Development of refined oil from Lake Victoria Nile perch (*Lates niloticus*) viscera

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Declaration

This thesis is my original work and has not been presented for an award in any other institution.

…..…………………………………………… Date:…………………………...……………

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Approval

This thesis has been submitted for examination with our approval as university supervisors.

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Professor M. W. Okoth

Dedication

This work is dedicated to my mentors; my parents, siblings, friends and tutors who have natured me over the years in various ways. They have instructed, challenged, encouraged and provided leadership towards my development. I recognize my parents for imparting discipline and resilience. My teachers at all levels from primary, secondary, undergraduate to graduate levels, have demonstrated a kind of commitment to my progress, akin to voluntarism! THANK YOU ALL.

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ABSTRACT

Oil was extracted from Nile perch viscera by varying the wet acid rendering method, then the oil characterized for yields and quality. Viscera were obtained from a local fish filleting plant in Nairobi. Temperature of the oil extraction was maintained at approximately $93^{o}C$. The acid concentration varied from 0 – 10% and the heating time from 5 – 25 minutes. The oil was evaluated for yield, free fatty acids, oxidative and color qualities to compare the effect of acid concentration and heating time. Statistical analysis ($p \leq 0.05$) was done using GenStat 13th edition software. There was significant difference in yield, with interaction between acid concentration and heating time, the yield generally declining after 15 minutes. The yield generally increased from 56.0 ± 4.7% at 5 minutes heating without acid to 77.4 ± 1.0% after 15 minutes heating in 2% acid solution, but again generally declining after 15 minutes heating in all acid concentrations. The yield generally declined as acid concentration increased. Heating for 15 minutes produced the highest yield, with no significant difference at 0% and 2% acid concentrations. There was significant difference in free fatty acids (%FFA), with interaction between acid concentration and heating time, the values generally increasing with increase in acid concentration and heating time from 0.46 ± 0.08% for 5 minutes without acid to 0.92 ± 0.08% for 25 minutes heating in 6% acid solution. Heating times of 5 and 15 minutes produced the lowest %FFA at 0% acid concentration, 0.60 ± 0.05% for 15 minutes being significantly higher than 0.46 ± 0.08% for 5 minutes. There was significant difference in peroxide value (PV) with interaction between acid concentration and heating time, magnitude decreasing from 5.51 ± 0.40 to 1.63 ± 0.37mEq O_2/kg oil as acid concentration increased. There was significant difference in color intensity, with acid concentrations. Heating time did not produce a significant difference, and so was the interaction between acid concentration and heating time. There was significant difference in color intensity between 0% and subsequent acid concentrations, with a general increase as acid concentration increased. Based on oil yield and quality, 0% acid and 15

minutes heating is the most suitable combination for mass production of crude oil from Nile perch viscera.

Bulk oil was extracted from the viscera at 93°C for 15 minutes by 0% acid wet rendering. The oil was neutralized, deodorized and winterized to obtain refined (low melting point - LMP) and the high melting point (HMP) fractions for analysis. The oil fractions were analyzed for omega – 3 fatty acids (eicosapentaenoic acid – EPA and docosahexaenoic – DHA), vitamin A, vitamin E, iodine value (IV), saponification value (SV), density, melting point (MP), and smoke point (SP). The crude fish oil was used as control. Statistical analysis ($p \leq 0.05$) was done in GenStat 13th edition software. The yield of winterized oil (low melting point fraction) was 45.6 ± 0.5% of deodorized, 39.8 ± 0.5% of crude and 19.6 ± 0.5% of raw material weight. There were no significant differences in density and vitamin E (tocopherol) content of the three fractions of oil. There were significant differences in the slip MP, SP, SV, IV and vitamin A (retinol) content of the three fractions of oil. There were significant differences in EPA and DHA contents of the crude, low melting point (LMP) and the high melting point (HMP) fractions of the oil. EPA and DHA values for the LMP fraction (7.02 ± 0.58 and 33.76 ± 4.04% w/w of total fatty acids) were highest, followed by crude (2.76 ± 0.31 and 9.5 ± 0.16% w/w of total fatty acids) and HMP having lowest (1.2 ± 0.39 and 4.07 ± 0.54). High vitamins A and E as well as omega – 3 polyunsaturated fatty acids (PUFA) contents and high SP make the crude and HMP fractions suitable for cooking. The high amount of LMP fraction has high content of omega – 3 PUFA, making it suitable for use in food, feed and nutraceutics.

The two fractions were studied for storage stability. Each fraction was stored at room temperature (19 – 23°C) for 20 weeks in transparent and amber colored glass containers. They were analyzed for %FFA and PV at the beginning of storage and an interval of a fortnight thereafter, to assess the effect of package color and storage duration. Statistical analysis of data was done at $p \leq 0.05$ using GenStat software, 13th edition. There was a general increase in FFA and PV, with significant differences between oil fractions and package color over storage time.

For FFA, there was no interaction between the oil fraction and package color while for PV there was significant interaction between the oil fraction and package color. FFA increased from 0.26% to 0.59% for LMP fraction in clear package, 0.26 – 0.43% for LMP in red package, 0.22 – 0.85% for HMP in clear package, and 0.22 – 0.69% for HMP in red package. PV increased from 0.50mEq O_2/kg oil to 11.65mEq O_2/kg oil for LMP fraction in clear package, 0.50 – 10.58mEq O_2/kg oil for LMP in red package, 1.01 – 9.94mEq O_2/kg oil for HMP in clear package, after 20 weeks; and 1.01 – 6.86mEq O_2/kg oil for HMP in red package after 14 weeks. The levels of FFA and PV of low melting point fraction in colored package remained within the CODEX limits of 0.3% and 5mEq O_2/kg oil respectively for refined fish oils up to 18 weeks. The FFA of high melting point fraction surpassed the limit after 6 and 10 weeks for transparent and colored packages respectively. The FFA of low melting point fraction in transparent package surpassed the limit after 8 weeks. Only low melting point oil fraction in transparent pack had PV above limit at 18 weeks. Therefore, colored package is the most suitable for both oil fractions studied. In terms of FFA and PV, both high and low melting point fractions can retain good quality at ambient temperatures for 10 and 18 weeks respectively.

ABBREVIATIONS AND GLOSSARY OF TERMS

AOAC: Association of Official Analytical Chemists

AOCS: American Oil Chemists Society

CCFO: CODEX Committee on Fats and Oils

FAO: Food and Agriculture Organization

MAAIF: Ministry of Agriculture, Animal Industry and Fisheries (Uganda)

Mesenterium: *Synonym of mesentery*; a membranous fold attaching an organ to the body wall [Saunders, 2003]; a double layer of peritoneum attached to the abdominal wall and enclosing in its fold a portion or all of one of the abdominal viscera, conveying to it its vessels and nerves [Farlex, 2012].

Pyloric caecum: This an organ with fingerlike projections located near the junction of the stomach and the intestines. Its function is not entirely understood, but it is known to secret enzymes that aid in digestion, may function to absorb digested food, or do both [*Fish & Wildlife Conservation Commission*].

