	17.01	20.0	22.80

A larger quantity (42.97%) of steric acid was adsorbed at a 5-hour interval by activated earth. About 39% steric acid was adsorbed by chitosan or activated carbon. Among polyunsaturated fatty acids, the highest loss was observed for C20:4 when chitosan was used as an adsorbent. About 28-29% of C18:3 and C20:1 was adsorbed by activated earth after 5 hours. Loss of combined omega-3 fatty acids (C18:3 and C22:6) during the five hour adsorption was 10.35%, 11.05% and 22.80, respectively by chitosan, activated carbon, and activated earth.

The differences in the fatty acids adsorption abilities of chitosan, activated carbon, and activated earth may be related to their effects on hydrogen bonding (Brown and Snyder, 1985; Hau and Nawar, 1985), competition for adsorption sites (Morgan et al., 1985), electrostatic field strength and intraparticles diffusion of molecules (Taylor and Ungermann, 1984), and hydrophobic interaction (Ruthven, 1984). C12 to C18 were more hydrophobic because of their longer carbon chain length as well as less polar character of the –COOH functional group (Toro-Vazquez and Rocha-Uribe, 1993).

5.3.2 Adsorption-Kinetics and Mass Transfer of Free Fatty Acids (FFA)

Figure 5.1 shows the effect of the contact (adsorption) time and the type of adsorbents (activated carbon, chitosan, and activated earth) on FFA. The FFA content adsorbed by adsorbents increased with the contact time. Chitosan adsorbed more FFA from crude catfish oil compared to activated carbon and activated earth (Figure 5.1). The calculated kinetic parameters are given in Table 5. 8. For FFA, the initial kinetic adsorption coefficient (c) was higher for chitosan than activated carbon and activated earth. Initial adsorption kinetic coefficient for chitosan was 0.084 $mlmg^{-1}min^{-1}$; 0.063 $mlmg^{-1}min^{-1}$ for activated carbon; and 0.025 $mlmg^{-1}min^{-1}$ for activated earth. Adsorption

capacities at saturation (q_s) confirmed the kinetic results in that chitosan was a better adsorbent than activated carbon and activated earth (Table 5. 8). Adsorption capacities at saturated (q_s) of FFA was 2.57 (mgg^{-1}) for chitosan and it was 2.37 (mgg^{-1}) for activated carbon and 2.07 (mgg^{-1}) for activated earth. The external coefficients (*ksA*) of chitosan were higher than that of activated carbon and activated earth. The *ksA* (mls^{-1}) was 0.068, 0.055, and 0.041 for chitosan, activated carbon, and activated earth, respectively. Intraparticular diffusion coefficients (k_w) were 0.006, 0.005 and 0.004 ($mgml^{-1}min^{-0.5}$) for chitosan, activated carbon and activated earth, respectively.

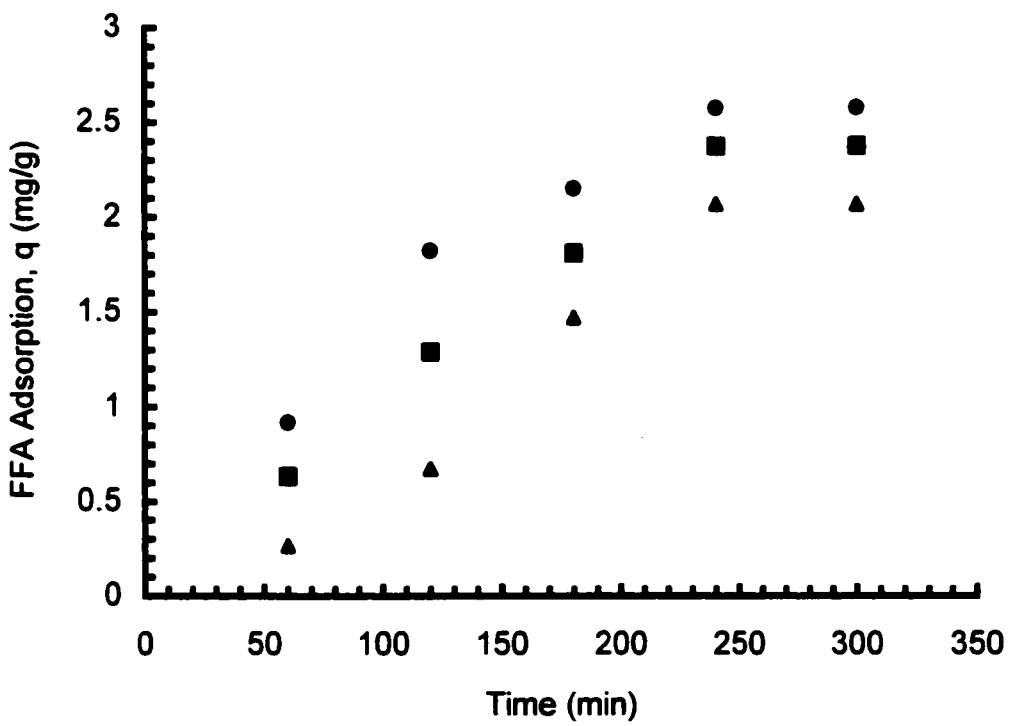

Figure 5.1. Free fatty acids adsorbed per g of adsobent (Chitosan •; Activated carbon, ■; Activated earth, ▲) was determined at different time intervals

Higher values of *c* and k_w for chitosan may be due to the faster intraparticular diffusion of free fatty acid through chitosan porosity network. We can represent the

experimental results by means of intrapartical diffusion coefficient diffusion model, i.e., $q = (Co-C)V/m$ as a function of $t^{0.5}$ (Morris and Weber, 1962; Mckay et al., 1980). The curves obtained with chitosan, activated carbon, and activated earth are given in Figure 5.2. The k_w can be also obtained from the slope the curves and this relationship allowed the possibility of describing the adsorption abilities of adsorbents on free fatty acids at different time intervals.

Table 5.8. FFA Adsorption kinetic parameters as affected by the types of adsorbents at 25°C

	ksA (mls^{-1})	k_w ($mgml^{-1}min^{-0.5}$)	q_s (mgg^{-1})	c ($mlmg^{-1}min^{-1}$)	kd (mlg^{-1})
Chitosan	0.068	0.006	2.57	0.084	32.91
Activated carbon	0.055	0.005	2.37	0.063	27.51
Activated earth	0.041	0.004	2.07	0.025	20.95

K_sA =External film mass transfer coefficient ($mgmin^{-1}$); K_w= Intraparticular diffusion cofefficient ($mgml^{-1}min^{-0.5}$); q_s = saturation adsorption capacity (mgg^{-1}); c = Initial adsorption kinetic coefficient ($mlmg^{-1}min$); *kd*=partition coefficient

The partition coefficient, *kd*, for chitosan was higher than activated carbon and activated earth (Table 5.8). The *kd* (mlg^{-1}) of chitosan was 32.91, 27.51 for activated carbon, 20.95 for activated earth. According to the definition of partition coefficients, a higher value of *kd* indicated a greater amount of FFA absorbed per gram chitosan.

Table 5.9 shows the relationship between water activity and contact time. Initial water activity of crude oil was 0.857 and it reached 0.668, 0.712 and 0.786 for chitosan, activated carbon, and activated earth, respectively, after 5 hours of adsorption.

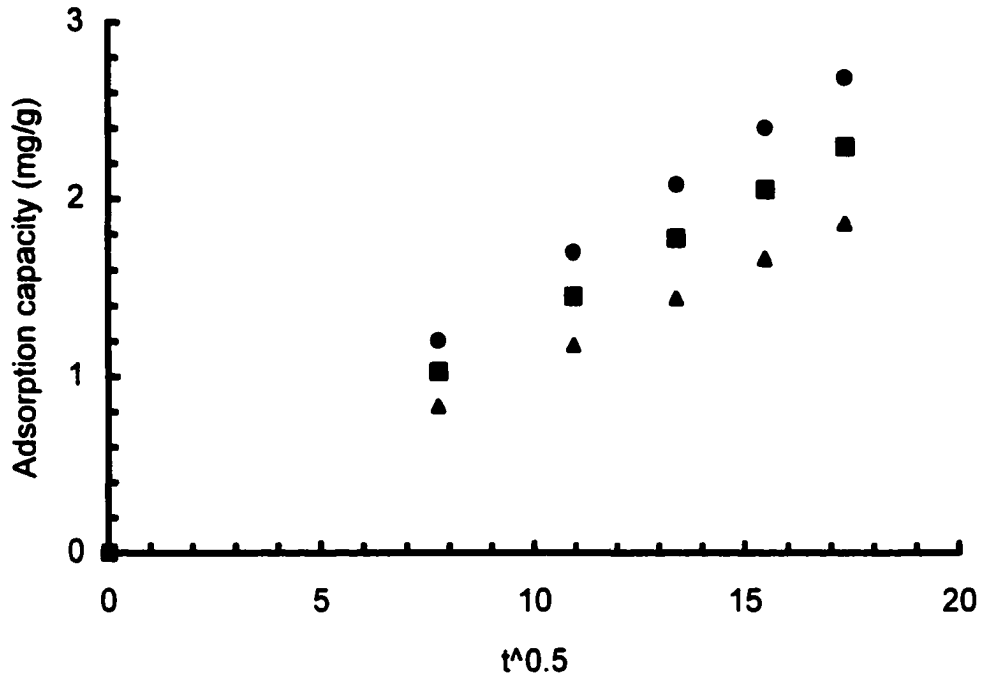

Figure 5.2 Adsorption capacity at $t^{0.5}$ Chitosan, •; Activated carbon, ■; Activated earth, ▲)

The overall result (Table 5.8 and 5.9) indicated that chitosan has a greater ability for adsorbing free fatty acid in crude catfish oil than the other two adsorbents.

Table 5.9. Water activity of residual oil during the adsorption periods

	Adsorption time (h)					
Adsorbents	0	1	2	3	4	5
Chitosan	0.857	0.767	0.705	0.680	0.679	0.668
Activate carbon	0.857	0.827	0.750	0.7363	0.717	0.712
Activated earth	0.857	0.847	0.838	0.831	0.786	0.786

Chitosan is a biopolymer, and its solid state exhibits complex network (Capitani et al., 2001). The network and brittle nature of chitosan makes it readily disperse in liquid (Piron et al., 1997). Therefore, dispersion of chitosan in catfish crude oil might have provided more surface area to adsorb greater amount of FFA than the other two adsorbents. Free doublets of chitosans nitrogen atoms were an excellent functional group for adsorbing impurities (Wan Ngah and Liang, 1999).

5.4 Conclusion

Loss of fatty acids generally increased with increased adsorption (contact) time. Combined omega-3 fatty acids (C18:3 and C22:6) were adsorbed up to 10.35%, 11.05%, and 22.8%, respectively, by chitosan, activated carbon, and activated earth after 5 hours. The FFA content adsorbed by adsorbents increased with increased contact time. Chitosan was more efficient in adsorbing FFA from crude catfish oil than actiavted carbon and activated earth. This study indicates that it is possible to use chitosan as an adsorbent for removal of FFA from crude catfish oil.

Nomenclature

q	Adsorption capacity (mgg^{-1})
c	Initial adsorption kinetic coefficient ($mlmg^{-1}min$)
KsA	External film mass transfer coefficient ($mgmin^{-1}$)
K_w	Intraparticular diffusion cofefficient ($mgml^{-1}min^{-0.5}$)
Kd	Partition coefficient (mlg^{-1})
C	Concentration of free fatty acids at time t
C_o	Initial concentration of free fatty acid

CHAPTER 6. REMOVAL OF FREE FATTY ACIDS AND OXIDIZED COMPONENTS BY ADSORBENTS THROUGH A FIXED BED COLUMN

6.1 Introduction

In general, edible crude oil contains free fatty acids, carotenoids, phospholipids, and peroxides which must be removed during oil refining processes to produce oil with acceptable shelf life and quality. The longer these components remain in the oil, the greater their negative effect on oil quality. Conventional oil refining is achieved through the following major steps: degumming, neutralization, bleaching, and deodorization. Bleaching is normally designed for pigment removal during oil refining. However, a number of studies showed that bleaching not only removes colored components, but also adsorb the impurities such as free fatty acids, oxidized products, and minerals from the oil (Toro-Vazquez, 1991; Toro-Vazquez and Rocha-Uribe, 1993; Henderdon, 1993; Boki et al., 1990; Brown and Synder, 1989; Nkpa et al., 1989; Palaniappan and Proctor, 1991; Proctor and Palaniappan, 1990; Mingyu and Proctor, 1993; Toro-Vazquez and Mendez-Montealvo, 1995; Riberio et al., 2001; Farook and Ravendran, 2000).

Activated earth is widely used in edible oil refining industries to remove pigments and impurities (Clark and Proctor, 1993; Proctor and Toro-Vazquez,1996). Activated carbon and chitosan may be alternative because these materials are stable at high temperature, have good adsorption capacities and humid conditions, and are not flammable. The physical and chemical characteristics of chitosan under activated carbon have been extensively investigated. They may serve as good adsorbents for effectively removing FFA. Chitosan is a biopolymer of linked β, (1→4), 2-amino-2-deoxy-D-glucose and 2-acetamidodeoxy-D-glucan. Chitosan bears hydroxyl and amino groups, which are excellent functional groups for the adsorption of a variety of

organometallic complex (Wan-Ngah and Liang, 1999; Piron et al., 1997; Peniche-Covas et al, 1992; Quignard et al., 2000; and Juang and Ju, 1998). Activated carbon is a very mature technology that is designed to help remove organic pollutants and odor that cause problem (Ahmedna et al., 1997; Bernardo et al., 1997; Migo et al., 1993; Castro et al., 2000). The carbons have nonpolar surfaces that are used to adsorb nonpolar molecules, especially hydrocarbons. They are manufactured from nut shells and rice hulls, among other agricultural wastes (Dai and Antal, 1999).

Proper design, operation of a fixed bed adsorption process, fixed-bed dynamics, and a breakthrough curve for removal of impurities of catfish oil have not been studied. Various mass-transfer models (Ruthven, 1984; Chatzopoulous and Varma, 1995; Wolborska et al., 1996; Levan et al., 1997; Slaney and Bhamidimarri, 1998; Wolborska, 1999) have been used to study the breakthrough curves for various pollutants. However, there is no such breakthrough curve study on removal of impurities of catfish oil in a fixed bed condition. Therefore, the objectives of this study were to determine the breakthrough curves of chitosan and activated carbon fixed beds for removal of impurities of catfish oil and to determine the optimum length of fixed bed column.

6.2 Theory

6.2.1 Breakthrough Curve

Breakthrough curve is determined by calculating the concentration versus time for fluid leaving the bed. When the concentration reaches some limiting permissible value, or break point, the flow is stopped or diverted to a fresh adsorbent bed. The break point is often taken as a relative concentration (C/Co) of 0.05. The following assumptions must be considered before determining the breakthrough curve and bed length of adsorbent:

(1) no chemical reactions occur in the column, (2) only mass transfer by convection is significant, (3) radial and axial dispersions are negligible, (4) the flow pattern is ideal plug flow, (5) the temperature in the column is uniform and invariant with time, (6) the flow rate is constant and invariant with the column position (Shearwood et al., 1975).

6.2.2 Concentration Patterns in Fixed Beds

For fixed-bed adsorption, the concentration in the fluid phase and the solid phase change with time and length of bed. At first, most of the mass transfer takes place near the inlet of the bed, where the fluid first contacts the adsorbent. After a few minutes, adsorbent near the inlet is nearly saturated, and most of the mass transfer takes place further from the inlet. The concentration gradient becomes S shaped. The region where most of the change in concentration occurs is called the mass-transfer zone (McCabe et al., 1993).

6.2.3 Ideal Adsorption Time

For a symmetrical curve, t^* is the time when C/Co reaches to 0.05. The amount of the adsorption through the bed and the effect of process variables on t^* can be obtained by a simple material balance.

For an ideal bed cross section, the solute feed rate (F_A) is the product of the superficial velocity Uo and the concentration (Co):

$$F_A = UoCo \qquad (1)$$

For an ideal break through curve, all the solute fed at time t^* is adsorbed, and the concentration on the solid phase has increased from the initial value (initial adsorbate loadin, *Wo*) to the equilibrium or saturation value (saturated adsorbate loading, *Wsat*).

$$UoCot^* = L\rho_b(Wst - Wo) \qquad (2)$$

where L and ρ_b are the length and bulk density of the bed, respectively. For fresh adsorbent, Wo =0.

In general, t^* is calculated using the following equation:

$$t^* = \int_0^{\infty} \left(1 - \frac{C}{Co}\right) dt \qquad (3)$$

The break point time (t_b) is always less than t^*, and the actual amount of solute adsorbed at the break point can be determined from the breakthrough curve up to the time t_b, as shown in equation (4).

$$t_b = \int_0^{t_b} \left(1 - \frac{C}{Co}\right) dt \qquad (4)$$

The length of unused bed from the breakthrough curve is determined by following equations:

$$Wsat = \frac{F_A t^*}{LP_b} \qquad (5)$$

$$Wb = \frac{F_A t_b}{LP_b} \qquad (6)$$

$$LUB = \frac{Wb}{Wsat} \qquad (7)$$

$$LNB = 1 - LUB \qquad (8)$$

where Wb is adsorbate loading at break point, LUB is length of used bed, and LNB is length of unused bed.

6.3 Materials and Method

6.3.1 Crude Catfish Oil

Catfish viscera was obtained in three batches (experimental replications) from the local fish processing plant in Baton Rouge, LA. The viscera was frozen at - 20°C until used. The slightly thawed viscera was finely ground in a Hobart Chopper Bowl (84181D) at 3450 rpm for 10 minutes. Water was added (water: ground viscear, 5:1 V/W) and the mixture was heated at 70°C for 15 minutes. The purpose of the heating step was to coagulate the protein of the catfish viscera, so that liquids and solids can be mechanically separated. During mild heating, fat cells are ruptured, releasing the oil into the liquid phase. The denatured proteins were separated from the liquid phase (oil and soluble particles) by filtration through cheesecloth and the cooked viscera were pressed manually to remove most of the liquid. The crude oil was separated from the water phase and other fish particles by centrifugation at 5,000 rpm for 30 minutes. Separated crude oil was stored at – 20°C until used. Three experimental batches were conducted

6.3.2 Adsorbents and Adsorbate

The activated bleaching earth (the American Oil Chemistry Society, Champaign, IL) and the crab chitosan with 80% degree of deacetylation (Vanson Inc, Redmond, WA) were used in this study. The CO_2 activated pecan shell carbon was supplied by USDA. Activated carbon was pretreated as explained by Riberio et al. (1995).

6.3.3 Experimental Fixed-bed Apparatus

Experimental apparatus used to measure the adsorption of crude catfish oil in a fixed-bed column is shown as Figure 6.1. It consisted of a fixed-bed adsorption column

made of glass. The internal diameter of column was 2.8 cm and the column length was 45 cm .

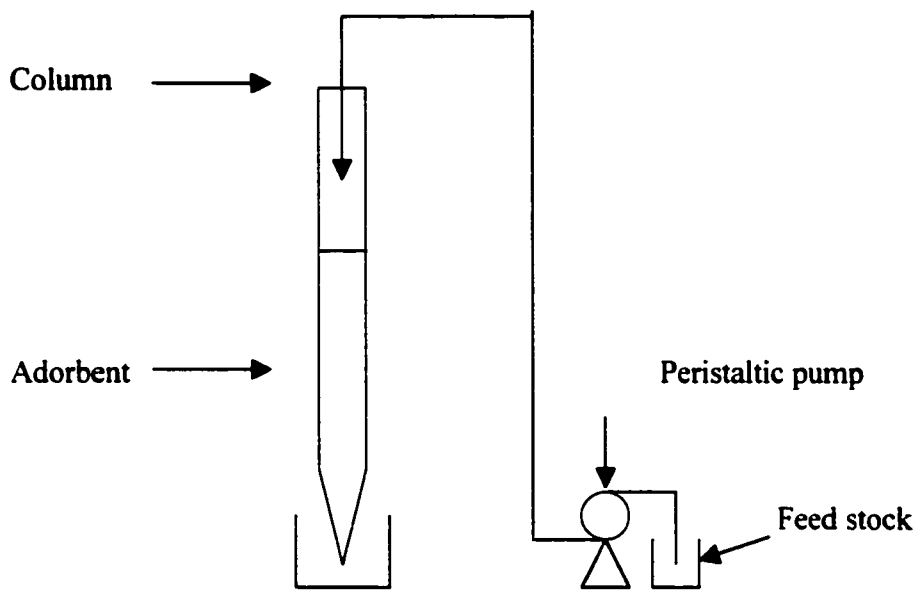

Figure 6.1 Experimental column

The column was attached to a peristaltic pump for catfish oil feeding. The column was packed with adsorbent (chitosan, activated earth and activated carbon) up to 17 cm. Crude catfish oil was fed to the fixed-bed column at a rate of 21.93g/h by means of the peristaltic pump. Discharged oil was collected every hour and the free fatty acid and peroxide values were analyzed. Experiments were repeated three times and average values were reported.

6.3.4 Free Fatty Acids (FFA) Analysis

FFA of crude oil and those collected after discharge from the column were analyzed according to the AOAC official method 940.28 (1990) and reported as mg of oleic/g of oil.

6.3.5 Peroxide Value

Peroxide values (PVs) of crude oil and those collected after discharged from the column were analyzed according to the AOAC official method 965.33 (1990). The PV values were reported as milliequivalent /kg of oil.

6.4 Results and Discussion

6.4.1 Breakthrough Curve for FFA

The activated earth bed did not allow crude oil to discharge even after 64 hour, and therefore, excluded from this experiments. Adsorbent parameters are given in Table 6.1. Feed rate of oil was 21.93 g/h. Flow rate of FFA through cross sectional area was 0.016 (g of FFA/cm^2.h). Crude catfish oil required approximately 3 h and 2 h to run through the 17 cm column packed with chitosan and activated carbon, respectively. Initial free fatty acid value of catfish oil was 4.54 mg oleic/g of oil.

Each fixed bed column was run till it reached C/Co=1 for free fatty acid. The C/Co ratio for free fatty acids reached 1.0 at 8 h and 7 h for chitosan and activated carbon was used as an adsorbent, respectively (Figures 6.2 and 6.3). Ideal adsorption time for a vertical breakthrough curve (t^*), where C/Co=0.5 was at 7.48 h for chitosan and 5.1 h for activated carbon. Break point time, *tb*, attained at C/Co =0.05, was at 5.93 h for chitosan and 2.01 h for activated carbon (Table 6.2).