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 BACKGROUND

Formerly called Nile perch, the Lake Victoria Nile perch (*Lates niloticus*) is a freshwater fish found in central Africa's lakes and rivers [*Turon et al., 2005*]. Nile perch, *Lates niloticus* (L) is among fish species introduced in the lake about the 1950s and 1960s, to convert the small bony abundant haplochromines to fish flesh of commercial importance and for sport fishing [*Njiru et al., 2008; Turon et al., 2005*]. Due to its ecological tenacity, this voracious predator rapidly multiplied and accounted for approximately two thirds of the lake's fish population by the early 1990s [*Turon et al., 2005*]. Today, Nile perch has become the backbone of the fisheries in the three East African countries, contributing more than 60% of the total landings (including the marine sector). The Lake Victoria fisheries constitute an important source of protein for local communities and foreign exchange earnings via exports. More than 90% of Nile perch landed is exported. Besides, employment opportunities have been created in the harvesting, processing and marketing sectors of the industry [*Njiru et al., 2008; Mkumbo et al., 2007; Turon et al., 2005*]. Nile perch is the most dominant species from Lake Victoria [*Mbatia et al., 2010b; Gumisiriza et al., 2009; Ogwok et al., 2008; Turon et al., 2005*]. It is usually processed into fillets which are mainly exported to Europe [*Turon et al., 2005*]. Nile Perch (*Lates niloticus*) products [*CCFO, 2013*] represent over 90 percent of Uganda's fishery exports, where consumers demand it for the presence of key omega-3-fatty acids [*MAAIF, 2011; MAAIF, 2010*]. Byproducts account for nearly 50% of the total fish mass [*Ogwok et al., 2008*]. Shoes, belts and purses are made from tanned perch hide; dried swim bladders are sent to England for filtering beer and wine and to Orient for making soup stock [*Turon et al., 2005*].

Fish oil has been considered as an available source of long-chain omega (ω)-3 PUFA, especially EPA (C20:5n3) and DHA (C22:6n3). These molecules have well documented nutritional value and health benefits [*Del Nobile et al., 2009; Ogwok et al., 2008; Gbogouri et*

al., 2006; Aidos et al., 2002]. DHA and EPA are essential for the development of fetal brain. Foods rich in PUFA are in general, essential for normal growth and proper body function [*Ogwok et al., 2008*]. Intake of fish oil, which is an excellent source of omega-3 fatty acids, has been linked to promotion of human health to fight against numerous diseases. EPA and DHA are believed to be the main protective components of fish oil that act against certain types of diseases. EPA and DHA also exhibit anti-inflammatory action and increase survival for people with autoimmune diseases. Clinical studies have revealed that polyunsaturated fish oil treatments relieve patients who are suffering from rheumatoid arthritis. EPA and DHA are successful at this because they can be converted into natural anti-inflammatory substances called prostaglandins and leukotrienes that help to decrease inflammation and pain. Moreover, omega-3 fatty acids of fish oil are reported to associate with the brain development, and important for the vision and the functions of reproductive system. It may be due to DHA which is a component of brain nerve synapses, in the retina of the eye and in the testes and sperms [*Kim and Mendis, 2006*]. However, the long chain polyunsaturated fatty acid (LC-PUFA) content varies between fish species and with location and season of capture [*Ogwok et al., 2009; Ogwok et al., 2008; Gbogouri et al., 2006*]. The content of the essential fatty acids in fish is dependent on fish species and size, physiological demand and varies from tissue to tissue. Lipids are primarily located in the subcutaneous tissue, belly flap, muscle tissue, liver, mesenteric tissue, and in the head [*Kołakowska et al., 2003*]. Fish liver and belly tissues are widely recognized for the high level of PUFA and vitamin A in their oil [*Ogwok et al., 2008*]. Other than the liver, belly oil is mainly found in pyloric ceaca and messenteria [*Jaquot, 1961*]. Heads [*Sahena et al., 2010b; Turon et al., 2005*], roes [*Bekhit et al., 2009; Falch et al, 2006c; Remme et al., 2005*] and skins [*Sahena et al., 2010a; Aryee and Simpson, 2009*] of other fishes also possess substantial amounts of high quality oil (*refer to appendices for fish anatomy*). So far, the utilization of fish-processing wastes has received increasing attention as a promising means to increase revenue for producers and to decrease the cost of disposal or management of those wastes. Fishery wastes can be used for

enzyme recovery, protein hydrolysate production, collagen, gelatin, and lipids extraction [*Kosseva, 2013*].

The bulk of the world's fish oil is manufactured by the wet pressing method [*Sathivel, 2011; Rubio-Rodríguez et al., 2010; FAO, 1986*]. Other methods include enzyme extraction [*Sathivel et al., 2009*], ensilage process [*Crexi et al., 2010*], use of supercritical fluids [*Sahena et al., 2010a; Sahena et al., 2010b*] and organic solvent methods [*Aryee and Simpson, 2009*]. The latter two methods are used largely for analytical purposes or as secondary stages to maximize yield after the established processes. *Rubio-Rodríguez et al.* [*2010*] have reviewed the wet pressing, enzyme and supercritical fluid extraction processes. Dry rendering is widely practiced by artisanal fish dealers [*Kabahenda et al., 2009*], but produces lower quality oil.

Oily tissues from Nile perch processing are currently underutilized among fishing communities in East Africa. Roes are sold and consumed locally without value addition yet they have substantial amount of oil. A lot of skins are also discarded or freely given away for animal feeding, despite containing high quality oil. The rural folk use the viscera oil to fry the local market fish and chips, domestic cooking, and lately to feed infants [*Kabahenda and Hüsken, 2009*], the method of extraction being rendering [*Kabahenda et al., 2009; Ardjosoediro and Neven, 2008; Josupeit, 2006*].

1.2 FISH OILS

Fish oils are rich in ω-3 long-chain polyunsaturated fatty acids (ω-3 LC-PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are important for human health [*De Leonardis, 2008*]. PUFAs have pharmaceutical properties since it has been shown that EPA may account for the low incidence of coronary heart disease. Therefore, supplementation of western diets with PUFAs has been suggested [*Guil-Guerrero, 2001; Cleland et al., 2006*]. Omega – 3 fatty acids with 20 carbon atoms (i.e. EPA and arachidonic acid) are precursors of eicosanoids, such as prostaglandins, leukotrienes, thromboxanes, prostacyclines, lipoxins and hydroxy-derivatives of fatty acids. These molecules are important

for several cellular functions like platelet aggregability, endothelial cell mobility, growth of smooth muscle cells, and synthesis of white blood cells, endothelial cells and leukotrienes which are important for chemotactic factors [*Spurvey, 2002*]. Studies have shown that a large intake of n-3 fatty acids is beneficial with regard to lowering blood pressure, reducing triacylglycerols and modulation of cell function and cell reactivity to external stimuli [*Valdimarsson and James, 2001*]. Fish lipids contain up to 40% of ω-3 (LC-PUFA) that contain five or six double bonds [*Kosseva, 2013*].

Compared to the high neutral lipid content in fish muscle, phospholipids are found in minor quantities. However, they are relatively abundant in offals such as viscera and fish heads. They are a lipid class of great interest as carriers of LC-PUFA instead of triacylglycerols (TAG) or PUFA ethyl esters. Herring by-products, offals from salmon processing, tuna and sardine heads, and bonito ovaries, resulting from the filleting operation, can be used as raw material to produce oil since they are relatively rich in PUFA. In salmon, the lipid content is higher in the heads than in the fillets. The head oil has low acid and peroxide values, and higher iodine value than that from fillets, thus showing a greater amount of PUFA. DHA is the major fatty acid, threefold higher than EPA. Marine lecithin extracted from fish heads obtained immediately after filleting represents a valuable source of LC-PUFA much sought-after by the food, nutraceutics, and cosmetics industries [*Gbogouri et al., 2006*]. The fatty acid profile for crude herring oil extracted from fresh herring (*Clupea harengus*) byproducts (heads, frames, skin, viscera, etc.) has been established [*Aidos et al., 2002*]. Fresh-water fish fatty acids exhibit the unique ability to elongate their carbon chains through enzymatic desaturation, contrary to what is observed in the fatty acids found in other strictly carnivorous fish species [*Namulawa, Mbabazi and Kwetegyeka, 2011*]. The major fatty acids in carp viscera oil are: oleic, palmitic, palmitoleic, linoleic, and linolenic, constituting about 67% of total fatty acids. Palmitic acid is the dominant saturated fatty acid (SFA), accounting for about 50% of all SFA. Oleic acid is the most abundant monounsaturated fatty acid (MUFA) for refined oils. Palmitoleic acid is the second most

abundant MUFA for crude, bleached and refined oils. High levels of oleic, palmitoleic, and arachidonic acids have been reported as a characteristic property of freshwater fish oils. Linoleic and linolenic acids are the dominant PUFA [*Crexi et al., 2010*].

In fish, PUFA are contained in phospholipids of cell membranes, where they are closely packed and surrounded with proteins. Thereby, their susceptibility to degradation by heating may differ from that of pure PUFA. Like many fish species, their content of EPA and DHA does not decrease under various ways of cooking compared to raw fish. As in raw fish, the nutritive value of different cooked fish species differ in about ten times. Canned fish are more valuable products concerning the PUFA content. To get the recommended daily dose, one needs to eat only 40g of canned saury [*Gladyshev, Sushchik and Makhutova, 2013*]. In menhaden oil, phospholipids account for 5.5% of the total lipid, dominated by phosphatidylcholine, with small amounts of phosphatidylethanolamine, phosphatidylserine, sphingomyelin and lysophosphatidylcholine [*Spurvey, 2002*]. In salmon oil, Phosphatidylcholine is the major phospholipid (55.7%), followed by phosphatidylethanolamine (14%). Phosphatidylserine, phosphatidylinositol, sphingomyelin and lysophosphatidylcholine account for less than 10.5% [*Gbogouri et al., 2006*]. Phospholipids in marine species are generally unsaturated and esterified mainly with EPA and DHA. Other minor components of marine oils are fat-soluble vitamins, wax esters and cholesterol. The main vitamins in marine oil are vitamins A, D and E [*Spurvey, 2002*].

In a study of frozen mackerel (*Scomber scombrus*), increasing lipid hydrolysis was observed during the frozen storage over 12 months at -20°C; no differences between whole fish and fillets were found for free fatty acid formation. However, leaner mackerel showed a higher hydrolysis development than its fattier counterpart. Fillet lipid oxidation was found to be higher than in whole fish. Fattier (November) mackerel showed a higher oxidation development than its leaner (May) counterpart in the case of fillet products. Fillets showed a shorter shelf-life than their whole fish counterparts [*Aubourg et al., 2005a*]. The most active lipoxygenases (LOX) are in the gill and skin of fish. The skin contains active LOX, carotenoids, and the melanin radical

trapping mechanism, which significantly affects fish lipid oxidation. In addition, the skin provides a physical boundary from light and oxygen for subcutaneous lipids. In general, skin lipids undergo more rapid oxidation than muscle lipids. Compared with other food lipids, fish lipids are very sensitive to photooxidation, with thermooxidation being less pronounced [*Kołakowska et al., 2003*]. Studies on herring have suggested presence of an enzyme which catalyses oxidation of the oil and its activity is apparently increased by the presence of sodium chloride [*Tsuchiya, 1961*]. Intense heating in presence of oxygen accelerates autoxidation. Even though rancidity often begins to be noticeable when the peroxide value is between 20 and 40mEq/kg, far low peroxide values have been reported to impart noticeable rancidity in fish and fish products [*Egan et al., 1981*]. While presence of oxygen is necessary for peroxide development, it has been suggested that hematin compounds enhance oxidation, particularly of linoleic acid and fish liver oils. Antioxidants e.g. tocopherols can be destroyed by such agents as heat, enzymes and salt. Lipid oxidation causes postmortem changes in fish muscle, which make the tissue themselves more susceptible to oxidation [*Opara et al., 2007*]. Marine lipids comprise highly unsaturated fatty acids that are known to be very prone to lipid oxidation. Based on biochemical lipid damage indices (FFA, PV, and TBA), rancidity development in chilled Coho salmon has been shown to be slow. A relatively high content of the endogenous antioxidant astaxanthin (AX) has been observed in the white muscle of the starting fish. It most likely contributes to the observed lipid stability, as it scavenges free radicals, so that protection against the very early stages of lipid oxidation is favored [*Aubourg et al., 2005b*]. Deodorization of fish oils at temperatures above 180^0C offers the activation energy required for polymerization (intra and inter) and geometrical isomerization of LC-PUFA [*Fournier et al., 2006*]. For example, deodorization increased *trans* FA content for oleic and linoleic acids in refined carp viscera oils due to the high temperatures used [*Crexi et al., 2010*]. Visceral lipid of steelhead trout is more stable than muscle lipid against oxidation. The pro-oxidant potency of carotenoids *in vivo* may

be inhibited by many unknown mechanisms as they may shift their antioxidant activity into pro-oxidant activity *in vitro*, such as in post-mortem fish [*Zhong et al., 2007*].