The curves shown in the Figures 6.2 and 6.3 are breakthrough curves. The area between the curve and a line at C/Co =1.0 was proportional to the total solute adsorbed. The mass-transfer zone for chitosan was narrow relative to the bed length and the break through curve was steeper than that for activated carbon (Figure 6. 2).

Table 6.1 Adsorbent parameters

Parameters	Values
Column internal diameter (cm)	2.8
Column length (cm)	17
Oil feed rate (g/h)	21.93
Initial FFA value mg oleic/g of acid	4.54
Flow rate of FFA per cross sectional area (g of FFF/cm^2.h)	0.016

Table 6.2 Experimental parameters for breakthrough curves

Adsorbent	Bed density (g/cm^3)	t^* (h)	t_b (h)	*Wsat* (g FFA/g adsorbent)	*Wb* (g FFA/g adsorbent)	*Wb/Wsat*
Chitosan	0.024	7.48	5.93	0.295	0.234	0.79
Activated carbon	0.079	5.1	2.01	0.061	0.024	0.39

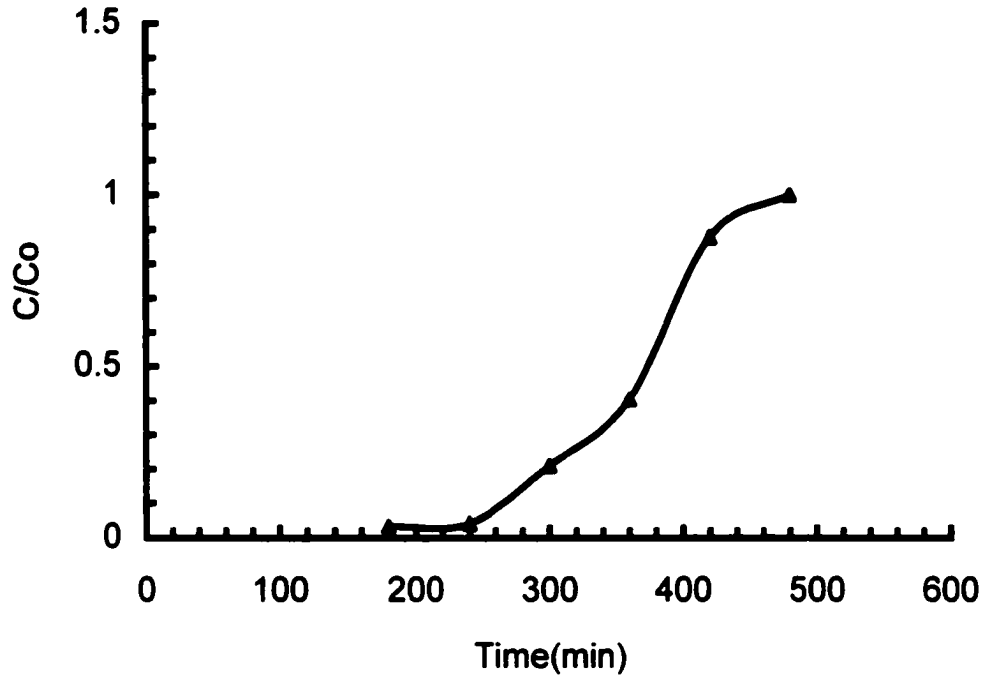

Figure 6.2 Breakthrough curve for FFA for a fixed-bed chitosan column

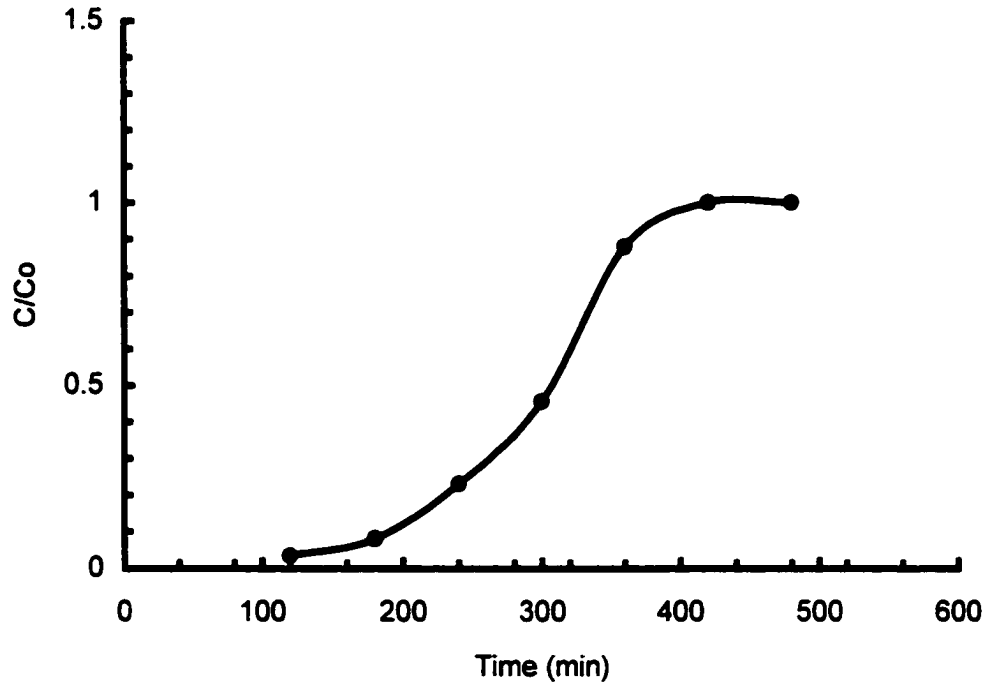

Figure 6.3 Breakthrough curve for FFA a fixed-bed activated carbon column

Figure 6.2 shows a narrow mass transfer zone and a steep breakthrough curve indicating that most of the adsorbent bed capacity was used and that chitosan is effective in adsorbing FFA from oil. Mass transfer zone for activated carbon was almost as long as the bed length and the breakthrough curve was greatly extended (Figure 6.3). Extended breakthrough curve indicated that half the adsorbent bed capacity was not used (Table 6.2). For chitosan, 79 (13.43) percent of the bed capacity was used, while 21 percent of the bed capacity was unused, which could be represented by a length of 3.6 cm of the original bed (17 cm). For activated carbon, 39 percent (6.63 cm) of the bed capacity was used, while 61 percent of the bed capacity was unused, which could be represented by a length of 10.4 cm of the original bed.

6.4.2 Adsorption Parameter for PVs

The C/Co ratio did not reach to 1.0 even after 7 h for both chitosan and activated carbon fixed columns. The initial peroxide value was 6.2 and the C/Co for chitosan at 7 h was 0.48 and 0.29 for activated carbon was (Table 6.3). This indicates that both activated carbon and chitosan were not saturated with the peroxide component of catfish even after 7-h. It means that adsorption for oxidized component in the mass transfer zone was not saturated.

The differences in the free fatty acids and oxidized products adsorption abilities of chitosan and activated carbon may be related to their effects on the hydrogen bonding (Brown and Snyder, 1985; Hau and Nawar, 1985); competition for adsorption sites (Morgan et al, 1985); electrostatic field strength, and intraparticles diffusion of molecules (Taylor and Ungermann, 1984); and hydrophobic interaction (Ruthven, 1984).

The network and brittle nature of chitosan contributes to the ability of chitosan to readily adsorb impurities from the liquid phase (Piron et al., 1997). The free doublets of its nitrogen atoms were excellent functional groups for adsorbing .

Table 6.3 Effects of adsorbents on peroxide value at different time interval (millieqiv/kg)

Time (min)	Chitosan	Activated Carbon
120	-	3.8
180	5.6	3
240	4.6	2.6
300	4.8	2.8
360	3	1.6
420	3	1.8

6.5 Conclusion

This study shows that chitosan can be used as an effective adsorbent for removal of free fatty acids (FFA) from crude catfish oil. Chitosan is also more effective than activated carbon for FFA removal of as indicated by a narrower mass transfer zone and a steeper breakthrough curve. Almost 80% of the bed capacity was utilized when chitosan was used as an adsorbent. Calculated length of unused bed from breakthrough curve and determination of total FFA fatty acids adsorbed up to the break point may information for catfish oil production industry.

CHAPTER 7. THERMAL DEGRADATION OF FATYY ACIDS AND CATFISH OIL FROM DIFFERENT PROCESSING STEPS

7.1 Introduction

From a quantitative point of view, fatty acids are the main constituents of both vegetable and animal fats, where they are mainly present as esters of glycerols. Their quantity and quantitative composition are characteristic of the different edible fats, and are responsible for the fat's bio-nutritional value. Fish oil is characterized by the presence of complex mixtures of polyunsaturated fatty acids with long carbon chains. Recently, interest in fish oil has grown rapidly, because such oil is believed to have certain beneficial effects on human health such as prevention of heart diseases (Simpoulos et al., 2000; Haglund et al., 1998; Herrmann, 1995; O'Keefe and Harris, 2000). Supplements of fish oil have also been linked to a decrease of arthritis (Kremer, et al., 1995). The use of fish oil is widely accepted in the pharmaceutical and food industries. Fish oil supplements contain polyunsaturated fatty acids, and they are often subjected to thermal treatments during the processing, storage, and preparation. Likewise, during processing, food oils are subjected to heat treatment. The ratio between saturated, monosaturated and polyunsaturated fatty acids in different oils makes them more or less resistant to thermal degradation. Thermogravimetry (TG) analysis is frequently used to study thermal degradation of wood, cellulose and related materials (Nguyen et al., 1981; Barl et al., 1986; Bergner and Albano, 1993; Krigstin et al., 1993;). TG has also been used to study the stability of oil (Gennaro et al., 1998) and to determine weight losses of whey powder system (Burin et al., 2000). A thermogravity analyzer is a balance which measures changes in sample weight. It monitors

continuously sample weights under a temperature program in a control atmosphere. This technique basically simulates the procedure of heating an oil in an oven at different time intervals. The purpose of this study were to understand the effects of temperature on weight loss of catfish oil from different purification steps and to study the effects of temperature on weight loss of individual fatty acids during the heating process using Thermogravimetry analysis.

7.2 Materials and Methods

7.2.1 Fatty Acids Used in the Study

Fatty acids samples were purchased from Sigma comapny, St. Louis, MO. Fatty acids, and their purities, and lot numbers are given in Table 7.1. All the unsaturated fatty acids used in this study were cis isomer.

7.2.2 Oil Samples and Sample Preparation

Catfish viscera was obtained from the local fish processing plant in Baton Rouge, LA. The viscera was frozen at - 20°C until used. The slightly thawed viscera was finely ground in a Hobart Chopper Bowl (84181D) at 3,450 rpm for 10 minutes. Water was added (water: ground viscera, 5:1 V/W) and the mixture was heated at 70°C for 15 minutes. The purpose of the heating step was to coagulate the protein of the catfish viscera, so that liquids and solids can be mechanically separated. During mild heating, fat cells are ruptured, releasing the oil into the liquid phase. The denatured proteins were separated from the liquid phase (oil and soluble particles) by filtration through cheesecloth and the cooked viscera were pressed manually to remove most of the liquid. The crude oil was separated from the water phase and other fish particles by

centrifugation at 5,000 rpm for 30 minutes. Separated crude oil was stored at – 20°C until used. Three experimental batches were conducted.

Table 7. 1. Fatty acids used in this study

Samples	Chemical Formula	Purity %	Lot number from Sigma Co.
Myristic acid	$C_{14}H_{28}O_2$	99	128H1242
Palmitic Acid	$C_{16}H_{32}O_2$	99	019H07011
Stearic Acid	$C_{18}H_{36}O_2$	99	068H5231
Arachidic Acid	$C_{20}H_{40}O_2$	99	079H1210
Palmitoleic Acid	$C_{16}H_{30}O_2$	99	087h5183
Oleic Acid	$C_{18}H_{34}O_2$	99	060K0703
Linoleic Acid	$C_{18}H_{32}O_2$	99	030K1162
Linolenic Acid	$C_{18}H_{30}O_2$	99	050K0723
cis-11,14-Eicosadienoic Acid	$C_{20}H_{36}O_2$	98	108H1076
Arachidonic Acid	$C_{20}H_{32}O_2$	90	040K1225
Cis-4,7,10,13,16,19-Docosahexaenoic Acid	$C_{22}H_{32}O_2$	98	080K5216

7.2.3 Degumming

Crude oil was removed from the storage at –20°C and 100g of the crude oil was taken for the degumming process and placed in a 600 ml beaker and heated to 70°C in a water bath. Three ml of citric acid (3%) was added to the oil, thoroughly mixed,and the

sample was heated at 70°C for one minute, then centrifuged at 5,000 rpm for 10 minutes to remove the impurities.

7.2.4 Neutralization

Sodium hydroxide solution (14 Baume; 13g NaOH; 0.25 normal NaOH) was added to 100 g of oil as explained in the AOCS method 9b-52Ca (1989) and the mixture was heated to 65°C for 30 minutes. The sample was cooled to room temperature and kept for six hours. The precipitated soap was removed by centrifugation at 5000 rpm for 10 minutes. Fifty ml of demineralized water was added to the oil to wash out remaining soaps from oil. This was repeated for three times. Remaining water was removed by centrifugation at 5,000 rpm for 10 minutes.

7.2.5 Bleaching

Neutralized oil was heated to 70°C in a water bath and was bleached with activated earth of 4% w/w (the activated bleaching earth of the American Oil Chemistry Society, Champaign, IL) at 70°C for 10 minutes. The activated earth with impurities was removed from the oil by centrifugation at 5,000 rpm for 30 minutes.

7.2.6 Deodorization

The laboratory distillation unit was used for deodorization. The unit consisted of a round bottom boiling flask with three outlets. One outlet was attached to a vacuum pump, another outlet was attached to a glass distillation column and the last outlet was sealed with a thermometer inserted. The flask was placed on a heating system. Bleached oil was added into the flask and heated to 100°C for 30 minutes under vacuum pressure at 65 cmHg. The temperature was maintained manually. The volatile products

were condensed with a circulated liquid cooling system around the distillation unit and distillate was collected.

7.2.7 Thermal Analysis

The crude oil and individual fatty acids were analyzed using the Hi-Res Modulated TGA 2950 Thermogravimetric Analyzer (TA) system equipped with a analytical balance thermobalance. Approximately 0.5-1 mg of each sample was added to a tared aluminum balance pan. The pan was placed in the furnace, which was attached to the thermobalance of TA, and the exact sample weight was determined. The sample was heated to 600°C at the rate of 5°C/min. Sample weight differences were automatically recorded by a Universal Analyzer Software and was plotted. All the graphs were normalized based on sample weight basis.

7.3 Results and Discussion

7.3.1 Weight Loss

Effects of temperature between 100°C to 500°C on weight (%) of individual fatty acids are given in Table 7.2. The thermograms for selected fatty acids C14:0 and C22:6 were presented to clearly demonstrate the effect of temperature on weight loss (Figures 7.1 and 7.2). All fatty acids had a weight loss more than 90% of their original weight at 250°C for C18:1 and C20:2. Saturated fatty acids were completely decomposed after 400°C. The resistance to temperature of fatty acids was dependent on chain length and degree of unsaturation. Weight reductions of saturated fatty acids from their original weights at 250°C for C14:0 C16:0, C18:0, C20:0 were 92.4%, 95.84%, 95.1%, and 95.07%, respectively. The intermolecular dispersion forces increased with increased

number of carbons in chains (Nawar, 1996); therefore, fatty acids with longer carbon chain that with a resisted temperature better than short chain.

Table 7.2. Remaining weight (%) of fatty acids during heating

Fatt yacids	Heating temperature (°C) 100	150	200	250	300	350	400	450	500
C14:0	99.95	97.19	45.81	7.96	5.77	0.45	0.03	-	-
C16:0	99.84	99.11	79.42	4.16	2.55	0.38	0.07	-	-
C18:0	99.91	99.51	89.65	4.90	2.62	0.36	0.09	-	--
C20:0	99.91	99.69	91.78	4.93	2.92	0.40	0.1	-	-
C16:1	99.83	98.27	74.45	1.19	0.83	0.16	0.052	0.01	-
C18:1	99.98	99.65	93.17	37.30	1.13	0.35	0.14	0.06	-
C18:2	99.95	99.27	86.76	3.97	2.65	0.96	-	-	-
C18:3	99.90	99.21	80.21	1.20	0.95	0.80	-	-	-
C20:2	99.73	96.98	79.83	21.77	15.44	10.24	7.7	2.62	-
C20:4	99.93	99.26	89.59	0.76	0.22	0.01	-	-	-
C22:6	99.83	99.18	91.12	5.22	2.19	1.64	1.12	0.50	0.40

(-not detected)

In the case of unsaturated fatty acids, the increase in the number of double bonds increased the resistance to thermal degradation especially after 350°C. Weight losses (%) at 250°C for C16:1, C18:1, C18:2, C18:3, C20:2, C20:4, and C22:4 were 98.81%, 96.27%, 96.03%, 98.8%, 78.23%, 99.24%, and 94.75%, respectively.

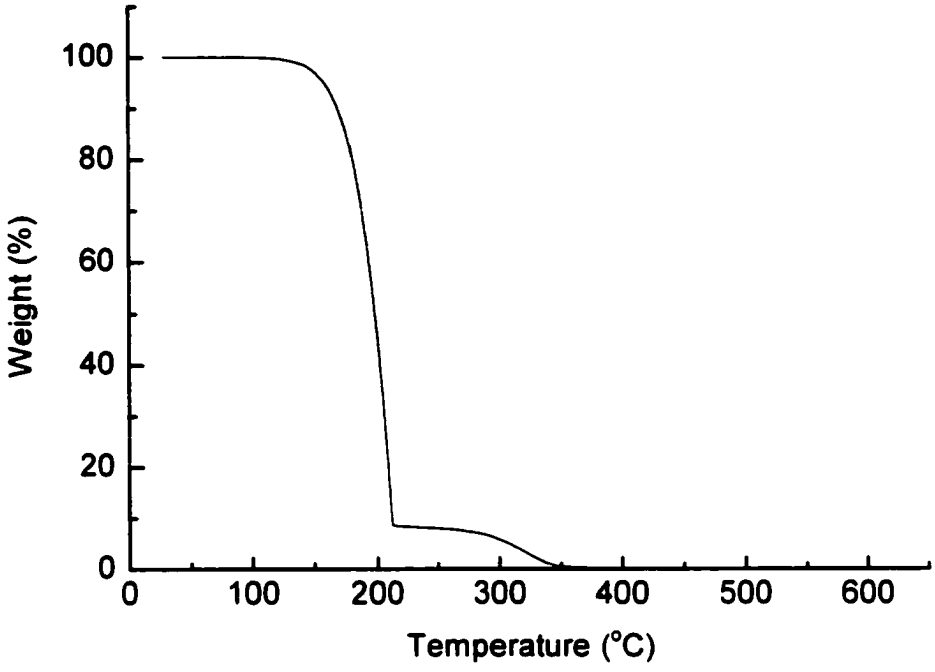

Figure 7.1. Thermogravimetric curve for a mystric acid

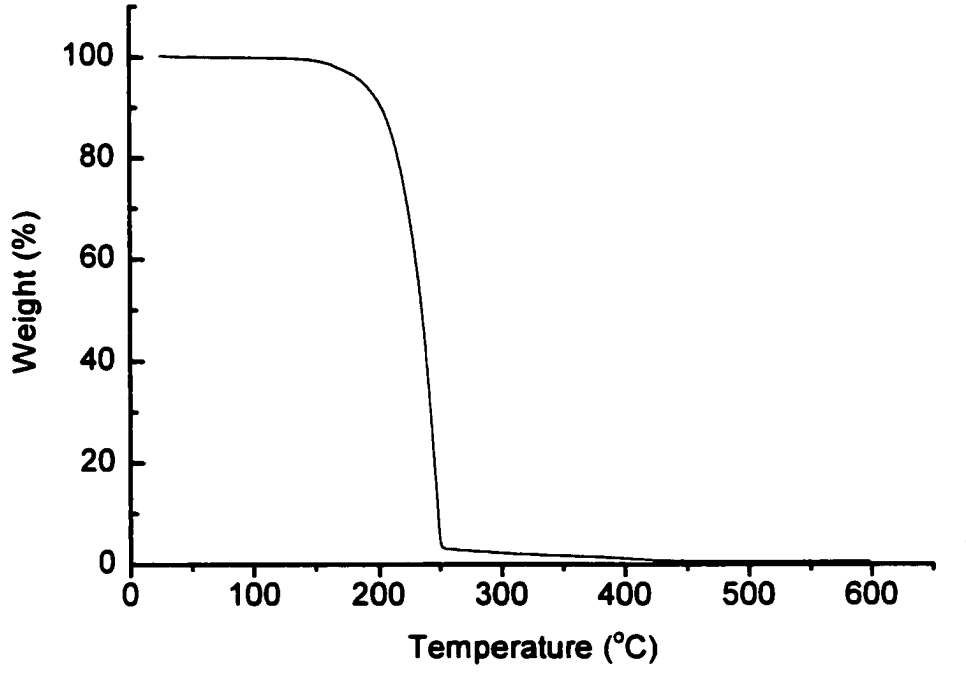

Figure 7.2. Thermogravimetric curve for a DHA

Comparing C16:1 with C16:0, more weight loss was observed for C16:1 at 250°C. Comparing C18:1 with C18:2, and C18:3, more loss was observed with increased double bonds. Highest weight loss was observed in C20:4 at 250°C. Four tenth percent weight of C22:6 still remained at 500°C.

Typical thermogravity curves for crude, degummed, neutralized, bleached, and deodorized catfish oil are shown in Figures 7.3, 7.4, 7.5, 7.6, and 7.7, respectively. Results (Table 7.3) also show weight loss of oils with increasing temperature.

Table 7.3. Remaining weight (%) of catfish oil during heating

	Heating temperature (°C)						
Process steps	200	250	300	350	400	450	500
Crude	99.34	96.30	94.63	86.92	25.12	0.54	0.40
Degummed	95.87	87.67	85.38	71.40	9.69	0.20	0.16
Neutralized	97.59	94.61	91.59	74.08	3.01	0.20	-[a]
Bleached	97.99	95.20	93.11	77.87	6.80	-[a]	-[a]
Deodorized	99.81	99.65	98.69	90.93	31.03	0.58	-[a]

[a] Not detected

Between 200-550°C, weight loss of oils increased with increased heating temperature, regardless of the processing steps. There were, however, no distinct weight losses noticed until over 350°C. At 550°C, all oil samples were decomposed. Significant weight reduction occurred at temperatures between 400°C to 450°C for all oils.