Eggs from various fish species contain an average lipid content of 25.2%. A significant part of the fatty acids are unsaturated (78.5-83.7%). PUFA content ranges from 17.7% to 25.5%, DHA (13.1%) being the major PUFA. The shark eggs contain higher amounts of vitamin E than cod liver oil [*Remme et al., 2005*]. Roes from three tuna species, skipjack (*Katsuwonous pelamis*), tongol (*Thunnus tonggol*) and bonito (*Euthynnus affinis*) have been shown to contain 3.29-5.68% lipid [*Intarasirisawat et al., 2011*]. Cod roes contain high levels of phospholipids (79% of the total lipids). The neutral lipids in roe consist of TAG, cholesterol and monoacylglycerols (MAG). The fatty acid composition in roe showed that more than 40% of the fatty acids are $\omega-3$ fatty acids. PUFAs make up nearly half of the amount of fatty acids [*Falch et al, 2006c*]. The lipids in milt and roe consist of more than 70% phospholipids, higher than what is found in viscera [*Falch et al, 2006a*]. The lipid class distribution is nearly uniform throughout the fish body. The belly flap contains the highest amount of TAG and white muscle and visceral tissue have the lowest levels [*Aursand et al., 1994*]. *Falch* [*2006*] found no seasonal differences in the groups of fatty acids in liver, but the viscera contained lower levels of PUFA during the winter catch. The lipid classes were highly different among the fractions, liver and viscera. Principal component analysis showed that it was possible to classify what organ the lipids originate from depending on their lipid class composition or fatty acid composition. Fatty acid composition of common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*) and tambacu, a hybrid of tambaqui (*Colossoma macropomum*) and pacu (*Piaractus mesopotamicus*), was evaluated by gas chromatography. With storage at -20°C, fatty acid composition did not present wide variations due to storage time and preparation method [*de Castro et al., 2007*]

Using Soxhlet extraction, ground fish skin gave the highest while viscera gave the lowest total oil yield from different parts of Indian mackerel (g oil extracted/100g of material), based on dry weight. The longer-chain fatty acids of fish flesh, skin, viscera and heads are members of the

ω-3 and ω-6 family. The greatest amounts of the ω-3 fatty acids, especially EPA and DHA, were obtained from fish skin, followed by flesh, heads and viscera [*Sahena et al., 2010b*]. Some Thai freshwater fish particularly catfish have been shown to possess high content of DHA and EPA [*Chedoloh, Karrila and Pakdeechanuan, 2011*]. Mature salmon roe has lower moisture content and higher protein, lipid and ash contents compared with immature salmon roe, although the nutritional quality of the oil fraction is not different [*Bekhit et al., 2009*].

1.3 NILE PERCH

Nile perch (*Lates niloticus*) is the most important fish species, commercially, for the East African Countries of Uganda, Tanzania and Kenya. It is mainly derived from Lake Victoria, which is shared by the three countries [*Namulema et al., 1999*]. A study on a possible cyanotoxin contamination by cyanobacteria revealed low concentration of microcystins in the muscle of Nile perch obtained from Murchison Bay along Lake Victoria. The contamination of Nile perch was found to be far lower than that of tilapia (*Oreochromis niloticus*), basing on all organs tested [*Nyakairu et al., 2010*]. The ability of the Nile perch stock to withstand high fishing pressure over the years, instead of collapsing as predicted is associated with the high turnover or the production/biomass ratio, which has also increased over the years. The dominance of juveniles in the population supports this argument. Because of the high fecundity and the high recruitment rates of the Nile perch stock, which is resilient to fishing, recruitment over-fishing may not be an immediate threat to the fishery. However, increasing the size at first capture will allow more fish to spawn [*Mkumbo et al., 2007*]. Many of the local people claim to be allergic to the fish and many are intolerant to its flavor. Referred to with apparent contempt as *Mbuta*, the Nile Perch is the least favorite fish of all. This has resulted in it attracting lower prices [*Awori, 2005*].

In the Lake Victoria region, Nile perch oil is mostly extracted from fat pads from visceral and belly flaps. There is fat also found under the skin and the skin itself. Artisanal processors and traders often melt the pad to obtain oil to use in frying other fish products. The oil is used to deep fry fish and its by-products as it is cheaper than commercial fats and oils such as vegetable oil.

Fish oil is not commonly used in cooking other products due to the strong fishy smell. However, street food vendors dealing in deep fried perch use it to deep fry sweet potato and cassava chips to complement the fish products. Nile perch is also the largest contributor of roes sold in the region. It is estimated that an average Nile perch matures and starts spawning at 3 years of age. Each produces about 16 million eggs at a time. At harvest, a mature female has an egg sack weighing about ½ kg [*Kabahenda and Hüsken, 2009*]. Prior to the establishment of filleting factories, oil was extracted from gutting mature Nile perch and scrapping pads inside and around the guts and maws using hands or a knife. The pad was then melted to obtain oil for deep frying. Filleting techniques have improved accessibility of fish oil especially from the belly flaps and skins. Locally produced crude Nile perch oil is valuable in processing of different low-value fish products. In the Lake Victoria region, it is the major oil used to deep fry fish products such as whole fish, frames, skins, heads, eggs, and fish balls made from trimmings. However, there is concern that deep frying may reduce the nutritive value of the oil and the fried products, since oil is subjected to high temperatures and is reused many times [*Kabahenda et al., 2009*].

1.4 NILE PERCH OIL

Work has been done on Nile perch oil from different anatomical parts to find out its quality. Whole *L. niloticus* heads have oil content of 15–18% of dry weight in one study [*Turon et al., 2005*]. Another study showed that fatty material weight from the belly cavity of Nile perch varied from 1.06% to 2.10% of body weight (wet weight basis). Mean weight was highest in the medium fish category and lowest in the small category. Extracted oil from the belly tissue was highest in the large fish category, while the small category had the lowest content. Lipid deposition in Nile perch belly flap seems to increase with increasing fish size [*Ogwok et al., 2008*].

Crude oil from the heads had low FFA and its oxidative status (measured by PV) was quite acceptable. The 12 major FA (> 1mol%) found in crude Nile perch oil included 14:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2, 18:3, 20:4, 20:5, 22:5 and 22:6. The saturated FA accounted for 39.9

mol%, comparable to contents observed in freshwater fish from tropical lakes. The pre-dominant FA was 16:0, accounting for about 60% of all saturated FA. Combined ω-3 PUFA (18:3, 20:5, 22:5 and 22:6) accounted for 16% of total FA. PUFA levels in the fish examined (57.3%) were higher than those observed in many tropical fish. A reported level of PUFA in tropical freshwater fish is between 5.8% and 29.5%. The distribution pattern of FA in the acylglycerols resembles that observed for tuna head oil [*Turon et al., 2005*].

In belly oil vitamin A content varies with fish size; oil from the large fish has the highest as vitamin A bioaccumulation occurs with age. The content of vitamin A in oil from the large fish is comparable to that reported for cod liver oil. Belly oil contains high contents of β-carotene and α-tocopherol; carotenes increase while tocopherols decrease with increase in fish size. This increase in carotene is partly attributed to bioaccumulation. The α-tocopherol content of the oils is related to diet, since fish cannot synthesize vitamin E. The carotene and tocopherol content in the belly oil is similar to values reported for ray (*Rhinoptera steindechneri*) liver oil. But the α-tocopherol content is higher than those reported for crude oil from cod liver and seal blubber [*Ogwok et al., 2008*].

The positional distribution of fatty acids in N. perch viscera oil is such that SFA, palmitic acid (51%) and myristic acid (58%) are high in *sn*-2 position while unsaturated fatty acids EPA, docosapentaenoic acid (DPA) and DHA are preferentially esterified in *sn*-1, 3 positions [*Mbatia et al., 2010b*]. Long-chain PUFA tend to be preferentially esterified at the 2-position in fish and invertebrate triacyl-*sn*-glycerol. The saturated fatty acids (SFA) and/or MUFA tend to be preferentially esterified at the *sn*-1 and/or *sn*-3 positions. In phospholipids, PUFA are also preferentially located at position-2. Freshwater fish contain less (n-3) PUFA than marine fish and the content of (n-3) PUFA increases with increasing latitude, which can be explained by adjustment of the membrane function to environmental constraints. Phospholipids, as structural lipids, are rich in LC-PUFA. Various organs of a species show similar fatty acid compositions to each other. The fatty acid profile of phospholipids can be used for identification (taxonomic)

purposes. However, the PUFA compositions of the total phospholipids of various species of fish and shellfish are not strikingly different, but the differences that do occur involve the n-3 PUFA, particularly DHA [*Kołakowska et al., 2003*].

Nile perch belly oil contains substantial amounts of palmitic, palmitoleic, stearic, oleic, DPA and DHA fatty acids. Fractions of myristic, linoleic, linolenic and EPA acids also exceed 1% of the total fatty acids. DHA, DPA and EPA are dominant among PUFA. The total PUFA is higher than most reported in tropical freshwater fish [*Ogwok et al., 2008*]. Total PUFA is close to that in Atlantic herring [*Ogwok et al., 2008; Aidos et al., 2002; Aidos, 2002*] and mackerel. DHA is higher than in marine catfish and standard menhaden oil. Total MUFA is generally higher than for freshwater fish. The proportion of individual fatty acids varies with fish size except stearic acid. PUFA:SFA ratios (exceeding 0.50) are higher than that for marine catfish but similar to that for crude herring oil [*Mbatia et al., 2010b; Ogwok et al., 2008; Aidos et al., 2002; Aidos, 2002*]. Considerable levels of ω-3 and ω-6 fatty acids exist, and their ratios (total ω-3)/(total ω-6) vary from 0.83 to 1.03. Nutrient intake estimates show that ratio close to 1:1 has significant health benefits. Based on this fact and high PUFA:SFA ratios, consumption of Nile perch oil can be considered important for human health, especially to stabilize or even lower blood cholesterol [*Ogwok et al., 2008*].

Oxidative resistance (measured by PV) of Nile perch belly oils increases with increase in fish size. This is due to lower level of unsaturation and possibly high content of β-carotene. Generally, fish oils rich in both β-carotene and α-tocopherol are known to possess high resistance to oxidative stress. These natural antioxidant vitamins have free radical quenching ability, capable of slowing down or terminating the onset of oxidative deterioration processes. There is formation of FFA if oil is subjected to accelerated oven test, partly attributed to the activity of residual lipases and phospholipases. Studies have shown that storage of oil at ambient temperature for a month is equivalent to one day of storage under Schaal oven conditions at

65°C. Based on this, crude Nile perch oil can generally be considered stable for at least five months of storage at ambient temperature, based on hydrolytic changes [*Ogwok et al., 2008*].

1.5 OIL EXTRACTION

Lipids from animal tissues are extracted largely by rendering. To maximize yield, pressing step is usually included after rendering, and sometimes solvent extraction may be included to obtain residual fat/oil from rendered tissues. Pressing squeezes out residual fat/oil from the rendered tissues, resulting in more oil and a compact cake (meal). Solvent extraction is usually done using Soxhlet method, employing appropriate solvent. Rendering is the extraction of fats and oils from animal tissues by heat. Dry rendering is where heat evaporates the water and fat/oil melts out. Wet rendering is where the tissue is heated without evaporation of its moisture or by addition of water/steam injection [*Hamilton and Bhati, 1980; Gunstone and Norris, 1983*].

In dry rendering of tallow, the raw materials are boiled in their own juices until most of the water has evaporated. When the solids contain about 10% moisture, the tallow and dried solids are separated. Typically, the material boils at about 100°C for about one hour. At some point during the cook, the temperature increases from 100°C to about 135°C over 30 minutes. Raw materials consisting of fat and bone contain less water and can be cooked in 55-90 minutes with a final temperature of 105°C to 125°C. Total cooking time depends on the amount of water in the raw material. In wet rendering, raw materials are heated in their own juices, with or without steam injection. Temperature can range from 60°C to 100°C. Continuous and semi-continuous systems use a short heating time of 5-20 minutes at about 95°C. The materials are comminuted and highly fluidized to achieve rapid heat transfer into the particles [*MLA, 1997; Anderson, 2006*]. The quality of products from wet rendering is claimed to be better than that from dry rendering. Since the tallow temperature does not exceed 100°C, it usually has a low bleached color even though the raw material may be dirty. No evidence suggests that the sterilization of meals from wet rendering systems is any less than from dry systems [*MLA, 1997*].

In a study on broiler breast skin, conventional oven was set at 176.7^{O}C. Increased rendering times increased the yield of fat up to an optimal rendering time of 40 minutes. Yield of skin fat from water cooking (ratio of skin to water was 1:2) showed a similar trend as the yield of fat was optimum after 40 minutes [*Sheu and Chen, 2002*].

1.5.1 Fish Oil Extraction

As stated earlier, the bulk of the world's fish oil is manufactured by the fishmeal process which is largely wet rendering also referred to as wet pressing [*Rubio-Rodríguez et al., 2010; FAO, 1986*]. Dry rendering is widely practiced by artisanal fish dealers [*Kabahenda et al., 2009*], but produces oil of lower quality [*Sparre, 1965*]. Extremes of pH and the high temperatures can cause lipolysis, resulting in FFA. FFA cause foaming, produce off-flavor, reduce oxidative stability, surface tension, smoke point and general quality of product. Some lipolysis also occur postmortem prior to rendering operations due to enzyme action, but these enzymes are usually inactivated by the operational temperatures [*McClements and Decker, 2008; Nawar, 1985; Dugan Jnr, 1976*].

Studies on solvent extraction have shown that Soxhlet method can work with hexane and petroleum ether although hexane gives higher yield. But Soxtec-hexane gives highest oil yield. For the various solvent systems, the FFA is quite low [*Aryee and Simpson, 2009*].

Enzyme extraction involves treating the material with protease to hydrolyze tissue protein and release oil. Extreme pH and temperatures accelerate protein hydrolysis but destroy the structures of EPA and DHA. Therefore, moderate reaction conditions are chosen e.g., pH = 3 at 20°C, with antioxidant added to decrease the oxidation of EPA and DHA [*Sun et al., 2002*].