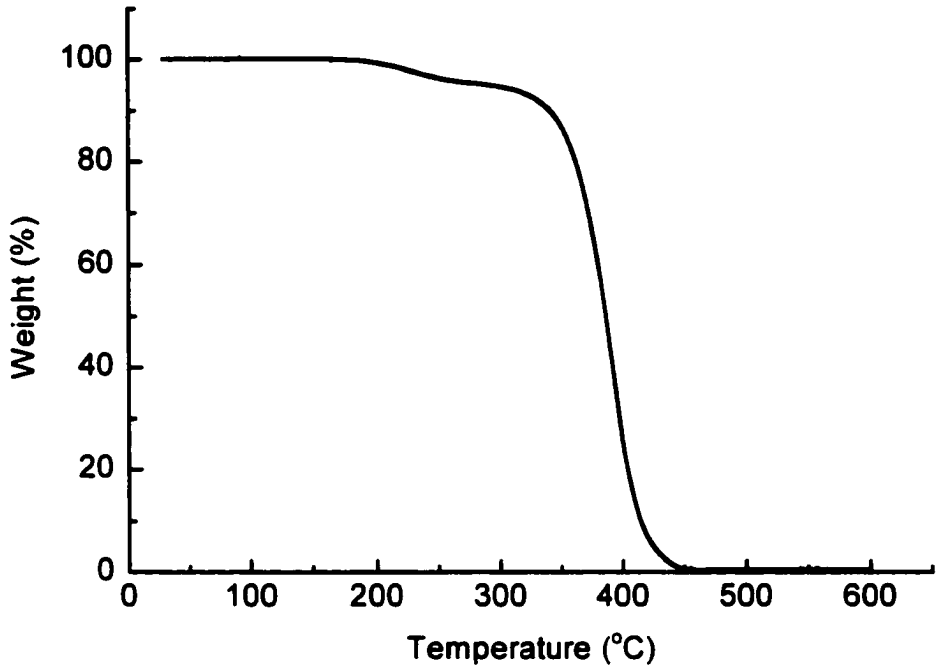

Figure 7.3 Thermogravimetric curve for crude catfish oil

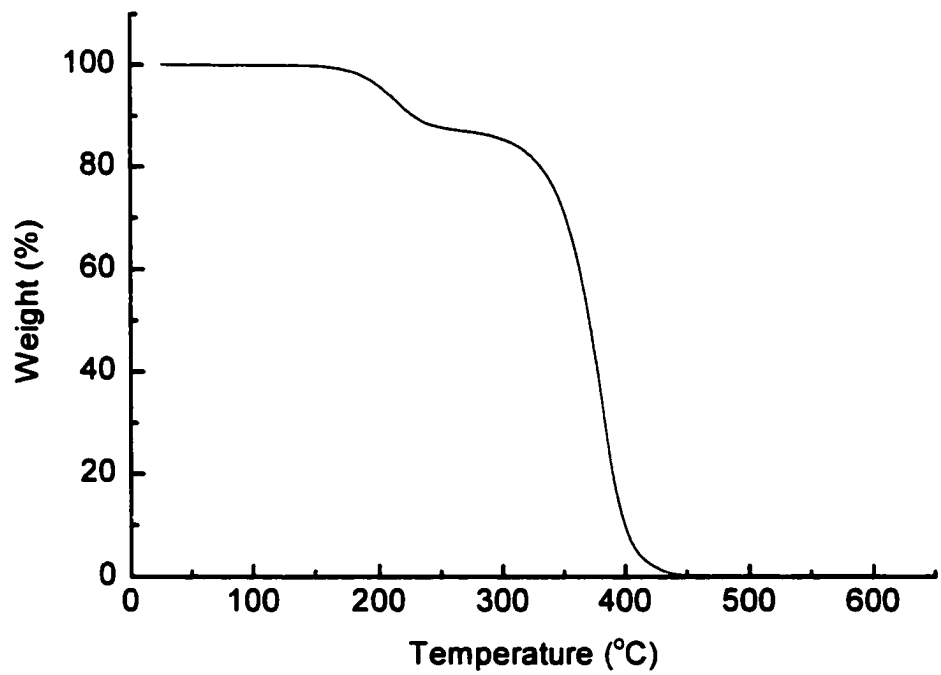

Figure 7.4 Thermogravimetric curve for degummed catfish oil

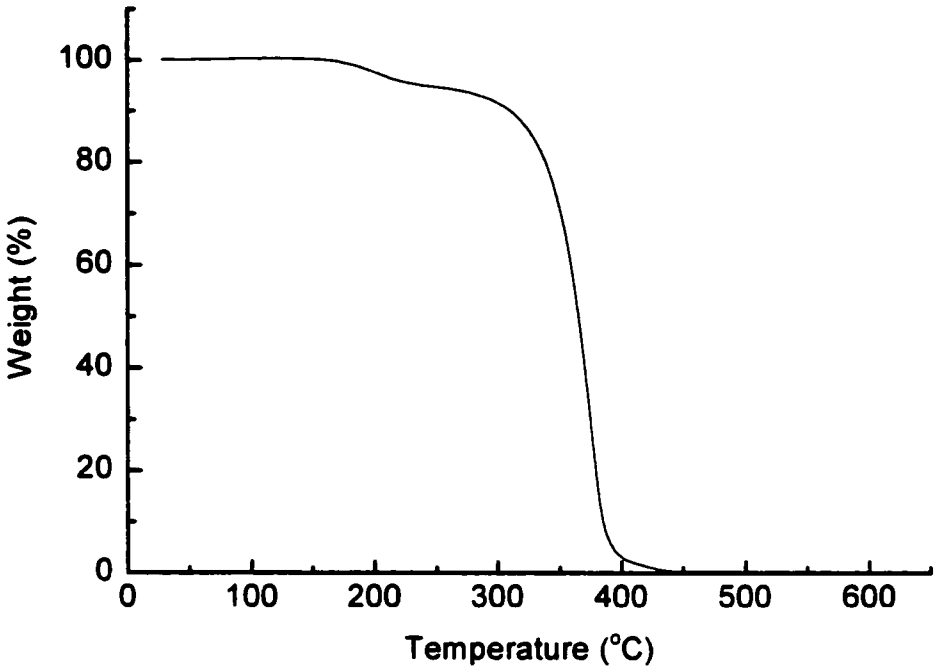

Figure 7.5 Thermogravimetric curve for neutralized oil

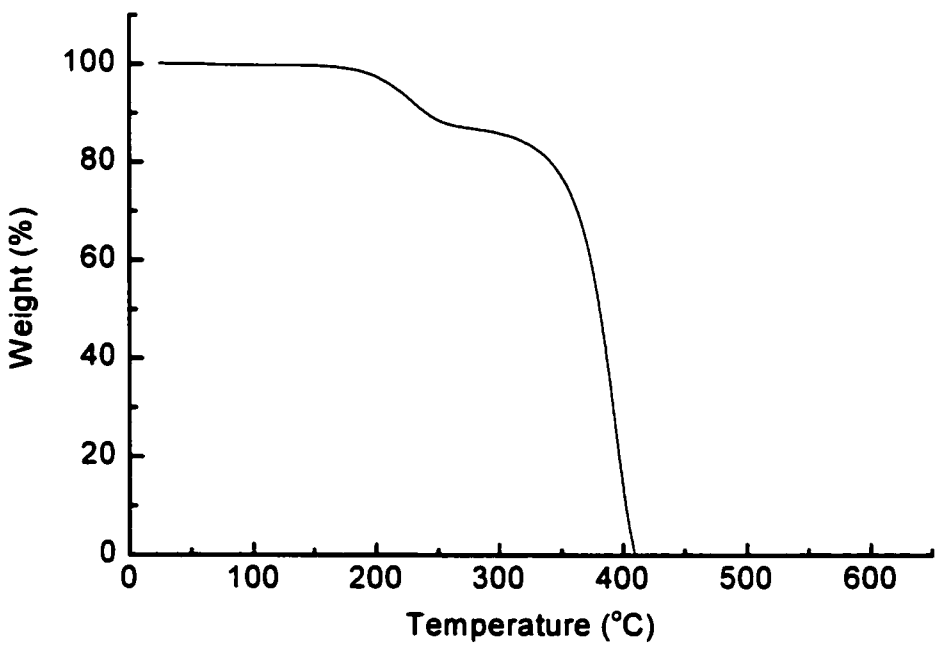

Figure 7.6 Thermogravimetric curve for bleached catfish oil

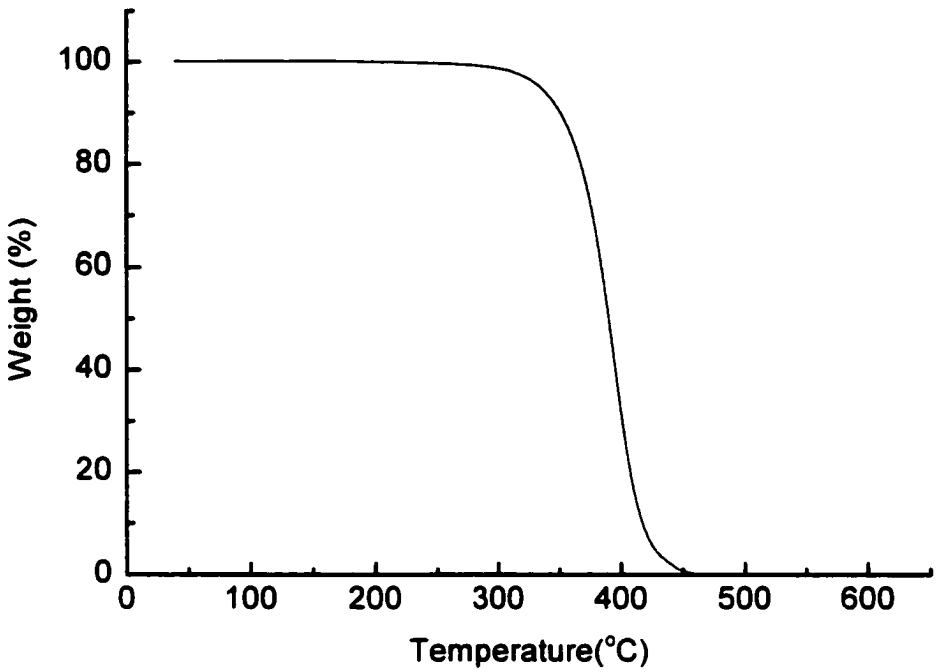

Figure 7.7 Thermogravimetric curve for deodorized catfish oil

Weight reduction (%) between 400°C -450°C was 74.85 to 99.46 for crude oil; 90.31 to 99.8 for degummed oil; 96.99 to 99.8 for neutralized oil; to 93.2 to 100% for bleached oil; 68.97 to 99.4 for deodorized oil. Crude oil contained a large quantity of impurities including phospholipids, free fatty acids, aldehyde, ketone, pigments, minerals, and water.

They all decomposed at various temperatures. During processing almost all impurities were removed from oil by the processing steps. According to previous investigations, insoluble and soluble materials are removed through the degumming step (List et al., 1993; Young, 1978; Dijkstra and Opstal 1989). Neutralization of crude oil with caustic soda is a step to remove free fatty acids. Bleaching removes soap, trace metals, sulphurous compounds, and part of the more stable pigments and pigments

breakdown products such as aldehydes and ketones (Young, 1978; Richardson, 1978; and Goebel, 1976). The purpose of deodorization is to remove residual fatty acids, aldehydes and ketones, which are responsible for unacceptable oil odor and flavor (Young, 1978; Gavin, 1977 and 1978; Zehnder, 1975). Therefore, the variation of decomposition observed between processing steps is reflected in Table 7.3 and Figures 7.3-7.7. Catfish oils with less impurities had more weight loss especially at 450-500°C.

7.4 Conclusion

All individual fatty acids (except C18;1 and C20:2) of catfish oil had a weight loss of greater than 90% heated up to 250°C. The resistance to heat of the fatty acids was dependent on chain length and degree of unsaturation. The weight loss of catfish oils increased with increased heating temperatures, regardless of the oil purification process. There was no distinct weight loss of catfish oils until the temperature was over 350°C. All oil samples were decomposed after 550°C. The information from this study is useful for the design of processing units and cost analysis, if catfish oils are to be used especially as cooking oils.

CHAPTER 8. DETERMINATION OF MELTING POINTS, SPECIFIC HEAT AND ENTHALPY OF CATFISH OIL FROM DIFFERENT PROCESSING STEPS

8.1 Introduction

There are five major processing steps involved in edible oil processing, i.e. extraction, degumming, neutralization, bleaching, and deodorization to produce oil from its raw material. During extraction and purification, oil is subjected to variations in temperature (e.g., evaporation, freezing, and chilling). Changes in the physical state of oil caused by temperature are known as phase transitions. The most commonly occurring phase transitions in oil are melting (solid-to-liquid) and crystallization (liquid to solid). Due to phase transitions, the overall physical and chemical properties of oil may drastically alter the quality of final products (Nawar, 1996). Information on the melting point and the transition temperature of oil is useful for designing and optimizing of oil processing conditions.

Phase transition is associated with enthalpy (Zhao and Yalkowsky, 1999) which explains whether an oil changes from one physical state to another state either by absorbing (endothermic) or releasing (exothermic) heat. Specific heat capacity is an important quantity which provides information about the amount of energy that must be supplied or withdraw to change temperature by a given amount.

The Differential scanning calorimetry (DSC) offers a simple means by which investigates the characteristic of melting and freezing points of fats. The influence of composition of fat, content of water, production materials, aging and heat treatment can be shown on the basis of DSC investigation. DSC has been used to investigate the thermal conductivity and specific heat (Buhri and Sing, 1994), melting and crystallization

(Kaisersberger, 1989 and 1990; Ritter et al., 2001), oil content (Aguilera and Gloria, 1997; Iannotta et al., 2001), residual moisture content (Tomassetti et al., 1996), wax coating (Ritter et al., 2001) and phase transition (Lai and Chao, 2000) of foods. It has been used for other areas including polymer phase transition (Fougnies et al., 1997) and crude oil combustion (Kok, 1998). Dollimore (2000, 1998 and 1996) thoroughly reviewed the overall application of DSC to several thermal analysis materials.

The study of the melting point, enthalpy, and specific heat capacity of catfish oil at each processing step is important for design and optimization of the processing of catfish oil in order to obtain good quality oil. Therefore, the objective of the study is to determine the melting points, enthalpy and specific heat capacity of catfish visceral oil at different purification stages.

8.2 Materials and Methods

8.2.1 Fatty Acids

Myristic acid (C14:0), palmitic acid (C16:0), stearic acid (18:1), arachidic acid (20:0), palmitoleic acid (16:1), oleic acid (18:1), linoleic acid (18:2) linolenic acid (C18:3), cis-11,14-eicosadienoic acid (C20:2), arachidonic acid (20:4), and cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6) were bought from Sigma Comapnay, St. Louis, MO. Purity of all the fatty acids was 99%, except purity of fatty acids was 98% for C20:2 and 90% for C20:4.

8.2.2 Samples and Sample Preparation

Catfish viscera was obtained from the local fish processing plant in Baton Rouge, LA. The viscera was frozen at - 20°C until used. The slightly thawed viscera was finely ground in a Hobart Chopper Bowl (84181D) at 3,450 rpm for 10 minutes. Water was

added (water: ground viscera, 5:1 V/W) and the mixture was heated at 70°C for 15 minutes. The purpose of the heating step was to coagulate the protein of the catfish viscera, so that liquids and solids can be mechanically separated. During mild heating, fat cells are ruptured, releasing the oil into the liquid phase. The denatured proteins were separated from the liquid phase (oil and soluble particles) filtration through cheesecloth and the cooked viscera were pressed by manually to remove most of the liquid. The crude oil was separated from the water phase and other fish particles by centrifugation at 5,000 rpm for 30 minutes. Separated crude oil was stored at – 20°C until used. Three experimental batches were conducted

8.2.3 Degumming

Crude oil was removed from the storage at –20°C and 100g of the crude oil was taken for the degumming process and placed in a 600- ml beaker and heated to 70°C in a water bath. Three ml of citric acid (3%) was added to the oil, thoroughly mixed,and the sample was heated at 70°C for one minute, then centrifuged at 5,000 rpm for 10 minutes to remove impurities.

8.2.4 Neutralization

Sodium hydroxide solution (14 Baume; 13g NaOH; 0.25 normal NaOH) was added to 100 gram of oil as explained in the AOCS method 9b-52Ca (1989) and heated to 65°C for 30 minutes. The sample was cooled to room temperature and kept for six hours. The precipitated soap was removed by centrifugation at 5,000 rpm for 10 minutes. Fifty ml of demineralized water was added to the oil to wash out remaining soaps. This was repeated for three times. Remaining water was removed by centrifugation at 5,000 rpm for 10 minutes.

8.2.5 Bleaching

Neutralized oil was heated to 70°C in a water bath and was bleached with 4% w/w activated earth (the American Oil Chemistry Society, Champaign, IL) at 70°C for 10 minutes. The activated earth with impurities was removed from the oil by centrifugation at 5,000 rpm for 30 minutes.

8.2.6 Deodorization

The laboratory distillation unit was used for deodorization. The unit consisted of a round bottom boiling flask with three outlets. One outlet was attached to a vacuum pump, another outlet was attached to a glass distillation column and the last outlet was sealed with a thermometer inserted. The flask was placed on a heating system. Bleached oil was added into the flask and heated to 100°C for 30 minutes under vacuum pressure at 65 cmHg. The temperature was maintained manually.

8.2.7 Esterification of Fatty Acid

Esterification of fatty acid was followed by the procedure described by Sathivel et al. (2001). About 1g of crude catfish oil and oil from each processing step was separately placed into a 50-ml vial with a taflon cap and a stirring magnet. Approximately 4 ml of methanolic sodium hydroxide (2g of NaOH dissolved in 100 ml methanol), 7 ml of boron trifluoride and 5 ml of heptane were added to the vial. The heptane solution containing FAMEs was recovered from the upper layer (neck) of the vial and dehydrated with 1.5g anhydrous sodium sulfate, and stored under nitrogen in teflon-capped vials at –20°C until analyzed.

8.2.8 Fatty Acids Analysis

The FAMEs were quantified by the Hewlett Packard 5890 Series II Gas Chromatograph equipped with a 7673A autosampler and interfaced to a 5970 mass selective detector (Agilent Technologies, Palo Alto, CA). The GC was equipped with an EZ-Flash fast temperature programmable column (Thermedics Detection, Inc., Chelmsford, MA). The column phase was RTX-2330 (90%-biscyanopropyl/10% phenylcyanopropyl polysiloxane) with the dimensions: 5 meter long, 0.25 mm inside diameter with a 0.2-μm phase thickness. One μL was injected using the inlet in a split mode. The head pressure was set at 2 psi and the split vent flow was 7 mL/m. The injector temperature was 260°C. Column flow rate at 2 psi was 0.68 mL/m. The column temperature was ramped from 50° to 260°C at 20°C/second and was held at 260° C for 90 seconds. Run length was 5 minutes. The transfer line temperature was 280°C. The MSD was operated with the selected ion monitoring mode. Fatty acids were identified with retention times obtained from the fatty acids methyl esters standards (Sigma Company, St.. Louis, MO). Three experimental replications (batches) were conducted, each with 3 extractions and 3 GC-injections per extraction. FA content was reported as mg/g dry-sample weight.

8.2.9 Differential Scanning Calorimetry (DSC)

All the experiments were conducted in triplicate using DSC 2920 Differential Scanning Calorimetry (TA Instruments – Water LLC, New Castle, DE). The melting point, enthalpy, and specific heat capacity for catfish oil from different processing steps, and the melting point and enthalpy for individual fatty acids were determined.

8.2.10 Melting Point and Enthalpy

About 0.5-1mg of samples was measured and put in a small aluminum sample vessel. The sample was then placed on the sample platform of the DSC and a similar same weight of empty aluminum vessel was placed on the reference platform. For determination of the melting point a linear heating rate of 5°C/min over a temperature range of –75°C to 125°C was used. Liquid nitrogen was used to chill the samples to a lower temperature. Duplicated experiments were conducted.

8.2.11 Specific Heat Capacity

For specific heat determination, approximately 0.9 mg of sample was weighed and placed in a small aluminum sample vessel and was placed on the DSC sample platform. Then same weight of an empty aluminum vessel was placed on the DSC reference platform. It was equilibrated at –75°C and then heated to 80°C at the rate of 2°C/min with modulated to $\pm$ 0.5°C every 40 seconds. All the graphs were normalized on sample a weight basis before the enthalpy and melting point were calculated. Duplicated experiments were conducted.

8.3 Results and Discussion

8.3.1 Fatty Acid Composition

The fatty acid composition of catfish oils from different processing steps is given in Tables 8.1. 12 fatty acids were found in catfish oil including C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:2, C20:4, and C22:6. Saturated fatty acids from crude, degummed, neutralized, bleached, and deodorized oils accounted for 34.9%, 31.51%, 31.21%l, 30.23%, and 24.45%, respectively. Steric acid was the predominant fatty acid accounting for 50% of total all saturated fatty acids. The total

unsaturated fatty acid present in catfish oil was relatively higher in quantity than total saturated fatty acid. It accounted for 65.12%, 60.78%, 59.43%, 56.74%, and 51.35% for crude oil, degummed oil, neutralized oil, bleached oil, and deodorized oil, respectively. Among unsaturated fatty acids, oleic acid was a predominant fatty acid accounting for 16.54% to 19.69%.

8.3.2 Melting Points of Fatty Acids

Melting points and enthalpy of the fatty acids are given in Table 8.2. There is an obvious relationship between chemical structure and melting points of fatty acids. Melting points of saturated fatty acids were 70.6°C, 67.6°C, 59.8°C, and 53.5°C for arachidic (C20:0), steric (C18:0), palmitic (C16:0), and mystric (C14:0), respectively. The melting point of saturated fatty acids increased with increased chain length (Table 8.2); this may be due to the intermolecular dispersion force which increased with the increased number of carbons in chains (Nawar, 1996; Shen and Alexander, 1999). In the case of unsaturated fatty acids, the increased number of double bonds decreased the melting point.

Melting points of C16:1, C18;1, C18:2, C18:3, C20:2, C20:4 and C22:6 were -0.9°C, -5.7°C, -13,0°C, -21.1°C, -8.3°C, -43.4°C, and -47.4°C, respectively. Comparing C16:0 with C16:1, an increase in double bond deceased the melting point up to 60.7°. Comparing C18:0 with C18:1, C18:2, and C18:3, there was a decrease in the melting point up to 61.9, 80.6, and 88.6 for increase in one, two and three double bonds. Lowest melting point (-47.4°C) was observed for C22:6.