Ensilage can involve either acid or fermentation [*Crexi et al., 2010; Rai et al., 2010*]. In fermentation ensilaging, sugar and salt are added to raw material and allowed to undergo fermentation for three days at about 37°C. This can be natural or by means of selected inoculated culture. Fermentation results in production of organic acid accompanied by pH reduction, hence suppressing spoilage microorganisms. In lactic acid fermentation, both intestinal and bacterial

proteases are active, causing hydrolysis of tissue protein resulting in release of oil. Acid ensilaging of fresh water fish viscera has been shown to have negative effect on visceral proteases. Fermentation may also result in formation of peptides possessing antioxidant activity which can prevent lipid oxidation [*Rai et al., 2010*]. Ensilage acid process involves comminution, acidification with glacial acetic acid (10% v/w) with an antioxidant, then silage at 25-35°C for about 15 days. The oil is then recovered by conventional means [*Crexi et al., 2010*].

Fishmeal process involves subjecting the raw material to grinding, cooking, screening and oil recovery [*Rubio-Rodríguez et al., 2010; Crexi et al., 2010; Aidos, 2002; FAO, 1986*]. The walls of the fat cells are broken down before the temperature reaches 50°C. The oil is then free, and can theoretically be separated from the solid material. Coagulation of fish protein is very rapidly completed at about 75°C. The problem, therefore, is primarily a question of heat transfer and temperature control to ensure a uniform, optimum temperature throughout the whole mass. Cooking is usually done at 95-100°C for 15-20 minutes, exact time-temperature combination depending on raw material quality and practical experience [*FAO, 1986*].

1.5.2 Effect of Extraction Processes on Fish Oil

Oil yield of 59% has been achieved from herring byproducts using wet pressing in a pilot plant, with possibility of higher yield [*Aidos, 2002; Aidos et al., 2001*], but pumping and separation speed affect quality [*Aidos et al., 2003; Aidos, 2002*]. In general, this traditional process leads to good results when using fish products or by-products with high-oil content such as herring, tuna, sardine, salmon, etc, but not so feasible for low oil content. Experiments with hake (*Merluccius capensis–Merluccius paradoxus*) skin and the stuck muscle showed that the oil–water emulsion obtained after cooking the fish by-products was fairly stable and difficult to separate. Recently, there has been a proposal for a new method to replace the cooking step by a pH adjustment to the iso-electric point of the protein of the raw material. This method proved promising for extracting oil from high-oil pelagic species [*Rubio-Rodríguez et al., 2010*].

Crude carp viscera oil obtained by ensilage acid has been shown to possess higher free fatty acid (FFA) values than that by fishmeal process [*Crexi et al., 2010*]. This is due to action of endogenous enzymes present in the viscera prior to acidification [*Falch, 2006; Falch et al, 2006a*]. Acid value (AV) and thiobarbituric acid (TBA) values of the fishmeal crude oil were higher than values of ensilage crude oil, due to the use of high temperatures during the fishmeal process, which potentially causes increased oxidation. Lovibond color was higher in ensilage crude oil than that obtained by fishmeal process. Oil pigmentation during ensiling is shown to be caused by the release of the acid hydrolysis product of hemoglobin, hem. In ensilage oil an increase in FFA content occurs, resulting in formation of lipid–protein complexes, and consequently increasing color [*Crexi et al., 2010*]. The oil yield from viscera of freshly harvested Indian major carps (*Rohu* and *Catla*) increased during fermentation and maximum recovery was observed on the 3rd day. After 1 day of fermentation nearly 75% of the total lipid in the viscera was recovered by centrifugation of the fermented mass. The yield increased to nearly 85% by 3rd day of fermentation. Lipase activity decreased during fermentation to almost a third on the 3rd day as the decrease in pH during fermentation influences activity of lipases [*Rai et al., 2010*].

In a study, it was demonstrated that extraction method affects recovery of the crude, FFA and peroxide value (PV) of catfish viscera oil. Enzyme extraction recovered the highest amount of crude oil, but the oil had higher FFA and PV values. Cold extraction recovered a lower amount of crude oil but the oil had lower FFA (except for wet rendering) and PV values [*Sathivel et al., 2009*]. Enzyme hydrolysis of salmon frames with proteases and the composition of the different fractions obtained after separation by centrifugation have been studied. Results show that ω-3 enriched oil can be extracted with a good recovery (about 77%) along with several interesting products such as peptides or essential amino acids [*Rubio-Rodríguez et al., 2010*]. During enzymatic hydrolysis of raw materials containing high amounts of lipids (10–30%), amount of added water has also been reported to have a significant influence on the amount of separated oil and emulsion fraction with the highest oil yield and lowest amount of emulsion

being attained in the absence of water [*Mbatia et al., 2010a*]. Carp crude oils obtained by the ensilage and fishmeal processes resulted in high unsaturated and polyunsaturated fatty acid contents, and ω3/ω6 ratios around [*Crexi, Souza-Soares and Pinto, 2009*]. Supercritical fluid extraction (SFE) has been done using supercritical carbon dioxide (SC-CO_2) which has also been shown to be a good solvent of natural fish oil. The extraction of sardine oil with SC-CO_2 is possible and the conditions to recover most of the oil (95%) without degradation of the ω-3 PUFA can be determined. Oil extraction using SC-CO_2 as extracting solvent over freeze dried hake gave rise to very good results [*Rubio-Rodríguez et al., 2010*]. SFE may be a useful method to prevent lipid oxidation, especially in fish oils rich in omega-3 fatty acids such as salmon oil, and, to reduce significantly the amount of certain pollutants such as some arsenic species (mainly polar derivatives). It has also been observed that SFE may involve the co-extraction of some endogenous volatile compounds soaked in the raw material, such as amines or short chain organic acids, when performed in a closed system, which reduce oil quality by increasing the fishy odor and the acidity. The success of a SFE method is therefore highly dependent on the quality and freshness of the raw material and, in some cases, coupling a subsequently deodorization step would be required. Fractionation of the extract in two separators after SFE is an easy way to enhance fish oil quality by reducing the amount of free fatty acids as well as some oxidation products [*Rubio-Rodríguez et al., 2012*].

1.6 FISH OIL REFINING

Neutralization is usually the first step in refining as degumming is not ordinarily carried out in fish oils processing, because they have a very low amount of phosphatides. In some refineries however, an acid pre-treatment, designed to hydrate gums and remove phosphorous and other trace metals, is applied to oil as it enters the alkali refining plant. Degumming has been shown to effectively reduce lead, copper, arsenic, and zinc in menhaden oil [*Sathivel, 2011*]. Neutralization is an alkali refining purifying treatment designed to remove free fatty acids, phospholipids and gums, pigments, insoluble matters, etc. from crude oil. An alkali solution is

added to crude oil and combines with FFA present in the oil to form soaps. The alkali most commonly employed for neutralization oils is caustic soda. However, it saponifies some of the neutral TAG, causing a higher purifying loss. Gums absorb alkali and are coagulated by hydration, much of the pigments are degraded, adsorbed on the gums or made water soluble by the alkali and the insoluble matters are drained with the other coagulated materials [*Sathivel, 2011; Wanasundara, 1996*]. Concentration of sodium hydroxide (NaOH) is 10% on average, amount depending on %FFA i.e. %NaOH = %FFA x 0.142 [*Gunstone and Norris, 1983*].