Table 8.1 Fatty acids profiles (%) of catfish oils from each processing step

Fatty Acids	Crude	Degummed	Neutralized	Bleached	Deodorized
C14:0	3.67	2.92	2.84	2.84	2.62
C16:0	12.72	11.38	11.3	10.1	9.9
C16:1	11.15	10.33	10.03	10.03	8.08
C18:0	17.89	16.62	16.47	16.39	12.05
C18:1	19.69	19.09	18.71	17.07	16.54
C18:2	18.71	17	16.47	15.57	15.49
C18:3	3.37	3.22	3.07	3.07	2.54
C20:0	0.82	0.62	0.61	0.58	0.58
C20:1	7.04	6.21	6.14	6.14	4.79
C20:2	3.07	2.84	2.84	2.77	2.1
C20:4	1.35	1.2	1.2	1.2	0.82
C22:6	0.9	0.9	0.9	0.9	0.9
Sat	34.9	31.51	31.21	30.23	25.45
Unsat	65.12	60.78	59.43	56.74	51.35
Omega-3	4.27	4.12	4.04	4.04	3.44

Table 8.2 Melting points and enthalpies of fatty acids

Samples[a]	Onset temperature (melting point °C)	Maximum temperature (°C)	Enthalpy (j/g)
C14:0	53.5	55.5	198.3
C16:0	59.8	63.9	212.8
C18:0	67.6	70.0	226.3
C20:0	70.6	75.8	236.9
C16:1	-0.90	1.64	125.8
C18:1	-5.7	15.2	152.2
C18:2	- 13.0	-4.1	119.1
C18:3	-21.0	-10.4	115.0
C20:2	-8.3	0.9	103.1
C20:4	-43.4	-38.4	113.3
C22:6	-47.4	-42.2	89.1

[a] Commercial samples

Table 8.3 Melting ranges and enthalpies of catfish oils from different processing steps

Process steps	Melting ranges (°C)	Enthalpy (j/g)
Crude	-46 to 20.4	73.9
Degummed	-46 to 11.4	73.5
Neutralized	-44.1 to 11.3	74.2
Bleached	-48.0 to 10	79.6
Deodorized	-51.4 to 7.3	84.7

8.3.3 Melting Points of Catfish Oil

Table 8.3 and Figures 8.1 to 8. 5 show a typical melting curve of crude, degummed, neutralized, bleached, and deodorized catfish oil. The melting points of catfish oil ranged from – 46 °C to 20.4°C for crude oil, -46 to 11.4°C for degummed oil, -44.1 to 11.3 °C for neutralized oil, -48.0 to 10.0°C for bleached oil and -51.4 to 7.3 °C for deodorized oil. Six (A, B, C, D, E, and F) distinct endothermic peaks were recognized in the DSC thermograms for crude catfish oil (Figure 8.1). These peaks are generally considered as melting points. Peaks E and F were not sharp indicating impurities.

Melting points of crude catfish oil for peaks A, B, C, D, E, and F were 20.4°C, -0.38°C, -5.6°C, -16.8°C, -23.5°C, and –46°C, respectively.

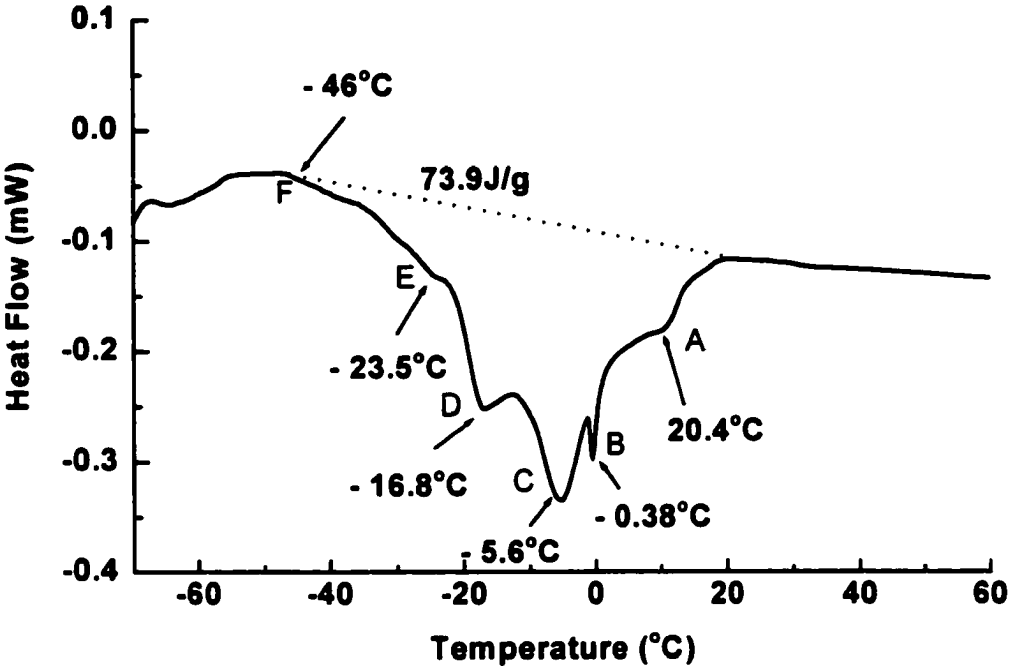

Figure 8.1 The DSC thermogram of crude catfish oil

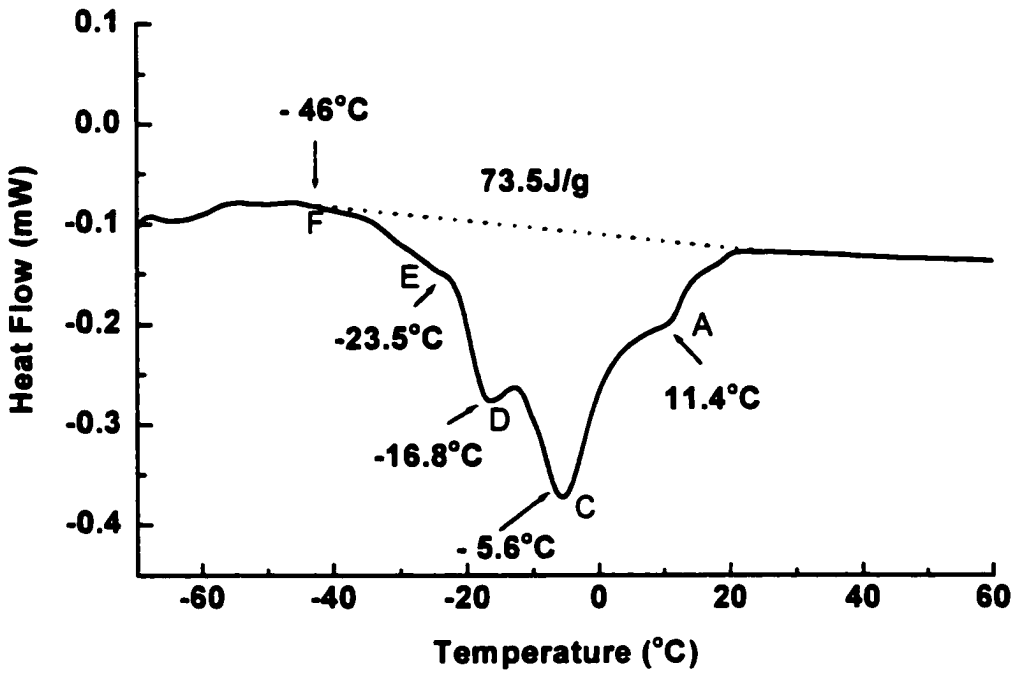

Figure 8.2 The DSC thermogram of degummed catfish oil

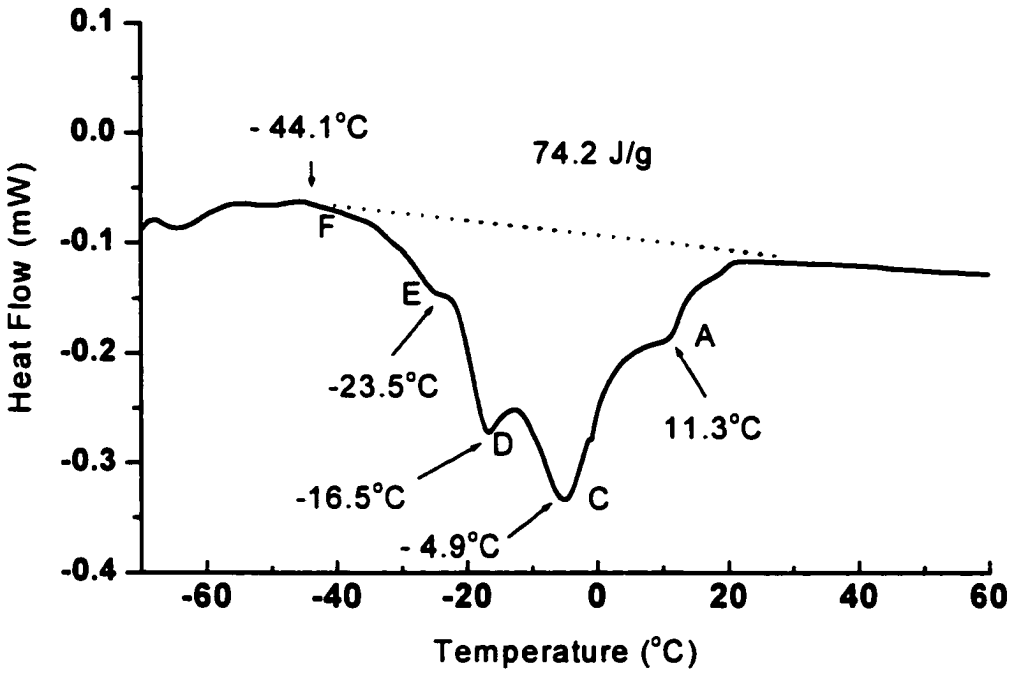

Figure 8.3 The DSC thermogram of neutralized catfish oil

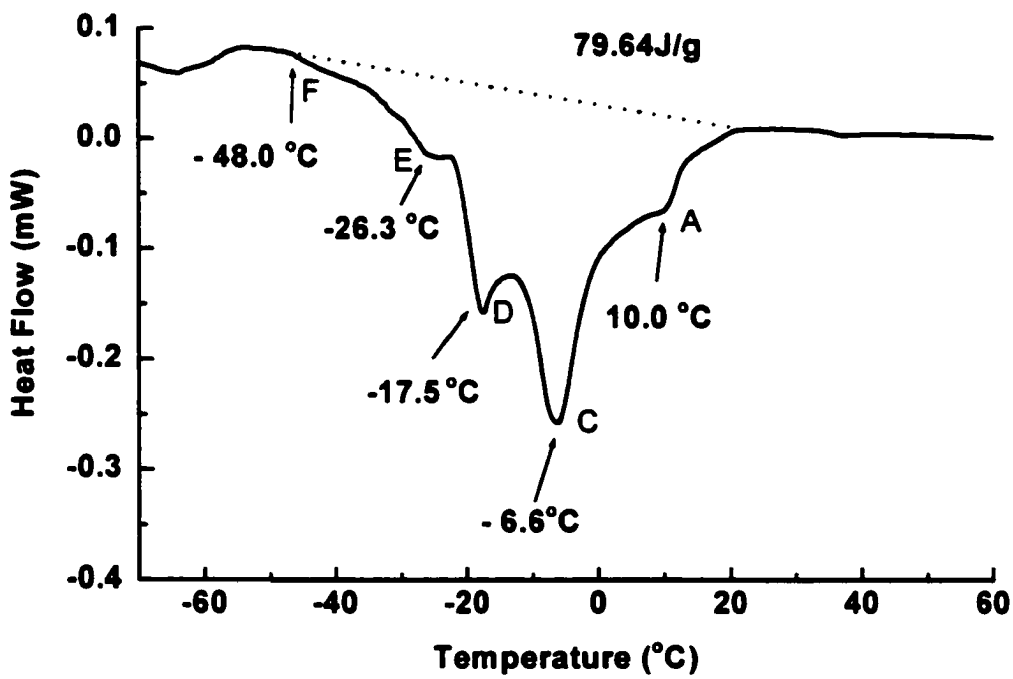

Figure 8.4 The DSC thermogram of bleached catfish oil

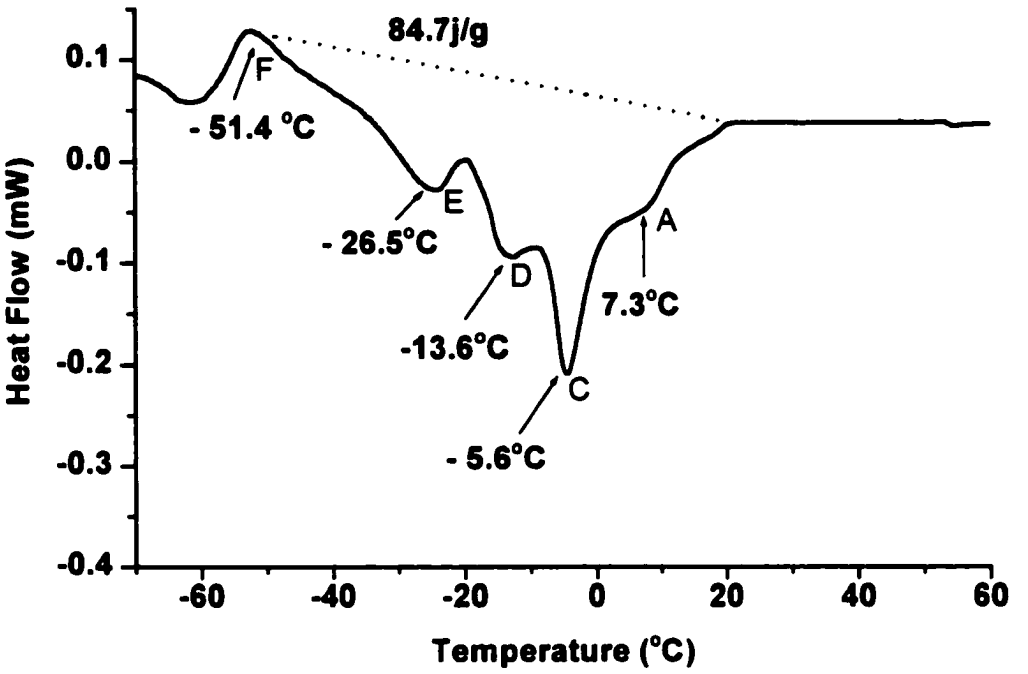

Figure 8.5 The DSC thermogram of deodorized catfish oil

Peak B was a small sharp peak and it might be a melting point of freezable water that remained in crude oil. Water melts closer to 0°C and it gives a very sharp peak (Berjak et al., 1992; Connor and Bonner, 2001). During the extraction of oil from catfish viscera, water was added and part of this water may have remained in the crude oil.

Figure 8.2 shows the DSC thermogram for degummed catfish oil with 5 peaks. The peak B observed for melting of freezable water in Figure 8.1 was not observed in Figure 8.2. This indicates that during the degumming process, water present in crude oil was removed. Other melting points showed similar values to those observed in crude oil, except for the melting point A, which decreased from 20.4°C to 11.4°C.

The neutralized oil also showed a similar pattern of DSC thermogram to the degummed oil; however, a very small peak B was observed (Figure 8.3). This peak may

be due to the remaining water used during neutralization to remove soap the bleached oil showed very sharp peaks (Figure 8.4). The peak B for freezable water was not observed. All melting points were lower than those of crude oil; melting point peaks A, C, D, E, and F were decreased from 20.4°C to 10°C,-5.6°C to -6.6°C, -16.8°C to –17.5°C, –23.5°C to –26.3°C , and -46°C to –48°C, respectively. The DSC thermogram of deodorized oil is given Figure 8.5. The peaks were sharper and narrower than oils from other processing steps. Melting point of A peak was reduced to 7.3°C compared with that (10°C) of the bleached oil. However, there was an increase in the melting point temperature for peak D (-13.6°C).

The DSC thermograms showed that there were significant changes in melting points of catfish oils obtained from different processing steps. Sharp melting points were observed in purified deodorized oil. Catfish oil is a chemically complex material and, therefore, the phase transition occurred over a wide range of temperature. The trends in melting points observed for the fatty acids reflected in the melting points of triglycerides in catfish oils. Catfish oil contained more than 60 % of total unsaturated fatty acids (Table 8.1) of which the majority had negative melting points. The negative melting points of catfish oil may have been attributed to these unsaturated fatty acids.

Crude catfish oil contains impurities such as phospholpids, free fatty acids, aldehydes, ketones, water, and pigments. Those impurities components melt uncharacteristically compared to pure fatty acids. Fatty acids have their own melting points; therefore, sharp peaks were not observed and a melting range was shorter than that of the oils. Figure 8.6 and 8.7 show the DSC thermogram of plamitic and DHA respectively. During the degumming, phospholpids, and water are removed (List et al.,

1993; Young, 1978; Dijkstra and Opstal, 1989). Free fatty acids are removed during neutralization, and pigments, minerals, free fatty acids, aldehyde, and ketones were removed during bleaching (Young, 1978; Richardson, 1978; Goebel, 1976). Off flavor components were removed during deodorization (Young, 1978; Gavin, 1977 and 1978; Zehnder, 1975). It can be clearly seen from Figures 8.1-8.5 that melting point peaks of catfish oils were sharper after each purification step that removed impurity from the oil.

8.3.4 Enthalpy

Figures 8.6 and 8.7 present the peaks of the thermograms obtained from C16:0 and C22:6 during the heating from –75 to 120°C. A typical parameter was the "onset" temperature, which represents the beginning of the melting processes.

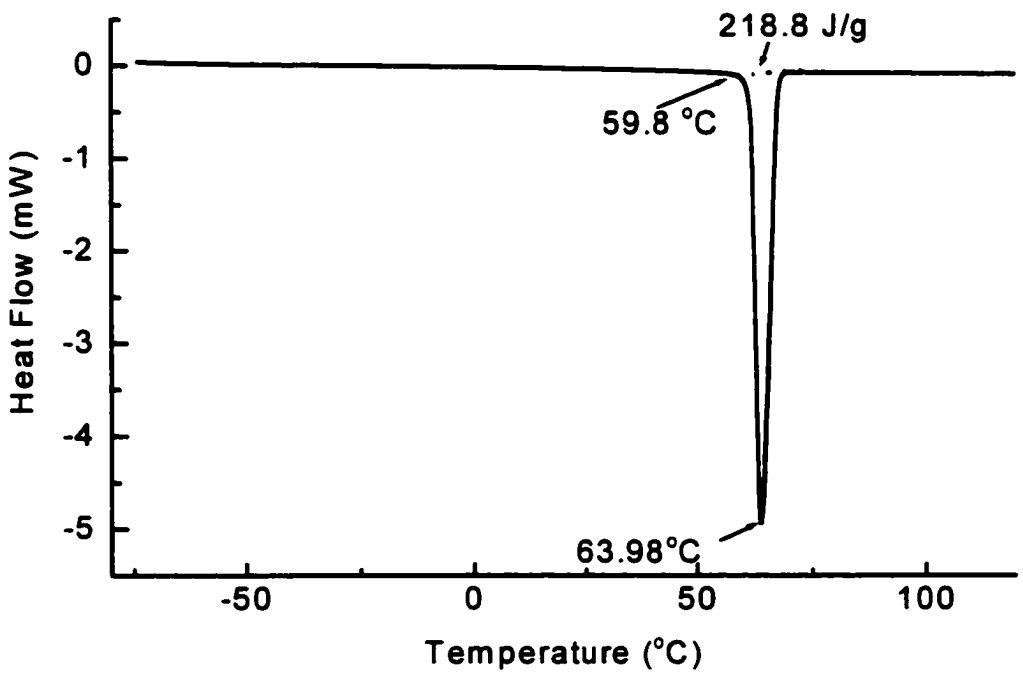

Figure 8.6 The DSC thermogram of palmitic acid

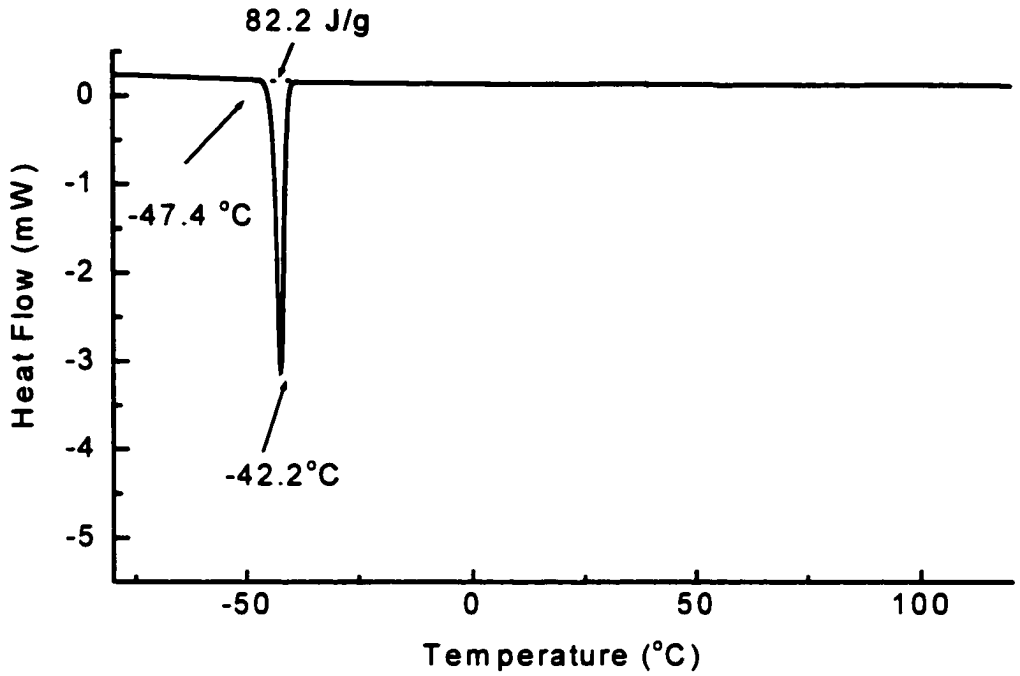

Figure 8.7 The DSC thermogram of DHA

It is defined as the intersection of the tangent at the first leg of the main peak with the base line (Ritter et al., 2001). The onset temperature for C16:0 and C22:6 was 59.8 ^{0}C and –47.4°C, respectively and integration of the total peak area yields an enthalpy of 218.8 J/g and 82.1 J/g for C16:0 and C22:0, respectively. Enthalpy of fatty acids increased with increased chain length (Table 8.2).

For saturated fatty acids, enthalpies were 198.3 J/g, 212.8 J/g, 226.3 J/g, and 236.9 J/g for C14:0, C16:0, C18:0, and C20:0, respectively. For unsaturated fatty acids, the enthalpy generally decreased with increased numbers of double bonds. Enthalpy of C16:1, C18:1, C18:2, C18:3, C20:2, C20:4, and C22:6 were 125.8 J/g, 152.2 J/g, 119.1 J/g, 115.0 J/g, 103.1 J/g, 113.3 J/g, and 82.1 J/g, respectively. Comparing C16:1 with C16:0, the enthalpy of 87.0 J/g was decreased for one double bond added. Comparing

C18:0, with C18:1, C18:2, and C18:3, the enthalpy progressively decreased for each double bond added. Lowest enthalpy (82.1 J/g) was observed for C22:6. However, C20:4 (113.3 J/g) showed higher enthalpy than C20:2 (103.1 J/g).

The enthalpies for catfish oils from different processing steps are given in Table 8.3. The enthalpy was 73.9 J/g for crude oil, 73.5 J/g for degummed oil, 74.2 J/g for neutralized oil, 79.6 for bleached oil, and 84.7 for deodorized oil (Table 8.2). The enthalpy of catfish oils increased after each purification step that removed impurities from the oils.

8.3.5 Specific Heat Capacity

For DSC, the specific heat capacity is determined from the equation: Q=mCp (dT/dt), where Q is the heat flow per unit time, m is the sample mass, Cp is specific heat capacity of material and dT/dt is the rate of change of the external temperature The temperature-dependent specific heat capacities of crude, degummed, neutralized, bleached and deodorized catfish oils are given in Figures 8.8-8 12.

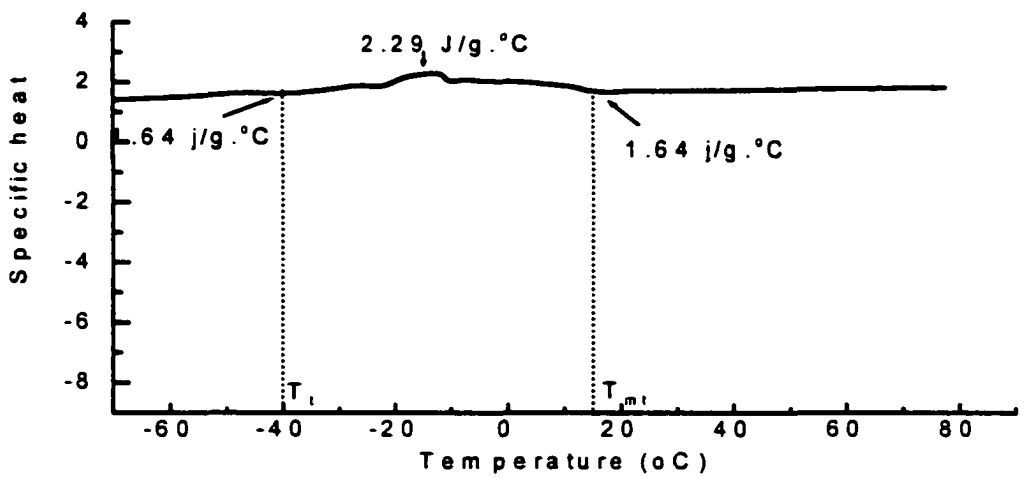

Figure 8.8 Specific heat capacity (J/g.°C) for crude catfish oil at various temperatures

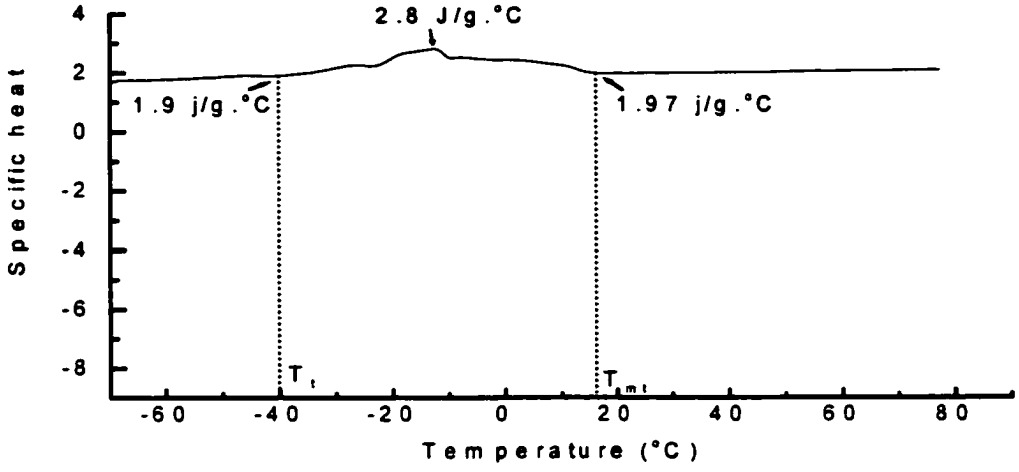

Figure 8.9 Specific heat capacity (J/g.°C) for degummed catfish oil at various temperatures

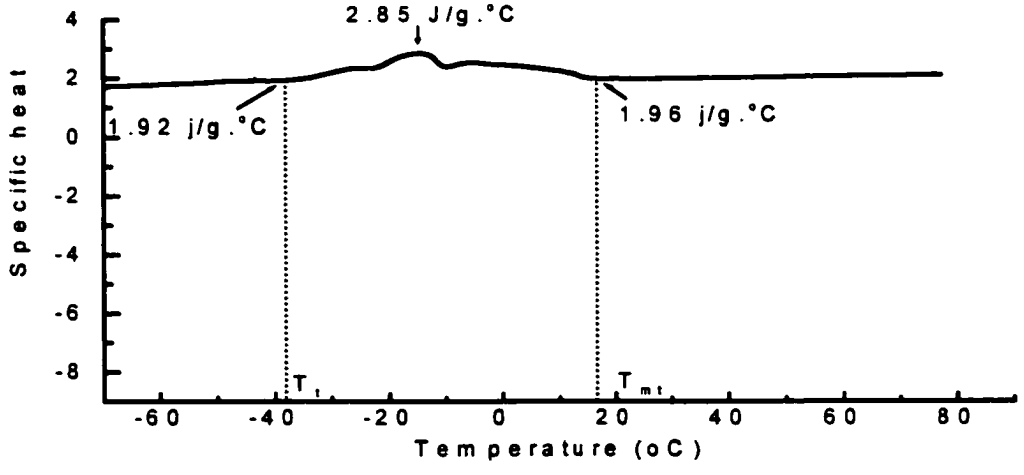

Figure 8.10 Specific heat capacity (J/g.°C) for neutralized catfish oil at various temperatures

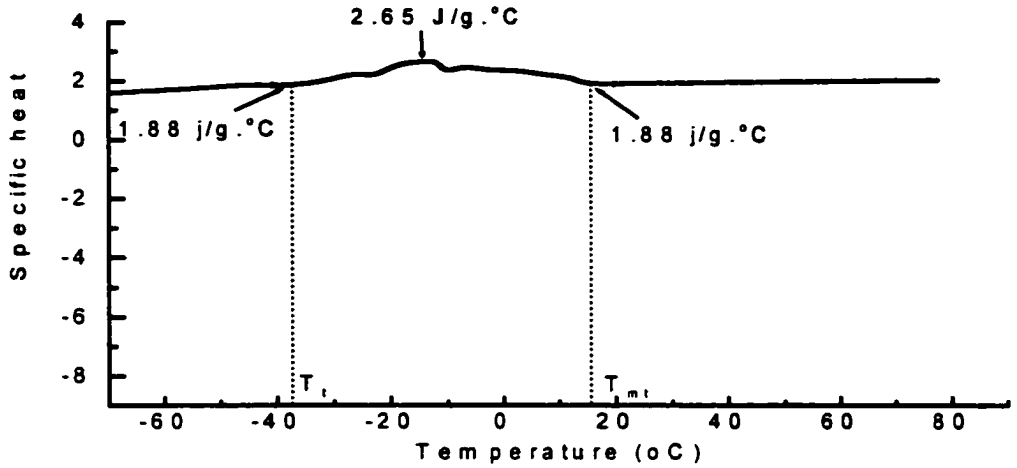

Figure 8.11 Specific heat capacity (J/g.°C) for bleached catfish oil at various temperatures

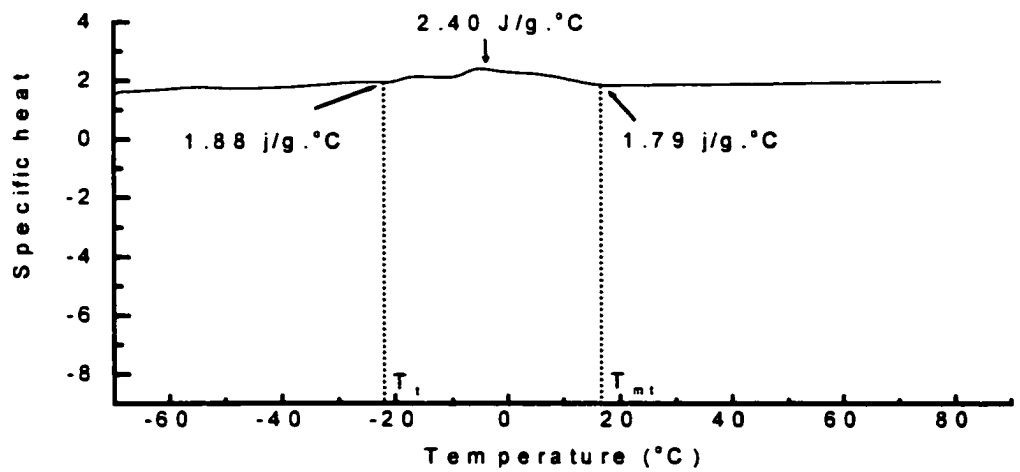

Figure 8.12 Specific heat capacity (J/g.°C) or deodorized catfish oil at various temperatures

The specific heat capacities (Cp) at 20°C for crude, degummed, neutralized, bleached, and deodorized oil were 1.64 , 1.96, 1.97, 1.88 and 1.79 j/g.°C, respectively. The specific heat capacity of the all oils was somewhat constant until closer to 40°C. The Cp increased with an increased temperature between the transition range (T_t) and the melting range (T_{mt}) of temperature of approximately 40°C to 19°C for oils. For

degummed, neutralized, bleached and deodorized, the variation of their specific heat with temperature showed the similar pattern tendency as with the crude catfish oil. There was a large difference between T_t to T_{mt} points for all oils from processing steps, which may have been due to the impurities.

8.4 Conclusion

The DSC thermogram indicated that significant changes in melting points of catfish oils was observed at different processing steps. A sharper melting point curve was observed in the purified deodorized catfish oil. The pattern of melting points observed for catfish oil was reflected by that of fatty acids, especially unsaturated fatty acids. The enthalpy of catfish oils increased after each purification step that removed impurities from the oil. There was not much difference for the specific heats of catfish oils from different processing steps.

CHAPTER 9. EFFECTS OF PROCESSING STEPS ON RHEOLOGICAL PROPERTIES OF CATFISH OIL

9.1 Introduction

Rheology is one of the most important properties of foods and food ingredients. It plays a major role on materials such as foods, cosmetics, and pharmaceuticals in determining suitability for particular applications (Sherman, 1970; McClements, 1998). Rheology of oil may be a determining factor in the performance of a product, such as margarine, salad oil, varnish or lubricant, of which the oil is a major ingredient. The knowledge of rheological prosperities of product help solve problems relating to the transfer or movement of bulk quantities of the liquid (Reild et al., 1987).

At low temperature, impurities of crude oil tends to precipitate on the wall of pipeline. Some precipitated solid particles in the bulk flow will increase viscosity of the oil, causing an increased pressure drop in the pipeline. Due to an increase in the viscosity of oil, the flow properties of oil will exhibit non-Newtonian behavior.

The fish oil processing involves a number of steps including degumming, neutralization, bleaches, and deodorization. Impurities, such as free fatty acids, protein, moisture, and pigments, are gradually removed from the oil from each processing step (Wiedermann, 1981). Removal of impurities may the flow properties of oil from non-Newtonian to Newtonian behavior.

Crude oil may be considered as a structured disperse system since a complex mixture of liquid hydrocarbons acts as a dispersion medium and aggregated impurities makes up the dispersed phase (Barry, 1971; Bower et al., 1999; Barnes, 1994 and 1995; Pal, 1992; Pal and Rhodes, 1989; Wasan et al., 1979). The impurities of crude oils

influences flow characteristic non-Newtonian properties (Pedersen and Rønningsen, 2000; Wetmann, 1943; Lakatos and Lakatos-Szabo, 1997 and Pal, 2000). The power law model has been used to describe flow prosperities of liquid based food products (Harper and El Sahrigi, 1965; Saravacos and Moyer, 1967; Rao et al., 1981; Dickie and Kokini, 1983; Hasson and Hobani, 1998; Hobani, 1988; Qiu and Rao, 1988; Khalil et al., 1989).

There is no satisfactory model for predicting viscosity of edible oils, which is shear rate dependent from different processing steps. Prediction and mathematical representation of flow characterization from different oil purification steps is very important for optimum design of unit operations and for better control of the final product quality. Therefore, the objectives of this study were to determine the effect of purification steps of oil on rheological properties of catfish oil, and to determine the applicability of different models for describing the rheological behavior of catfish oil.

9.1.1 Theory

9.1.2 Notation

A	constant
B	constant
C	constant
K	consistency coefficient
dV_x/dY	shear rate
n	shear rate index
τ_0	yield stress
τ_{xy}	shear stress

η apparent viscosity

η_c Casson higher shear limiting viscosity

η_p Bingham consistency coefficient

9.1.3 Rheological Models

For a Newtonian fluid, the shear stress (τ_{xy}) (equation 1) is directly proportional to the shear rate, (dV_x/dY), where η is apparent viscosity.

$$\tau_{xy} = \eta\left(\frac{dV_x}{dY}\right) \qquad \text{(eq 1)}$$

Viscosity(η) is defined as a ratio of shear stress to shear rate (equation 1). The viscosity of a Newtonian fluid is independent of shear rate and apparent viscosity is determined as a ratio of shear stress and shear rate (Steffe, 1996). The viscosity of a pseudoplastic fluid decreases with increasing shear rate, while the opposite is observed for a dilatant fluid. A pseudoplastic fluid without a yield stress(τ_o) is most often described by a simple two-parameter power function model.

The Herschel-Bulkley model (HB) (equation 2) is a generalized power law equation with a yield stress. The Herschel-Bulley (HB) model is one of the most extensively used equations to describe the flow characteristics of shear thinning fluids for over a wide range of shear rate (Steffe, 1996).

$$\tau_{xy} = \tau_o + K\left(\frac{dV_x}{dY}\right)^n \qquad \text{(eq 2)}$$

where τ_{xy} = shear stress, τ_o =yield stress (Pa), n = a shear rate index, K= consistency coefficient (Pa.s^n) and dV_x/dY=shear rate.

A Bingham plastic fluid is similar to a Newtonian fluid because there is a linear relationship between shear stress and shear rate after application of threshold shear stress. This threshold shear stress is called the yield stress of the fluid. The behavior of Bingham plastic fluid can be mathematically expressed:

$$\tau_{xy} = \tau_o + \eta_p\left(\frac{dV_x}{dY}\right) \qquad \text{(eq 3)}$$

where τ_o = the Bingham yield stress (Pa) and n = the shear rate index. The η_p is referred to as the Bingham plastic consistency coefficient (Pa.s). Like pseduo- plastic fluids, the Bingham fluid exhibits decreasing viscosity with increasing shear rate (shear thinning). As with the Herschel-Bulkley model, the Casson model (equation 4) is another common rehological fluid model, and it takes into account both the nonlinearity of the flow curve and the existence of a yield stress. The Casson model has been used to described the flow behavior of liquid with suspension particles (Rao et al., 1981; Barabosa-Canovas and Peleg, 1983; Dervisolglu and kokini, 1986; Qiu and Rao, 1988; Missaire et al., 1990 and Rao, 1977). It was initially developed by Casson (1959) for pigment suspension (Mackey, 1989):

$$\tau_{xy}^{0.5} = \tau_o^{0.5} + \eta_c^{0.5}\left(\frac{dV_x}{dY}\right)^{0.5} \qquad \text{(eq 4)}$$

Where τ_0 = yield stress (Pa), and η_c = higher shear limiting viscosity (Pa.s).

9.2 Materials and Methods

9.2.1 Samples and Sample Preparation

Catfish viscera was obtained in three batches (experimental replications) from the local fish processing plant in Baton Rouge, LA. The viscera was frozen at - 20°C until used. The slightly thawed viscera was finely ground in a one horsepower Hobart Chopper Bowl (84181D) at 3450 rpm at for 10 minutes. Water was added (water: oil, 5:1 V/W) and the mixture was heated at 70°C for 15 minutes. The purpose of the heating step is to coagulate the protein of the catfish viscera, so that liquids and solids can be mechanically separated. During mild heating, fat cells are ruptured, releasing the oil into the liquid phase. The denatured proteins were separated from liquid phase (oil and soluble particles) by filtration through cheesecloth and the cooked viscera were pressed by manually to remove most of liquid. The crude oil was separated from the water phase and other fish particles by centrifugation at 5,000 rpm for 30 minutes. Separated crude oil was stored at – 20°C until used. Three experimental batches were conducted. Crude menhaden oil was supplied by the Omegaprotein Inc, Reedville, VA. Both catfish and menhaden crude oils were purified as explained below.

9.2.2 Degumming

Crude oil was removed from the storage at –20°C and 100g of the crude oil was taken for degumming process and placed in a 600 ml beaker and heated to 70°C in a water bath. Three ml of citric acid (3%) was added to the oil, thoroughly mixed,and the sample was heated at 70°C for one minute, then centrifuged at 5000 rpm for 10 minutes to remove the impurities.

9.2.3 Neutralization

Sodium hydroxide solution (17-20 Baume; 13-17g NaOH/100mL; 3.1-4.2 normal NaOH was added to degummed oil and heated to 65°C for 30 minutes. The sample was cooled to room temperature and kept for six hours. The precipitated soaps were removed by centrifugation at 5000 rpm for 10 minutes. Fifty ml of demineralized water was added to the oil and to wash out remaining soaps from oil. This was repeated for three times. Remaining water was removed by centrifugation at 5000 rpm for 10 minutes to remove the impurities.

9.2.4 Bleaching

Neutralized oil was heated to 70°C in a water bath and was bleached with activated earth of 4% w/w (CS Z1077) at 70°C for 10 minutes. The activated earth with impurities was removed from the oil by centrifugation at 5,000 rpm for 30 minutes.

9.2.5 Deodorization

The laboratory distillation unit was used for deodorization. The unit consisted of a round bottom boiling flask with three outlets. One outlet was attached to a vacuum pump, another outlet was attached to a glass distillation column and the last outlet was sealed with a thermometer inserted. The flask was placed on a heating system. Bleached oil was added into the flask and heated to 100°C for 30 minutes under vacuum pressure at 65 cmHg. The temperature was maintained manually. The volatile products were condensed with a circulated liquid cooling system around the distillation unit and distillate was collected.

9.2.6 Free Fatty Acids Analysis

Free fatty acid (FFA) contain was determined in three replications by titration according to the AOAC official method 949.28 (1990) and FFA was expressed as oleic acid.

9.2.7 Water Activity

Water activity of oil was measured in three replications by use of water activity meter (AW sprint, novasina, Swiss) at 25°C.

9.2.8 Mineral Analysis

Minerals of catfish oil from different processing steps were determined in three replications by acid digestion method involving CEM innovators in microwave technology. A 0.5 g oil sample was separately placed in the vessel and 6 ml of HNO_3 was added the vessel and sealed. The sealed vessel was placed into the turntable. The heating program was run the until the digestion process complete. Then sample was cooled for 5 minutes and digested solution was transfer to flask with a filtration step. Absorption spectrophotometer was used to analyzed the Ca, Fe, Mg, and P.

9.2.9 Rheological Properties

The rheological properties of catfish oils were measured using a dynamic shear rheometer with a concentric cylinder measurement cell (Constant Stress Rheometer, CS-10, Bohlin Instruments, Cranbury, NJ). The diameter of the cylinder was 2.5 cm, and the diameter of the outer cylinder was 2.75 cm. Samples were placed in the temperature controlled measurement vessel and allowed to equilibrate to the required temperature (25°C) for 5 minutes prior to making the measurements. The shear stress of sample was measured from 0 to 1200s^{-1}. All rheological measurements were conducted at 25$\pm$0.1°C

and the average value from triplicate samples were reported . The standard deviation for (not shown) for rheological measurements were < 10%.

9.2.10 Flow Curve

Flow curves were generated using the data obtained from shear rate and shear stress experiments. Shear rates were gradually increased up $1200s^{-1}$, whereas shear stress was progressively increased (up to 53.3 Pa) corresponding to shear rate values. The experiments were conducted in triplicate, each samples with 2 measures at $25\pm0.1^{\circ}C$. The rheological equations, such as Newtonian, Hershel-Bulkey, Casson, and Bingham plastic models, were used to fit to the shear-rate and shear stress data. The model fitting of Newtonian, Hershel-Bulkey, Casson, and Bingham plastic models to the experimental data was carried out using a curve fit program CurveExpert 1.3 (Copyright Daniel Hyams), a comprehensive curve fitting system for window. The bets fitted model was based on the prediction coefficient (R^2).

9.2.11 Yield Stress, Power Law Behavior, and Apparent Viscosity

Yield stress was also calculated from the plots corresponding to (i) Hershel-Bulkey, (ii) Modified Casson, and (iii) Bingham plastic models. Casson equation was used to predict the apparent viscosity at different shear rate, and that was then compared with the experimental viscosity.

9.3 Results and Discussion

9.3.1 Flow Parameters

Commercial processing of fish oil involves the following major steps: extraction of crude oil, degumming, neutralization, bleaching, and deodorization. Rheological properties of catfish oil were determined for each purification processing step and

comparison was made with that of menhaden oil. Tables, respectively, 9.1, 9.2, 9. 3, and 9.4 presents the flow parameters of Newtonian, Herschel-Bulkey, Bingham plastic, and Casson models for catfish oil. It can be seen that yield stress for catfish oil at different processing steps determined by the Bingham model was the highest compared with other models (Tables 9.1, 9.2, 9.3, and 9.4). Similar results were observed for menhaden oils (Tables, 9.5, 9. 6, 9.7,and 9.8). Without exception yield stress and consistency coefficient of catfish and menhaden oils decreased after processing.