Deodorization has been considered as a unit process that finally establishes the oil flavor characteristics that are most readily recognized by the consumer. Steam deodorization is possible because of the great differences in volatility between the TAG and the substances that give oils and fat their natural flavors. It is essentially a process of steam distillation where the volatile compounds are stripped from the non-volatile oil. Due to current harvesting and processing practices, high concentration of PUFA, and other contaminants, crude fish oils are easily subjected to deterioration. This severe deterioration changes the flavor quality. Off-flavors arise from metabolite contaminants, fish oil protein spoilage, or oxidation of the fish oil itself. Analysis has found that many odor compounds 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 affect the flavor quality. Therefore, undesirable odors and volatile components should be removed during refining and deodorization to obtain food grade oil with good quality. Since FFA are more volatile than glycerols, it is also possible to remove them from the oil by high temperature steam distillation. Hence this process is called “physical refining” [*Sathivel, 2011; Wanasundara, 1996*]. However, fish oils are not normally physically refined because they are too unstable. The highly unsaturated TAG would polymerize during the distillation and produce a rapid flavor reversion after refining. A cation strong-acid microporous resins process for the refining of fish oil for human consumption has been explained. Conventional refining processes

require high temperature treatment that damage ω-3 fatty acids in fish oil [*Sathivel, 2011; Crexi et al., 2010; Wanasundara, 1996*]. On the other hand, resins that do not require high temperature, are of consistent quality and can be regenerated [*Sathivel, 2011*]. Furthermore, heat treatment involved bleaches pigments such as carotenoids [*Gunstone and Norris, 1983*]. Oil is sparged with steam at 170–250ºC under reduced pressure to remove the oxidation products responsible for off-flavor. At higher temperatures (>220ºC) there is isomerization of *cis* to *trans* bonds so highly unsaturated oils should be deodorized at the lowest possible temperature. This applies especially to fish oils with their highly unsaturated long-chain PUFA which should not be heated above 180ºC [*Gunstone, 2008; Fournier et al., 2006; Wanasundara, 1996*]. Therefore, modifications of conventional deodorization process have been sought to deodorize marine oils. A pilot-scale procedure has been developed for deodorizing fish oil by high vacuum distillation at low temperatures (below 150°C) and produced a bland oil without destroying the long-chain PUFA [*Wanasundara, 1996*]. Polyunsaturated fatty acids only retain their important nutritional properties if they remain in the all-*cis* forms [*Gunstone, 2008; Fournier et al., 2006*].

Fractionation or winterization operations in the processing of edible oils are basically the separation of oils into two or more fractions with different melting points. Melted oils are directly cooled and allowed to form crystals, and the crystalline mass is separated from the remaining fraction [*Sathivel, 2011; Rajah, 1996*]. For therapeutic purposes, the natural sources may not provide the necessary amount of ω – 3 fatty acids and hence production and use of n-3 concentrates may be required. The n-3 concentrates may be produced in the free fatty acid, simple alkyl ester, and acylglycerol forms. To achieve this, physical, chemical, and enzymatic processes may be employed for concentrate production. The available methods suitable for this purpose, on an industrial scale, are: low-temperature crystallization, fractional or molecular distillation, urea complexation, chromatography, supercritical fluid extraction, and enzymatic splitting, etc. These procedures have been used, albeit to different extents, by the industry to prepare concentrates that are often sold in the ethyl ester form or re-esterified with glycerol to be

offered as TAG to the market. However, it has been demonstrated that acylglycerols are more stable than their corresponding ethyl esters. Regardless, the modified oils need to be stabilized using synthetic or preferably natural antioxidants [*Shahidi and Alasalvar, 2011*]. Three major commercial processes are available for the fractionation of fats. These combine the crystallization and separation processes [*Rajah, 1996*]. Among methods that concentrate PUFAs as acylglycerols without prior hydrolysis, are solvent fractionation, winterization, and molecular distillation. Concentrations of up to 30% EPA and DHA are feasible using these methods. However, higher levels (65% to 80%) are attainable by processes combining either hydrolysis or esterification with methods such as supercritical fluid extraction, urea complexation, and molecular distillation. Concentrations beyond 90% are possible with high-performance liquid chromatography [*Sahena et al., 2009*]. SC-CO_2 provides technical viability of fractionating fish oils with lower contents of EPA and DHA in the TAG molecules [*Lopes et al., 2012*]. The objective of winterization processes is to remove trisaturated and disaturated glycerides, waxes, and other non-triglyceride constituents. It is a slow process, and the entire winterization process from start to finish takes up to six days. If a solvent such as hexane is added, the oil viscosity is reduced. Fish oils are cooled in the presence of solvent yielding high-melting crystals that are separated by filtration [*Sathivel, 2011*]. Dry fractionation of oils and fats also known as low temperature crystallization is a pure physical process which separates high melting triacylglycerols from low-melting triacylglycerols by crystallization from the melt [*Wanasundara, 2011; Deffense, 2000*]. Apart from blending, it is the cheapest process in oils and fats processing [*Deffense, 2000*]. It involves cooling to 13°C for 6-12 hours and further to 7°C without addition of chemicals then filtering [*Hamilton and Bhati, 1980; Gunstone and Norris, 1983; Hoffman, 1989*]. Poor quality crystals are formed as a result of rapid cooling; the crystals group together and form clumps within which part of the liquid phase is occluded. Hence there is a decrease in olein yield of up to 10%. It has been reported that a low yield could also result from intersolubility and the formation of mixed crystals [*Rajah, 1996*]. Crystallization is a mild

procedure and especially suitable for PUFA, but the separation of PUFA from one another works less satisfactorily than the separation of unsaturates from saturates [*Wanasundara, 2011*]. Other research, however, showed that cooling rate and temperature has little influence on the yield and contents of EPA and DHA, suggesting that the use of ambient temperatures would offer a practical choice for large-scale separation and production of ω – 3 PUFA concentrates [*Shahidi and Wanasundara, 1998*]. Since chemical methods partially destroy the natural all *cis* ω-3 PUFA structure [*Halldorsson, Kristinsson and Haraldsson, 2004*], considerable attention is being given to microbial lipases as EPA and DHA are resistant to hydrolysis by commercial lipases [*Ferraro et al., 2010*].

1.7 PROBLEM STATEMENT

In fish processing [*CCFO, 2013*], viscera are generally considered waste products and often discarded [*Sun et al., 2002*]. Fatty materials from Nile perch belly flaps are presently discarded or sold at very low price [*Ogwok et al., 2008*]. Lipid waste is sold in Tanzania for use as food or fuel [*Gumisiriza et al., 2009*]. Fish oils from fatty species contain high levels of vitamin A, a natural antioxidant. Based on its high content of vitamin A and essential fatty acids, particularly DHA, DPA and EPA, Nile perch oil is beneficial to human health [*Ogwok et al., 2008*]. A survey of fish processing wastewater has indicated that the solid fish waste consists of viscera among others. The fish processing wastewater contained a very high concentration of lipids. The fish waste utilization methods mainly include local sale of solid fish residues such as fish frames used as food by the local community. However, the residues are sold at low prices that cannot pay off their handling costs. High lipid content of fish processing wastewater leads to the formation of thick fat layers, covering the surfaces of the treatment ponds [*Gumisiriza et al., 2009*] and is therefore a potential contributor to environmental pollution.