Shear rate index, n, obtained by Herschel-Bulkey was varied between from 0.758 to 0.904 for catfish oil (Table 9.2), whereas it was range from 0.696 to 0.789 for menhaden oil (Table 9.6). The highest shear rate index was (0.904) observed in deodorized catfish oil, while Herschel-Bulkey model for menhaden oil showed that highest shear rate index (0.789) for bleached oil. Both oils generally showed a gradual decrease in consistency coefficient after each purification step.

Table 9.1 Flow parameters for catfish oil using the Newtonian model (eq 1)

Processing	η (Pa.s)	R^2	SE
Crude	0.049	0.951	4.68
Degummed	0.036	0.978	2.5
Neutralized	0.034	0.979	2.3
Bleached	0.029	0.978	1.89
Deodorized	0.026	0.985	1.52

η = apparent viscosity ;R^2 = prediction coefficient; SE = standard error

Table 9.2 Flow parameters for catfish oil using the Herschel-Bulkley model (eq 2)

Processing	τ_o (Pa)	N	K(Pa.s^n)	R^2	SE
Crude	2.73	0.794	0.1794	0.995	1.65
Degummed	0.365	0.758	0.184	0.999	0.538
Neutralized	0.946	0.819	0.109	0.998	0.697
Bleached	0.816	0.819	0.093	0.997	0.806
Deodorized	1.063	0.904	0.047	0.997	0.766

τ_o = shear stress; n = shear rate index; K= consistency coefficient; R^2 = prediction coefficient; SE = standard error

Table 9.3 Flow parameters for catfish oil using the Bingham model (eq 3)

Processing	τ_o (Pa)	η_p (Pa.s)	R^2	SE
Crude	4.79	0.042	0.991	2.08
Degummed	2.41	0.033	0.992	1.56
Neutralized	2.42	0.030	0.995	1.44
Bleached	2.08	0.026	0.993	1.11
Deodorized	1.40	0.024	0.995	0.911

τ_o = shear stress; η_p = Bingham consistency coefficient; R^2 = prediction coefficient; SE = standard error

Table 9.4 Flow parameters for catfish oil using the modified Casson model (eq 4)

Processing	τ_o (Pa)	η_c(Pa.s)	R^2	SE
Crude	2.27	0.028	0.998	1.12
Degummed	0.952	0.025	0.998	0.80
Neutralized	0.776	0.023	0.998	0.74
Bleached	0.670	0.0198	0.996	0.84
Deodorized	0.658	0.0139	0.996	0.75

τ_o= yields stress;η_c= Casson higher shear limiting viscosity; R^2 = prediction coefficient; SE=standard error

Table 9.5 Flow parameters for menhaden oil using the Newtonian model (eq 1)

Processing	η	R^2	SE
Crude	0.038	0.962	3.35
Degummed	0.039	0.964	3.31
Neutralized	0.036	0.977	2.52
Bleached	0.034	0.981	2.13
Deodorized	0.032	0.977	2.25

η = apparent viscosity ; R^2 = prediction coefficient; SE = standard error

Table 9.6 Flow parameters for menhaden oil using the Herschel-Bulkley model (eq 2)

Processing	τ_o (Pa)	n	K(Pa.s^n)	R^2	SE
Crude	0.505	0.709	0.265	0.998	0.623
Degummed	0.137	0.696	0.296	0.999	0.610
Neutralized	0.769	0.785	0.147	0.998	0.646
Bleached	0.543	0.789	0.136	0.998	0.657
Deodorized	0.176	0.752	0.169	0.999	0.505

τ_o = shear stress; n = shear rate index; K= consistency coefficient; R^2 = prediction coefficient; SE = standard error

Table 9.7 Flow parameters for menhaden oil using the Bingham model (eq 3)

Processing	τ_o (Pa)	η_p	R^2	SE
Crude	3.10	0.034	0.988	1.97
Degummed	3.47	0.033	0.990	1.81
Neutralized	2.70	0.032	0.995	1.28
Bleached	2.34	0.030	0.999	1.22
Deodorized	2.01	0.029	0.991	1.41

τ_o = shear stress; η_p = Bingham consistency coefficient; R^2 = prediction coefficient; SE = standard error

Table 9.8 Flow parameters for menhaden oil using the modified Casson model (eq 4)

Processing	τ_o (Pa)	η_c (Pa.s)	R^2	SE
Crude	1.42	0.023	0.997	0.940
Degummed	1.37	0.023	0.983	0.997
Neutralized	0.925	0.024	0.998	0.685
Bleached	0.765	0.023	0.998	0.713
Deodorized	0.789	0.021	0.997	0.739

τ_o= yields stress; η_c= Casson higher shear limiting viscosity; R^2 = prediction coefficient; SE = standard error

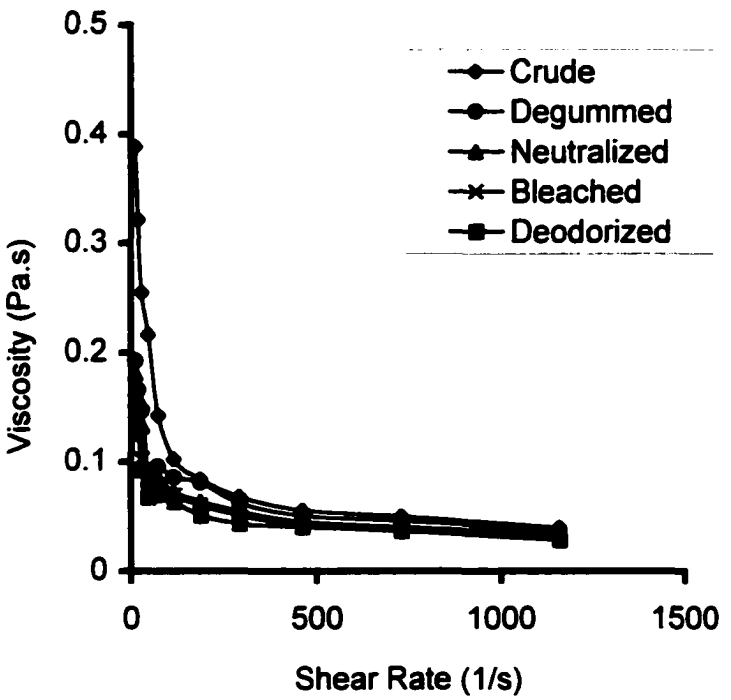

Figure 9.1 Viscosity profiles of catfish oil at different processing steps

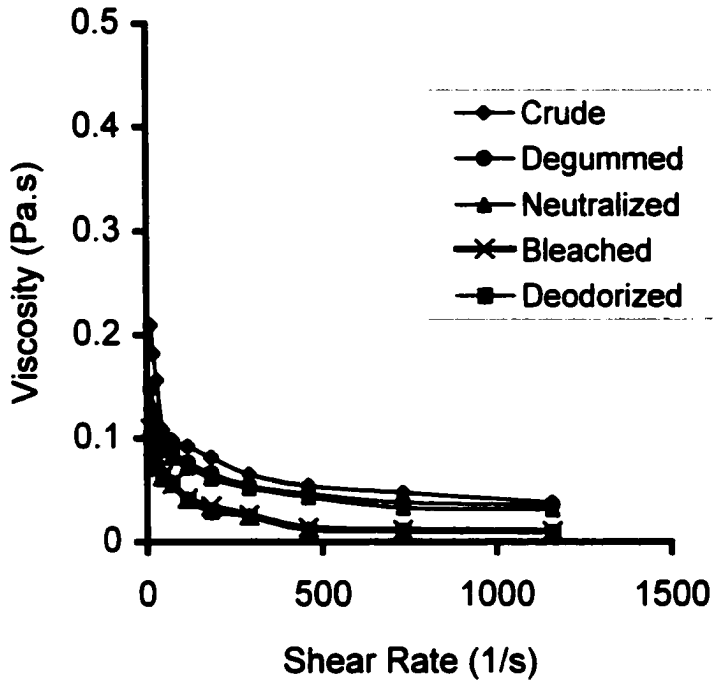

Figure 9.2 Viscosity profile of menhaden oil at different processing steps

It clearly illustrated that the oil after removal of impurities from each processing step, the flow behavior of oils satisfied from non-Newtonian closer to Newtonian. The viscosity profiles are shown in Figures 9.1 and 9.2. Both oils illustrated the shear-thinning nature.

9.3.2 Model Fitting

The regression coefficients (R^2) for 4 models were over 0.95 for all processing steps for both oils (Tables 9.1-9.8). Discrepancy in prediction by different models appeared to be minor. Figures 9.5 to 9.14 indicated that predication by all models were nearly superimposed. The prediction coefficient of Herschel-Bulkey, Bingham plastic,

Casson, and Newtonian models for crude catfish oil was 0.995, 0.991, 0.998, and 0.951, respectively, whereas 0.998, 0.988, 0.997,and 0.962 was for Herschel-Bulkey, Bingham plastic, Casson, and Newtonian models, respectively for crude menhaden oil. The Newtonian model was less fitted for crude oil compared to the other 3 models. Both Herschel-Bulkey and Casson models adequately described the flow behavior of both oils at different processing steps. It was reported that the Casson model takes into account both the nonlinearly of the flow curve and the existence of a yield stress which is often observed with oil samples (Pedersen and Rϕnningsen, 2000). Furthermore, the Casson model works well at both lower higher shear rates (Kirsanor and Remizor, 1999). In most situations, crude oil is transferred from a cold storage tank to processing plants. It would be highly beneficial to have a non-Newtonian viscosity model for crude oils. Therefore, the Casson model equation was chosen to predict the viscosity of both crude catfish and menhaden oils.

To determine the viscosity of catfish oil, the Casson equation may be rewritten as follow:

$$\tau_{xy}^{0.5} = \tau_o^{0.5} + \eta_c^{0.5}\left(\frac{dV_x}{dY}\right)^{0.5}$$

$$\tau_{xy} = \left(\tau_o^{0.5} + \eta_c^{0.5}\left(\frac{dV_x}{dY}\right)^{0.5}\right)^2$$

$$\tau_{xy} = \tau_0 + 2\tau_o\eta_c^{0.5}\left(\frac{dV_x}{dY}\right)^{0.5}\left[\eta_c^{0.5}\left(\frac{dVx}{dy}\right)^{0.5}\right]^2$$

$$\tau_{xy} = \eta\left(\frac{dV_x}{dY}\right)$$

$$\eta\frac{dVx}{dy} = \tau_0 + 2\tau_o\eta_c^{0.5}\left(\frac{dV_x}{dY}\right)^{0.5} + \eta_c\left(\frac{dVx}{dy}\right)$$

$$\eta = \frac{\tau_o}{\frac{dVx}{dy}} + 2\tau_o\eta_c^{0.5}\left(\frac{dV_x}{dY}\right)^{-0.5} + \eta_c$$

$$\eta = A + \frac{B}{\sqrt{\frac{dVx}{dy}}} + \frac{C}{\frac{dVx}{dy}}$$

$$A = \eta_c, B = 2\tau_o\eta_c^{0.5}, C = \tau_o$$

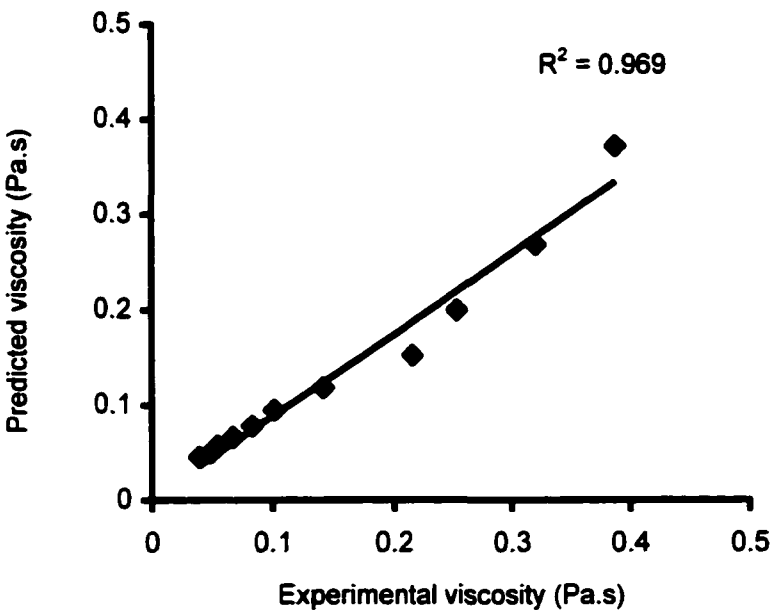

Figure 9.3 Predicted apparent viscosity from the Casson model in contrast to experimental apparent viscosity of crude catfish oil

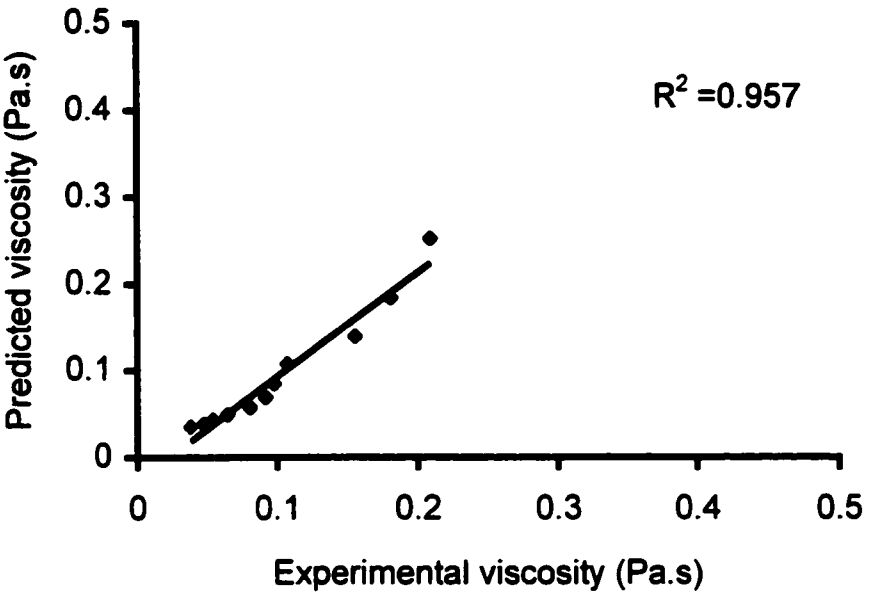

Figure 9.4 Predicted apparent viscosity from the Casson model in contrast to experimental apparent viscosity of crude menhaden oil

Figure 9.3 and 9.4 illustrated the fit between the predicted apparent viscosity using the Casson model and experimental viscosity for crude catfish and menhaden catfish oils. The degree of fit is good as shown by the distribution along the 45° line. The prediction coefficient (R^2) was 0.969 for crude catfish oil and 0.957 for crude menhaden oil. The predicted apparent viscosity by the Casson model agreed satisfactorily with the experimental apparent viscosity.

Figures 9.5 to 9.14 showed corresponding plots of shear stress versus shear rate for catfish and menhaden oils for each processing step. The gradually increasing linearity of flow curves was observed due to removal of impurities from each processing step.

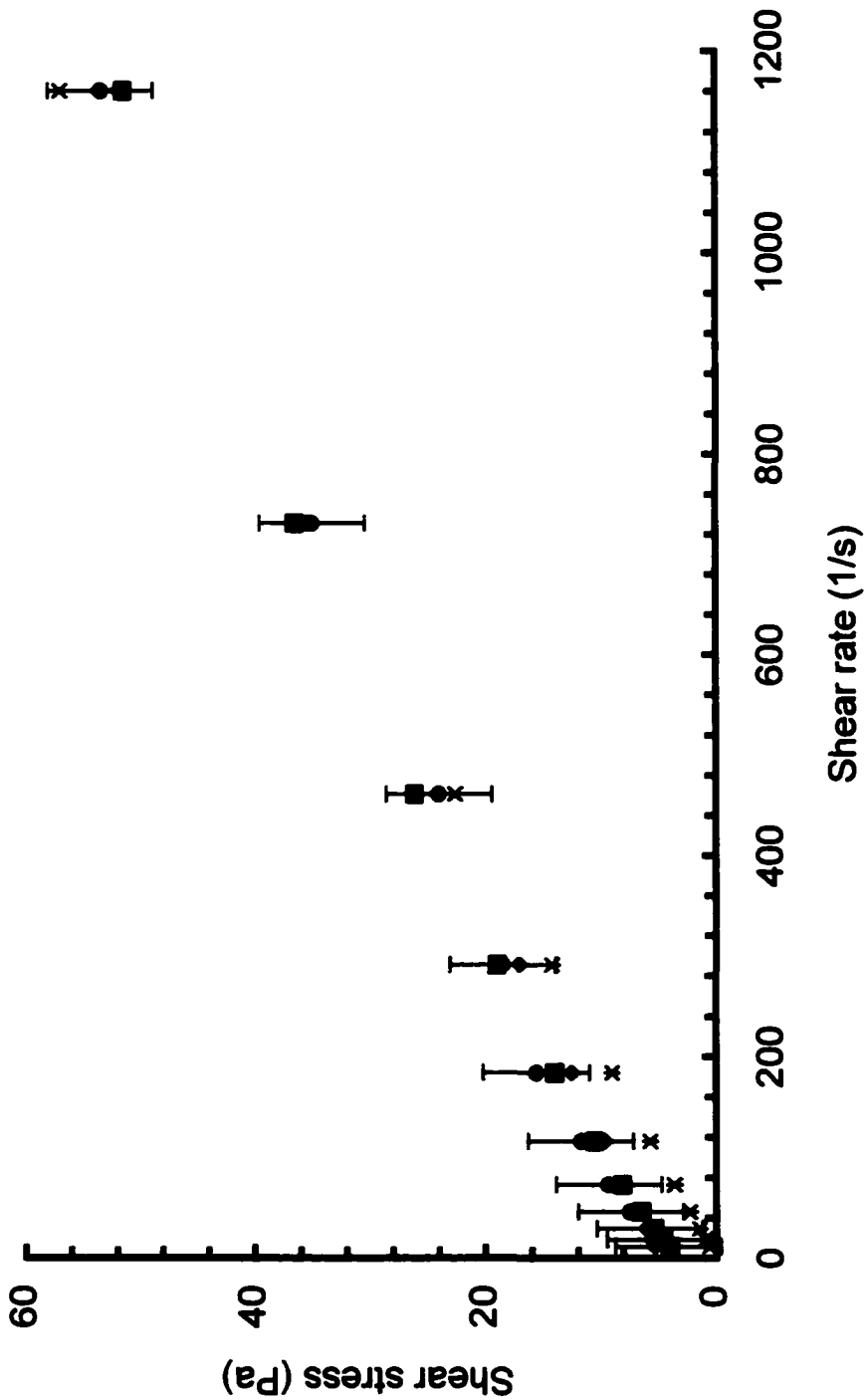

Figure 9.5. Rheological properties of crude oil at 25°C models (Experimental, ●; Hershel-Bulkey, ■; Bingham plastic, ◆; Casson, ▲; and Newtonian, ✱)

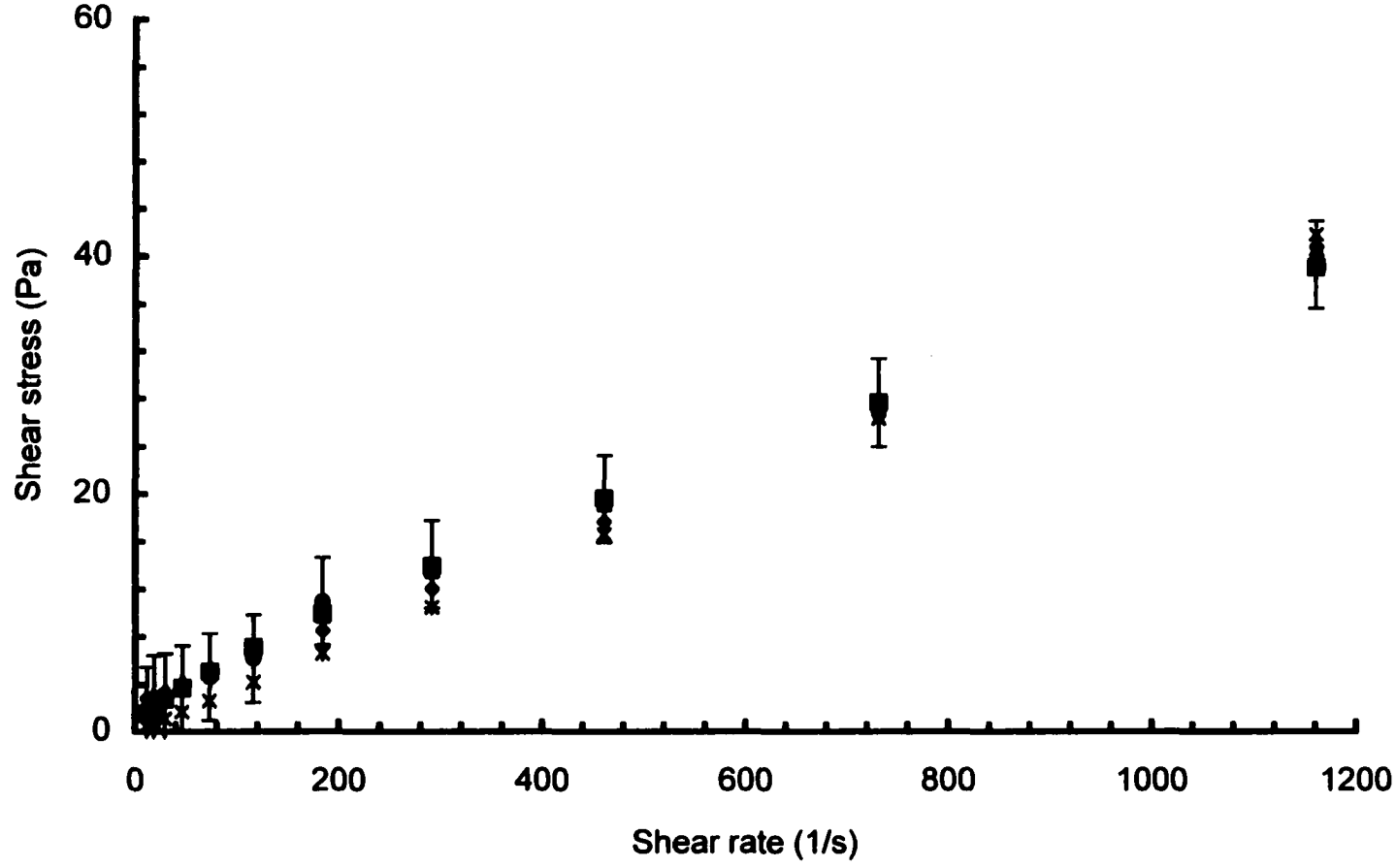

Figure 9.6. Rheological properties of degummed catfish oil at 25°C (Experimental, ●; Hershel-Bulkey, ■; Bingham plastic, ◆;Casson, ▲; and Newtonian, ✱) .

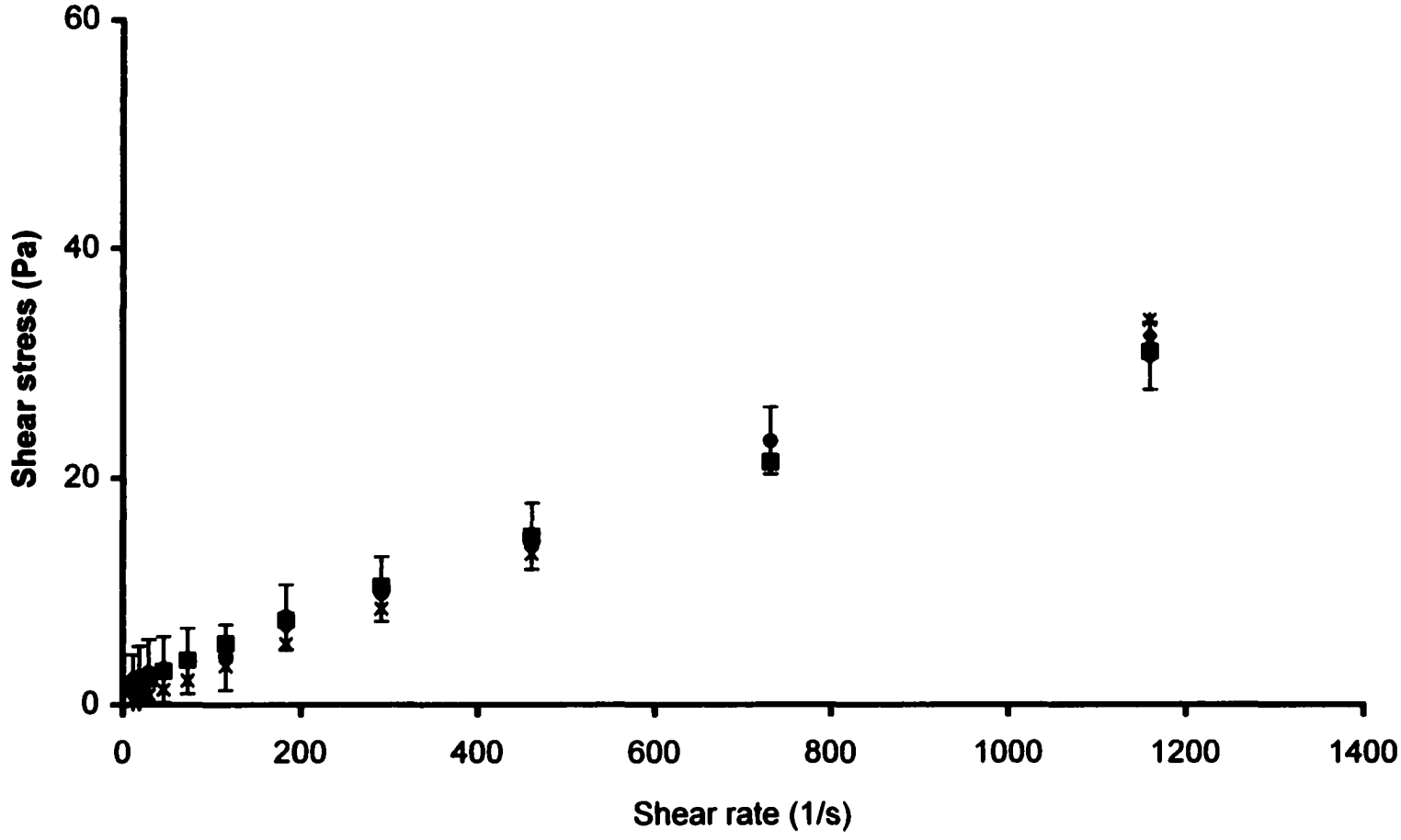

Figure 9.7. Rheological properties of neutralized catfish oil at 25°C models (Experimental, ●; Hershel-Bulkey, ■;Bingham plastic, ◆; Casson, ▲;and Newtonian, ✱)

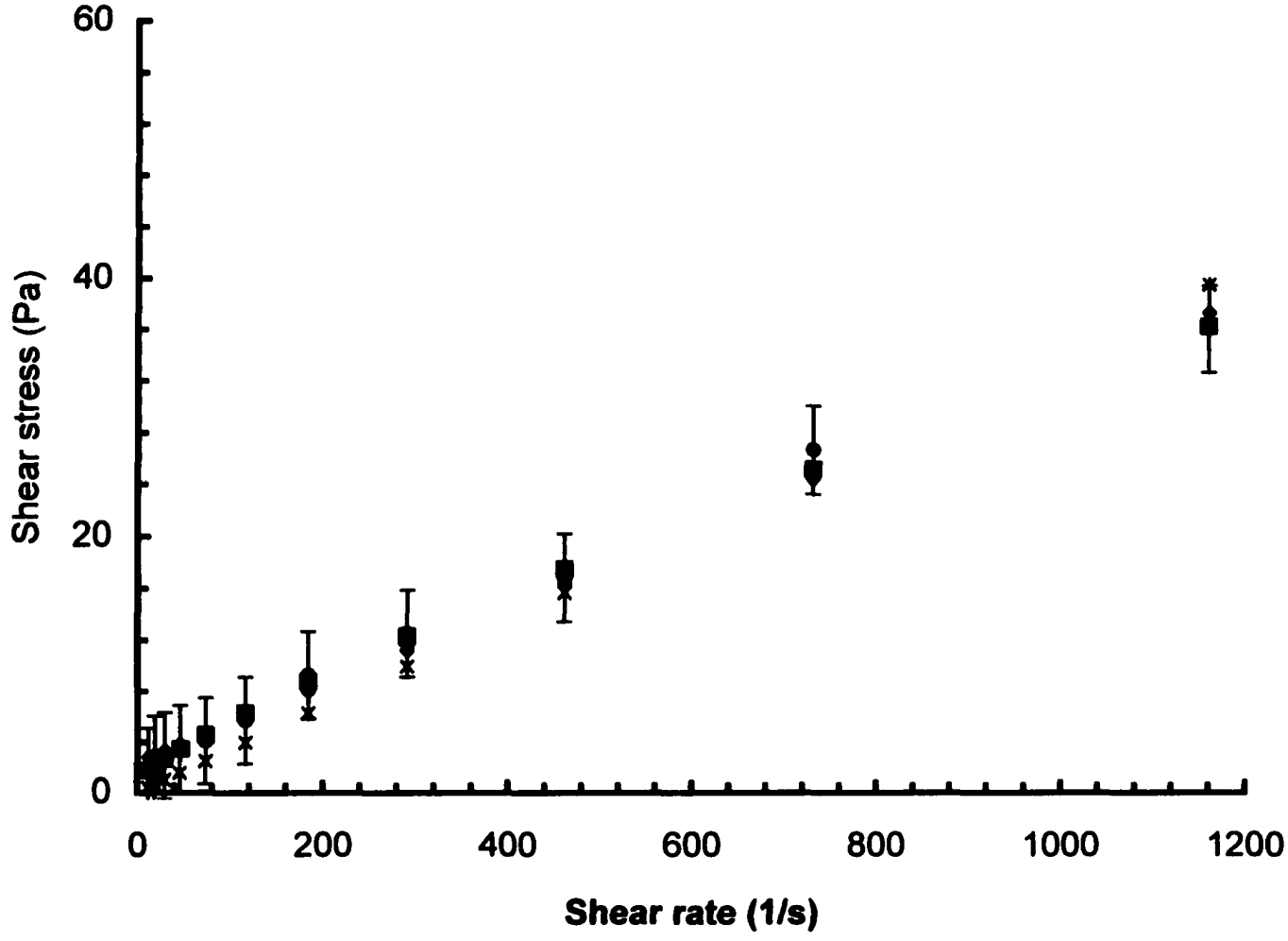

Figure 9.8. Rheological properties of bleached catfish oil at 25°C (Experimental, ●; Hershel-Bulkey, ■; Bingham plastic, ◆;Casson, ▲; and Newtonian, ✱)

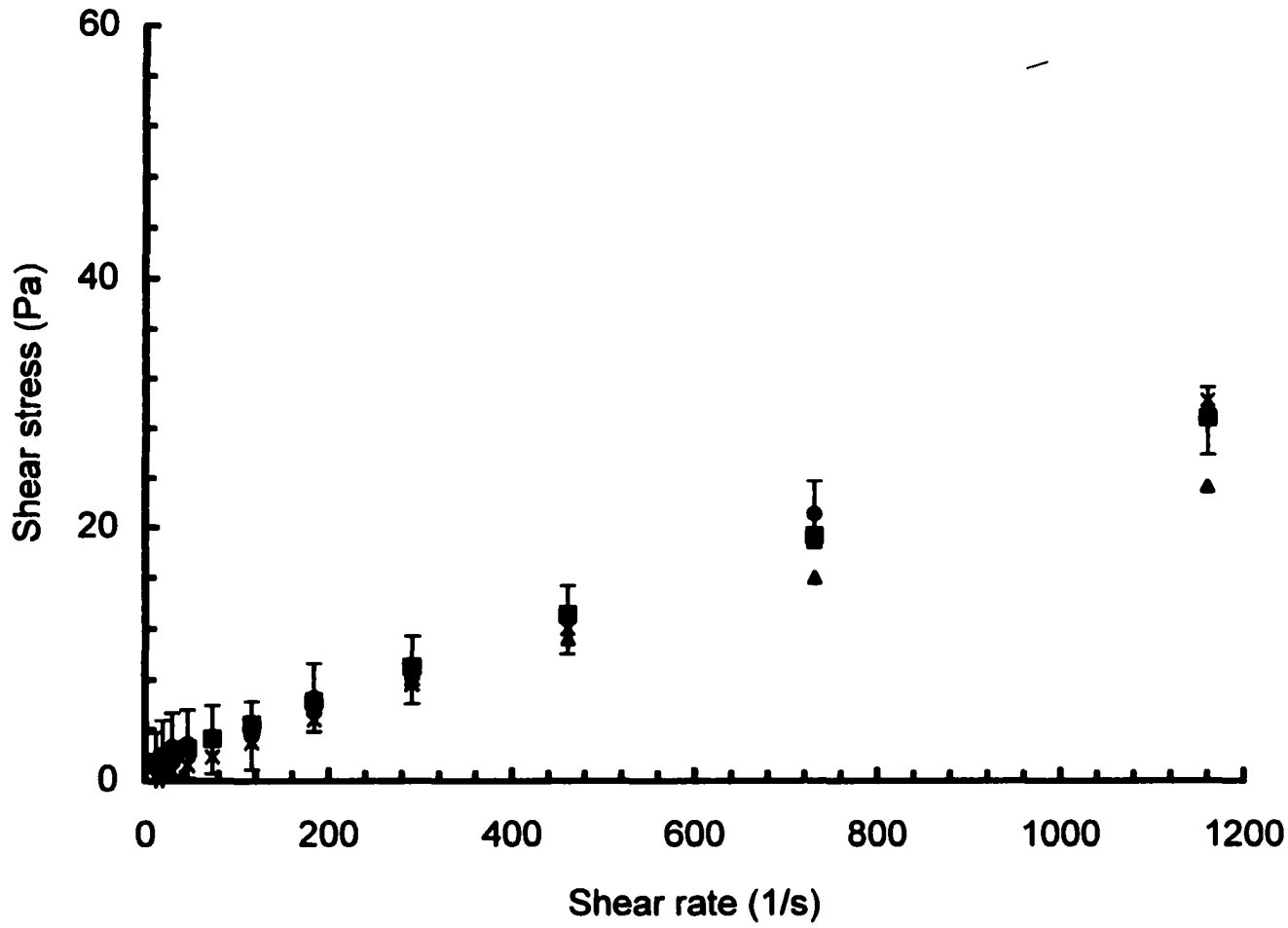

Figure 9.9. Rheological properties deodorized catfish oil at 25°C (Experimental, ●; Hershel-Bulkey, ■; Bingham plastic, ◆; Casson, ▲ ;and Newtonian, ✱)

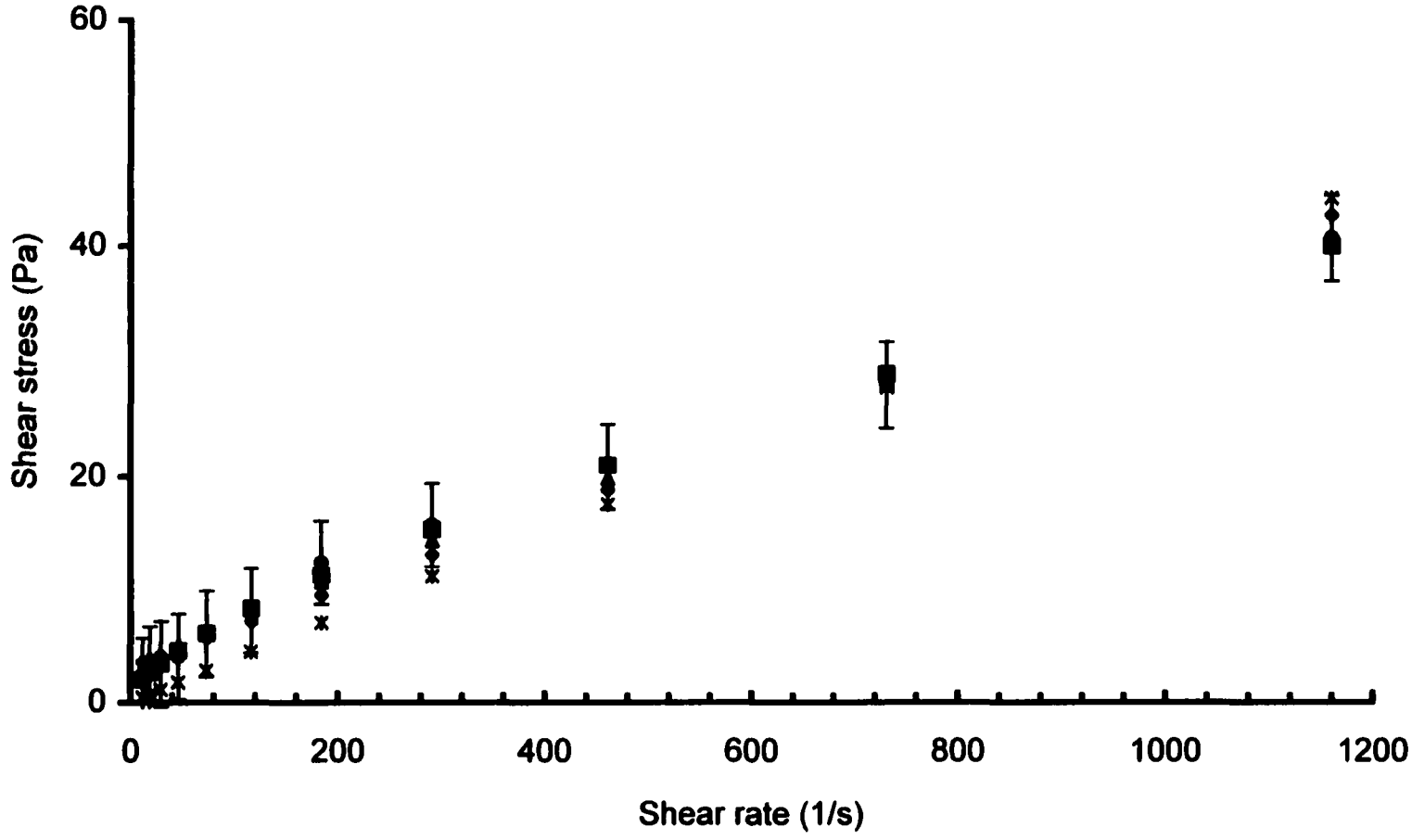

Figure 9.10. Rheological properties deodorized menhaden oil at 25°C (Experimental, ●; Hershel-Bulkey, ■; Bingham plastic, ◆;Casson, ▲; and Newtonian, ✱)

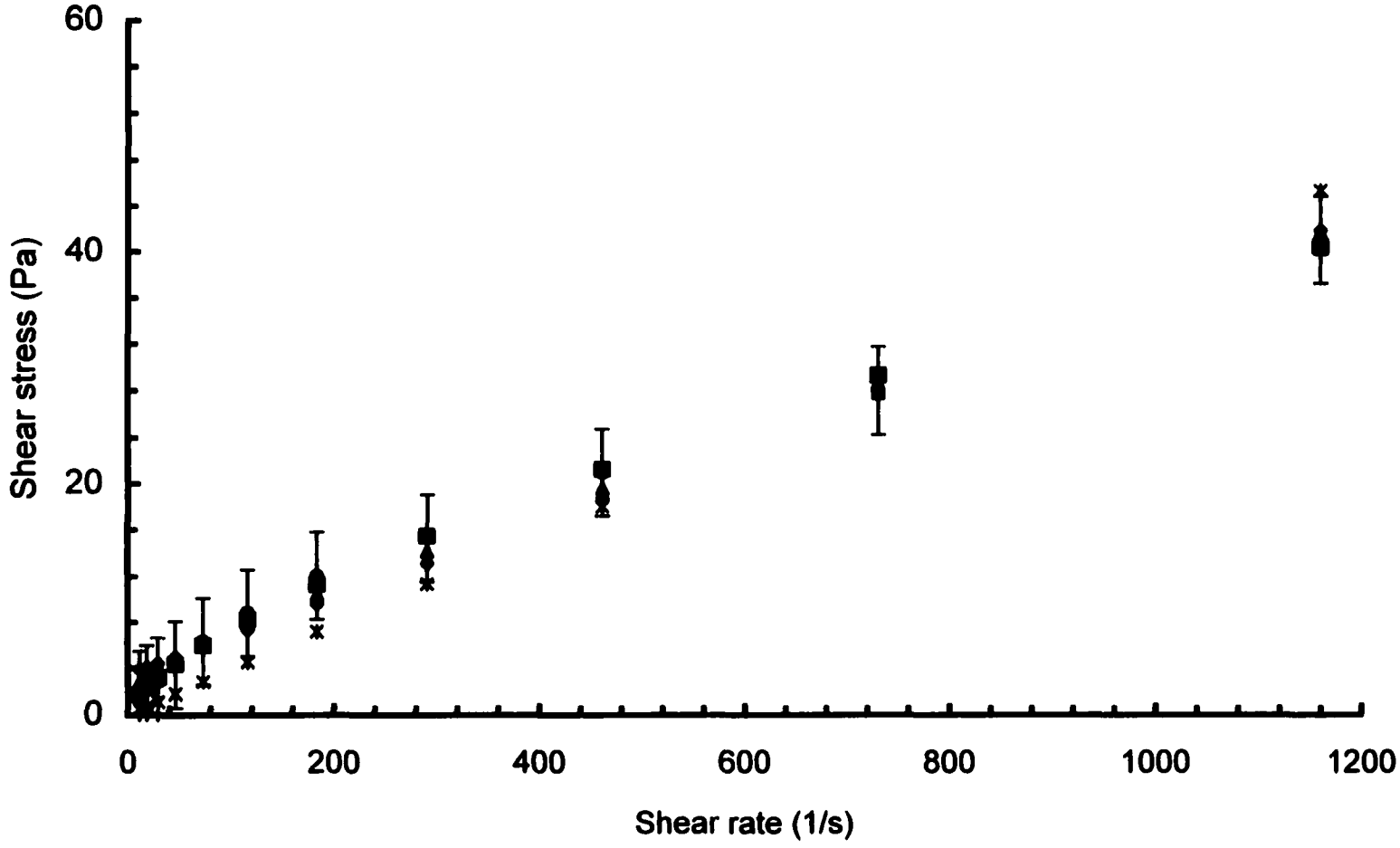

Figure 9.11. Rheological properties degummed menhaden oil at 25°C (Experimental, ●; Hershel-Bulkey, ■; Bingham plastic, ◆; Casson, ▲; and Newtonian, ✱)

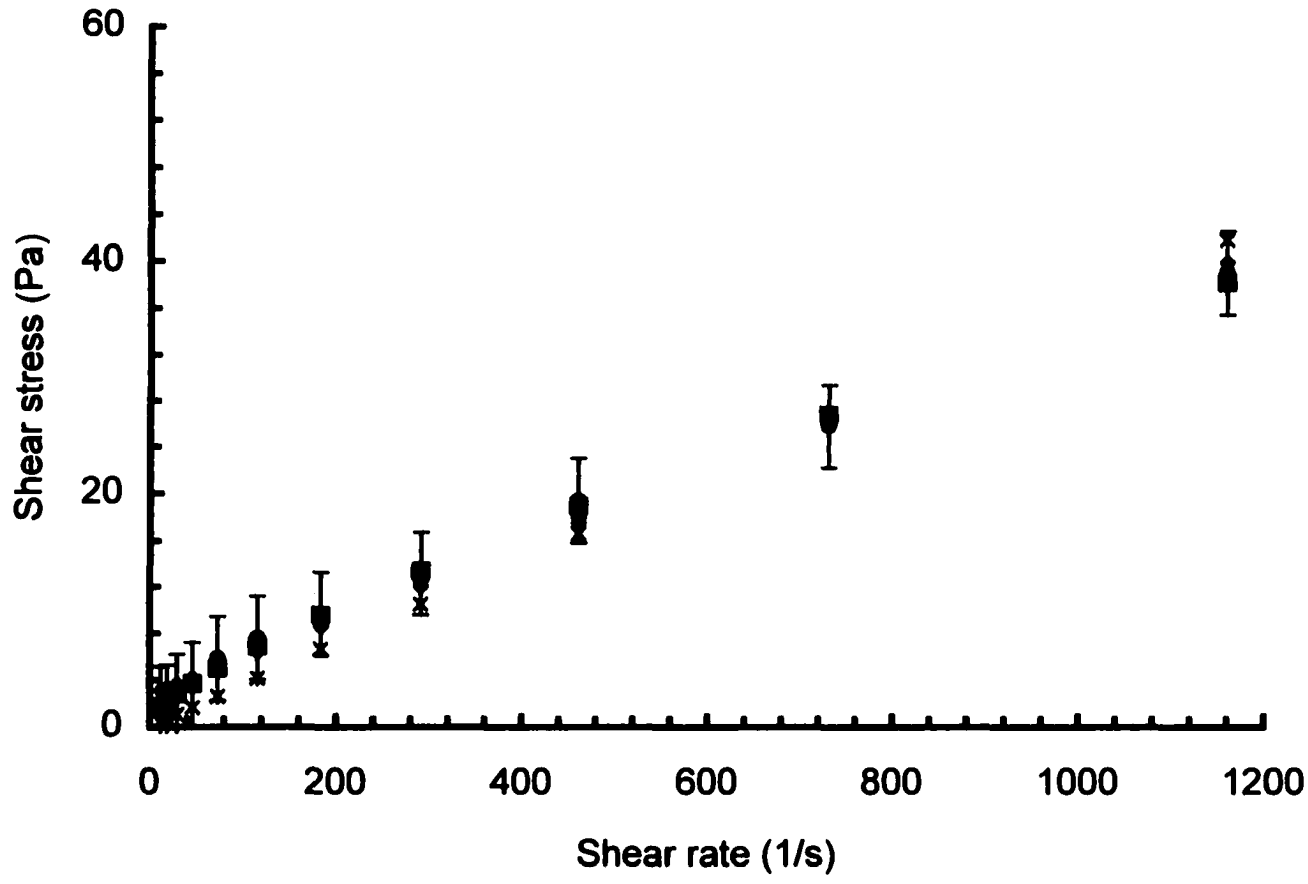

Figure 9.12. Rheological properties neutralized menhaden oil at 25°C (Experimental, ●; Hershel-Bulkey, ■; Bingham plastic, ◆;Casson, ▲; and Newtonian, ✱)

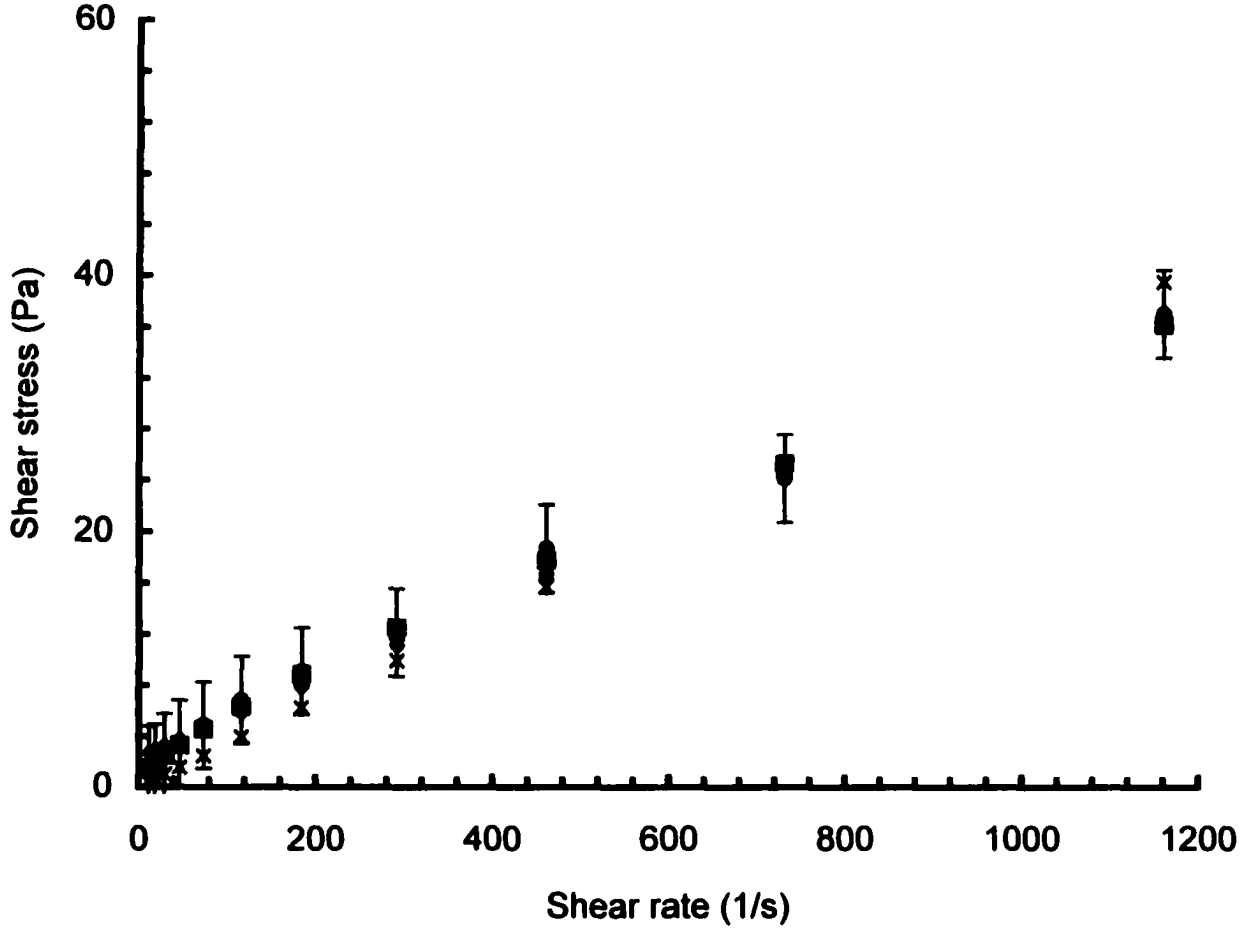

Figure 9.13. Rheological properties bleached menhaden oil at 25°C (Experimental, ●; Hershel-Bulkey, ■; Bingham plastic, ◆; Casson, ▲; and Newtonian, ✱)

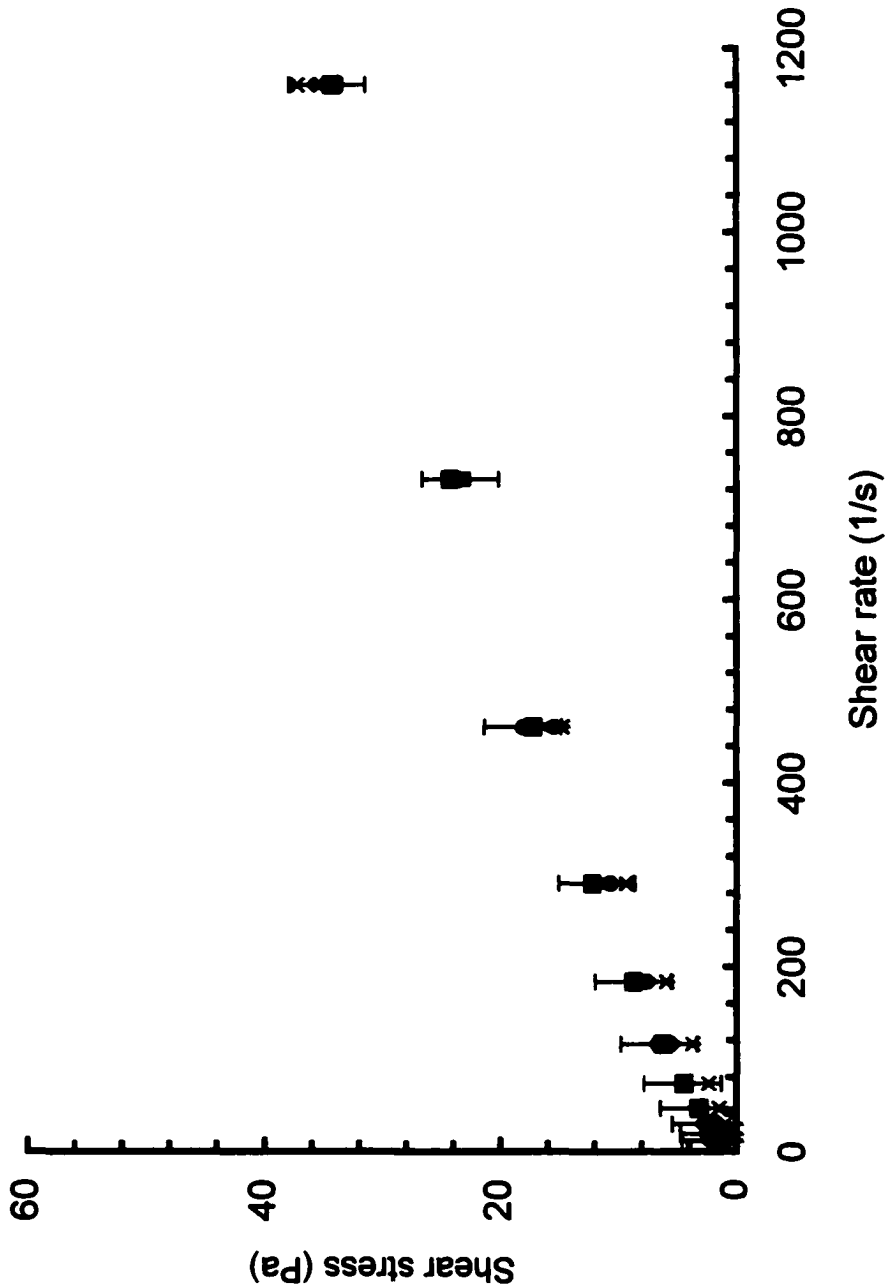

Figure 9.14. Rheological properties deodorized menhaden oil at 25°C (Experimental, ●; Hershel-Bulkey, ■; Bingham plastic, ◆;Casson, ▲;and Newtonian, ✱)

The plot suggests that the both crude oils behave like pseudoplastic fluids with a yield point (i.e., Casson or Herschel-Bulkley) at room temperature. These results also support the changes in flow characters from non-Newtonian to Newtonain due to removal of impurities from each processing step.

Wiedermann (1981) reported that crude oils consist of soluble impurities such as phospholipids, mineral metal complex (notably iron, calcium, and magnesium), free fatty acids (FFA), peroxides and their breakdown products were highly interactive with oil. The interaction between oil and impurities depends on size, shape, nature of intermolecular forces, chain length, present or absent of side chains, nature of polar groups, and existence of hydrogen bonding of molecules present in impurities.

The interaction between oil and impurities may be attributed to the formation of the aggregated colloidal dispersion system and often shows strong shear-thinning characteristics when shear force is applied to the system. Structural integrity of crude oils is disrupted when the shear is force applied (Weiss and McClements, 2000; Howe and Clarke, 1997). Both crude catfish and menhaden oils exhibited more shear thinning characteristic than did the oils after each processing step. Results obtained for free fatty acids and minerals support the above explanation. Ca, P, Mg, and Fe were presented in crude catfish oil but they were not detectable after neutralization process (Table 9.9). Free fatty acids were gradually reduced in subsequent processing steps. The oil which contains larger amounts of free fatty acids may require more shear forces to flow the oil (Teeter and Cowan, 1956). Degumming, neutralization, bleaching, and deodorization processing steps remove impurities.

Table 9.9 Characterization of catfish oil at different processing steps

Properties	Crude	Degummed	Neutralized	Bleached	Deodorized
Free fatty acid	4.53	4.28	4.25	3.80	3.25
Water activity	0.838	0.756	0.696	0.651	0.555
Magnesium (ppm)	4.99	4.64	-	-	-
Calcium(ppm)	10.7	-	-	-	-
Iron (ppm)	0.64	-	-	-	-
Phosphorus (ppm)	107.6	99.2	-	-	-

- (trace amount)

Gums and metal complex are removed by degumming or chemical refining; free fatty acids are removed by neutralizations; oxidation products are removed from bleaching (Wiedermann, 1981)

9.4 Conclusion

Rheological properties of catfish oil and menhaden oils at different processing steps: crude, degummed, neutralized, bleached, and deodorized were investigated. The Herschel-Bulkly, Bingham, Casson, and Newtonian model were used to fit to the experimental rheological data without exception, yield stress and consistency coefficient of both oils decreased due to removal of impurities from each processing step. After removal of impurities from each processing step; the flow behavior of oils shifted from non-Newtonian closer to Newtonian. The Casson model was chosen to predict the viscosity. The prediction coefficient (R^2) for crude oils were greater than 0.95.

Information from this study will be useful for the transfer or movement of bulk quantity of fish oil.

CHAPTER 10. SUMMARY AND CONCLUSIONS

Catfish industry is the largest aquaculture industry in North America. Approximately 260 and 240 million pounds of processed catfish were, respectively, sold by catfish processors in 1997 and 1998. Most catfish products are as sold either fresh or frozen fillets or whole-dressed fish with little "added value." The yield of fillets is between 45-55 % of the total weight of live catfish. Between 45- 55% of viscera, head, skin, intestine and slurries are generated as byproducts or processing waste. A whole catfish viscera consists of liver, digestive track (intestine and stomach), gallbladder and visceral storage fat, and the average weight is 265g, approximately 10% by weight of a live catfish. The average weight of catfish liver, gallbladder, digestive track and visceral storage fat is 65g, 8g, 90g and 80g, respectively. Our study showed that the crude fat content of the whole catfish viscera is 30-35% (wet basis). To the best of our knowledge, very little or no interest has been paid by the industries to add value to catfish viscera, which may very well as an excellent source of health promoting oil. Multimillion pounds of visceral oil that could be recovered from processing wastes have been wasted.

Omega-3 fatty acids have been claimed to help maintain heart and vascular health in human. Our study indicated that catfish visceral oil contains omega-3 fatty acids. A number of studies have shown that omega-3 fatty acids reduce the risk of arthritis, asthma, lupus, psoriasis, crohn's diseases, and are vital for the skin cells, keeping them moist and healthy. Numerous researches have also shown that omega-3 fatty acids may play a critical role in brain development of the fetus and infant, and are vital to healthy maintenance and function of the body. Omega-3 fatty acids are

particularly important during the last three months of pregnancy, and during an early infancy stage for proper development of eyes, brain, and nerves. Since the unborn baby cannot make its own omega-3 fatty acids, its mother must meet its needs.

Oil-riched fish and fish oil supplements are the most readily available source of omega-3 EPA and DHA. For general good health, taking two fish meals per week or 150-200 mg of omega-3 from fish oil or cod liver oil daily supplements will help maintain effective brain and body function. For heart disease prevention, two oil-riched fishmeals weekly or 400 mg of omega-3 from fish oil daily supplements will help maintain healthy blood flow.

The overall Ph.D. research goals were (1) to utilize and add value to catfish processing wastes and (2) to extract and purify oil recovered from catfish processing wastes for pharmaceutical uses. The specific research objectives were separately divided into nine major aspects, thus the dissertation was written accordingly. The First Chapter gives a comprehensive review of catfish processing wastes, utilization of processing wastes, health benefit of omega-3 fatty acids, and common extraction and purification steps of fish oil. The Second Chapter was devoted to characterization of fatty acids profiles of catfish oil recovered from the whole or portioned visceral parts. The fatty acids in the oils recovered from different visceral parts were identified and compared to those of fillets and nuggets. About 34% crude fat (wet basis) could be recovered from the whole catfish viscera, which is currently considered as processing waste and has no market value. The recovered catfish visceral oil was characterized by a high level of polyunsaturated fatty acids, which are similarly found in catfish fillets. The total ω-3 fatty acids of oils recovered from the whole and portioned viscera ranged from

4.3-20.9 mg/ g (dry weight basis). Results indicated that the whole catfish viscera might serve as a good source of edible oil that contains health-promoting fatty acids. Value addition to catfish processing wastes would economically impact the entire catfish industry.

The extraction procedure (cooking and physical separation) and purification steps (degumming, neutralization, bleaching, and deodorization) of catfish oil from viscera were elaborately described in the Third Chapter. The oil collected from each processing step was characterized for its chemical properties. Free fatty acids, peroxide value, CIE-L*a*b* color, and water activity of oil were measured. Material balances were designed to determine loss of total oil and individual fatty acids throughout the whole purifying process. Crude Menhaden oil was purified using our procedure and its quality was compared with that of the catfish visceral oil. The yield of deodorized catfish oil was 65.7% compared with 76.3% of deodorized menhaden oil. Major yield loss took place during the degumming process. The combined omega-3 fatty acids content of deodorized catfish oil was about 5 times less than that of the menhaden oil. The percent loss at 19.3, 27.77 and 21.24% was, respectively, observed for omega-3, saturated and unsaturated fatty acids after the deodorization process. Free fatty acid, water activity, and some minerals were decreased during processing. Bleaching removed pigment, thus resulting in oils with greater lightness and less yellowness.

The Forth Chapter involved development of a rapid method to analyze fatty acids profile of oils recovered from ground viscera containing high moisture. Gas chromatographic (GC) analysis is commonly used to determine fatty acid profiles of lipids in biological materials, which normally requires methyl esterification of fatty acids

(FAMEs). Organic solvents commonly used for fat extraction include chloroform, dichloromethane, hexane, toluene, benzene and methanol or a mixture of them. After extraction, the solvent is evaporated from the mixture by mild heating under nitrogen gas and it is a multi-step, time-consuming, labor-intensive procedure, not suitable for handling a large number of samples, and it may lead to introduction of contaminants and loss of esters. Therefore, it may not be practical in laboratories where a number of tests are required. The objective of this study was to develop a rapid microwave–assisted method for fatty acids analysis. The microwave heating power (%) and time (sec) required for maximal fatty acids recovery were determined. This research investigate the feasibility of preparing FAMEs from microwave-heated samples without solvent extraction. Catfish liver with about 75% moisture content was used for demonstration. Heating at 100% power for 80 sec yielded the highest recovery of DHA (C22:6 ω-3) and arachidonic acids (C20:4 ω-3). Fatty acid profiles of microwave-heated samples were different. C20:4, C16:0, and C20:0 are discriminating fatty acids. Recovery of C20:4, C16:0, and C20:0 of all microwave-heated samples was lower than that of the control, while higher recovery was observed for C20:1, C18:2, C16:1, and C22:6 at 80-100% power for 60-80 sec or 60% power for 80 sec. In addition to speed and ease of use, the advantages of the microwave-assisted technique were no solvent consumption and low energy consumption. The total process took a few minutes compared with several hours if done using conventional fat extraction methods. This technique also generated less chemical wastes.

In Chapter Five, the adsorption of free fatty acids (FFA) was studied. The adverse effect of adsorption on fatty acids was also investigated. Loss of fatty acids

generally increased with increased adsorption (contact) time. Combined omega-3 fatty acids (C18:3 and C22:6) were adsorbed up to 10.35%, 11.05%, and 22.8%, respectively, by chitosan, activated carbon, and activated earth after 5 hours. The FFA content adsorbed by adsorbents increased with increased contact time. Chitosan was more efficient in adsorbing FFA from crude catfish oil than activated carbon and activated earth. This study indicated that it is possible to use chitosan as an adsorbent for removal of FFA from crude catfish oil prior to a series of purification steps.

In Chapter Six, a column was designed to study the removal of impurities (e.g., free fatty acids, color) at different time intervals. The experimental data were used to determine the fixed-bed column capacity of each adsorbent. This study showed that chitosan could be used as an effective adsorbent for removal of free fatty acids (FFA) from crude catfish oil. Chitosan is also more effective than activated carbon for FFA removal as indicated by a narrower mass transfer zone and a steeper breakthrough curve. Almost 80% of the bed capacity was utilized when chitosan was used as an adsorbent. Calculated length of unused bed from the breakthrough curve and determination of total FFA fatty acids adsorbed up to the break point may provide very important information for the fish oil production industry.

In Chapters Seven and Eight, thermal properties such as melting points, specific heat capacity, enthalpy, and boiling points of catfish oils were studied using DSC and TGA. All individual fatty acids (except C18:1 and C20:2) of catfish oil had a weight loss of greater than 90% when heated up to 250°C. The resistance to heat of the fatty acids was dependent on chain length and degree of unsaturation. The weight loss of catfish oils increased with increased heating temperatures, regardless of the oil

purification process. There was no distinct weight loss of catfish oils until the temperature was over 350°C. All oil samples were decomposed after 550°C. The information from this study is useful for the design of processing units and cost analysis, if catfish oils are to be used especially as cooking oils.

The DSC thermogram indicated that significant changes in melting points of catfish oils were observed at different processing steps. A sharper melting point curve was observed in the purified deodorized catfish oil. The pattern of melting points observed for catfish oils was reflected by that of individual fatty acids, especially unsaturated fatty acids. The enthalpy of catfish oils increased after each purification step that removed impurities from the oil. There was not much difference for the specific heat capacity of catfish oils from different processing steps.

The Ninth Chapter demonstrated rheological properties of catfish oil collected from each purification step and comparison was made with that of menhaden oil. The Herschel-Bulkley, Bingham, Casson, and Newtonian model were used to fit the experimental rheological data. Without exception, yield stress and consistency coefficient of both oils decreased due to removal of impurities from each processing step. After removal of impurities from each processing step, the flow behavior of oils shifted from non-Newtonian closer to Newtonian. The Casson model was chosen to predict the viscosity. The prediction coefficient (R^2) for crude oils were greater than 0.95. Information from this study will be useful for the transfer or movement of bulk quantity of fish oil.

From this Ph.D. dissertation research, it is possible to produce catfish oil from processing wastes with the quality intended for pharmaceutical uses. Producing catfish

oil from processing wastes supports the "total resource utilization" and "value-added new product" concept. The research findings will benefit the whole catfish industries and will set a good model for fish oil recovery from other fish species as well.

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VITA

Subramaniam Sathivel, son of Mr. Subramaniam and Mrs. Latchumie, was born in Dickoya, Sri Lanka. He completed his higher secondary school in 1986 from Highlands Central College, Hatton, Sri Lanka. In 1987, Sathivel entered University of Peradeniya, Sri Lanka, and completed a Bachelor of Science with Honors in Agricultural Engineering in 1993.

He was later appointed as an instructor in the Department of Agricultural Engineering, Faculty of Agriculture, Sri Lanka for six months. In 1994, he joined the Open University of Sri Lanka as a lecturer and worked till 1996. During his job, Sathivel excelled in the research area of Food Processing and Engineering.

In 1996, Sathivel received Overseas Technical Training award to pursue a Master of Science degree in Food Technology (Process Engineering) and he completed his master degree at University of Reading, United Kingdom in 1997. He was accepted at Louisiana State University to pursue a Doctoral Degree in Food Science with a focus on Food Process Engineering in 1998. He received the National IFT fellowship for 2001. His research interest has focused on extraction, purification, and production design of catfish visceral oil. Currently, he is a candidate for the degree of Doctor of Philosophy, which will be conferred in December, 2001. Being at the right place and at the right time, Sathivel has been hired as an Assistant Professor in Seafood Processing and Engineering, at University of Alaska, Fairbanks.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Subramaniam Sathivel

Major Field: Food Science

Title of Dissertation: Production, Process Design and Quality Characterization of Catfish Visceral Oil

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

October 30, 2001