1.8 JUSTIFICATION

There are 150 – 200 metric tons of visceral fatty waste from Kenyan fisheries handling establishments [*Fisheries Department, 2013; 2009*]. The utilization of the otherwise fish

processing wastes such as viscera is essential for value addition. Upgrading byproducts would also reduce fish processing waste problems and increase revenue. Nile perch has high belly material weight and oil yield. The belly oil has high content of vitamin A, essential fatty acids (particularly DHA, DPA and EPA) and natural antioxidants [*Ogwok et al., 2008*]. Acid ensilaging provides prospect for improving fish oil extraction. In line with the foregoing background, prospect was envisaged of producing nutritious fish oil from Nile perch viscera. This is hoped will be alternative to existing imported commercial product range such as cod liver oil that are sources of $\omega - 3$ fatty acids. Consequently, studies were carried out to explore the possibility of improving the conventional wet rendering method of extraction in terms of yield and quality, by acidification. Further studies were done to establish the physico-chemical characteristics as well as storage quality of the refined Nile perch viscera oil.

1.9 OBJECTIVES

1.9.1 General

To develop refined Nile perch viscera oil.

1.9.2 Specific

1.9.2.1 To optimize acidic wet rendering for extracting Nile Perch viscera oil.

1.9.2.2 To determine the physico-chemical characteristics of refined Nile perch viscera oil.

1.9.2.3 To determine the storage stability of refined Nile perch viscera oil.

CHAPTER 2

2.0 OPTIMIZATION OF ACID ENSILAGING METHOD FOR THE EXTRACTION OF OIL FROM NILE PERCH (*Lates niloticus*) VISCERA

ABSTRACT

Oil was extracted from Nile perch viscera by varying the wet acid rendering method, then the oil characterized for yields and quality. Viscera were obtained from a local fish filleting plant in Nairobi. Temperature of the oil extraction was maintained at approximately 93°C. The acid concentration varied from 0 – 10% and the heating time from 5 – 25 minutes. The oil was evaluated for yield, free fatty acids, oxidative and color qualities to compare the effect of acid concentration and heating time. Statistical analysis of data was carried out at $p \leq 0.05$ using GenStat 13th edition software. The results showed that there was significant difference in yield, with interaction between acid concentration and heating time. The yield generally increased from 56.0 ± 4.7% at 5 minutes heating without acid to 77.4 ± 1.0% after 15 minutes heating in 2% acid solution, but again generally declining after 15 minutes heating in all acid concentrations. The yield generally declined as acid concentration increased. Heating time of 15 minutes produced the highest yield, with no significant difference at 0% and 2% acid concentrations, giving yields of 75.8 ± 6.9 and 77.4 ± 1.0% respectively. There was significant difference in free fatty acids (%FFA), with interaction between acid concentration and heating time, the values generally increasing with increase in acid concentration and heating time from 0.46 ± 0.08% for 5 minutes without acid to 0.92 ± 0.08% for 25 minutes heating in 6% acid solution. Heating times of 5 and 15 minutes produced the lowest %FFA at 0% acid concentration, 0.60 ± 0.05% for 15 minutes being significantly higher than 0.46 ± 0.08% for 5 minutes. All the %FFA values were within permitted limits for crude fish oil, however. There was significant difference in peroxide value (PV) with interaction between acid concentration and heating time, magnitude decreasing from 5.51 ± 0.40 to 1.63 ± 0.37mEq O_2/kg oil as acid concentration was increased.

Heating times of 15 and 25 minutes produced lower PV for 0% acid concentration, with no significant difference between the heating times. All the PV were nevertheless within permitted limits (3 – 20mEq O_2/kg oil) for crude fish oil. There was significant difference in color intensity, with acid concentrations. Heating time did not produce a significant difference, and the interaction between acid concentration and heating time was not significant. There was significant difference in color intensity between 0% and subsequent acid concentrations, the color intensity generally increasing as acid concentration was increased from 0.17 ± 0.13 absorbance for 15 minutes without acid to 1.11 ± 0.37 absorbance for 5 minutes heating in 6% acid solution. On the basis of oil yield and quality, 0% acid and 15 minutes heating is the most suitable combination for mass production of crude oil from Nile perch viscera.

2.1 INTRODUCTION

Nile perch (*Lates niloticus*) is a freshwater fish of central Africa's lakes and rivers [*Turon et al., 2005*]. It is among fish species introduced into Lake Victoria about the 1950s and 1960s, to convert the small bony abundant haplochromines to fish flesh of commercial importance and for sport fishing [*Njiru et al., 2008; Turon et al., 2005*]. Due to its ecological tenacity, it rapidly multiplied to dominance of about two thirds of the lake's population by early 1990s [*Turon et al., 2005; Ogwok et al., 2008*]. Today, it is the backbone of the East African fisheries, contributing above 60% of the total landings (including the marine sector). The Lake Victoria fisheries are an important source of protein for local communities and foreign exchange earnings via exports. More than 90% of Nile perch landed is exported, creating employment opportunities in the harvesting, processing and marketing sectors of the industry [*Njiru et al., 2008; Mkumbo et al., 2007; Turon et al., 2005*]. Nile perch is processed into fillets, mainly exported to Europe [*Turon et al., 2005*]. Byproducts are nearly 50% of the total fish mass [*Ogwok et al., 2008*]. Among the byproducts, the viscera are considered wastes and often discarded [*Sun et al., 2002*]. The fatty tissues from the Nile perch belly flaps are also discarded or sold cheaply [*Ogwok et al., 2008*].

Fish oil is a rich source of long-chain ω-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and have high nutritional value and health benefits [*Del Nobile et al., 2009; Ogwok et al., 2008; Gbogouri et al., 2006; Aidos et al., 2002*]. DHA and EPA are essential for fetal brain development. Foods rich in PUFA are essential for normal growth and proper body function [*Ogwok et al., 2008*]. The essential fatty acid (EFA) content depends on fish species, size and physiological demand, and within the same fish, varies from tissue to tissue. Fish liver and belly tissues are widely recognized for high PUFA and vitamin A contents in their oil [*Ogwok et al., 2008*]. Other than the liver, belly oil is mainly found in pyloric ceaca and messenteria [*Jaquot, 1961*]. Heads [*Sahena et al., 2010b; Turon et al., 2005*], roes [*Bekhit et al., 2009; Falch et al, 2006c; Remme et al., 2005*] and skins [*Sahena et al., 2010a; Aryee and Simpson, 2009*] of other fishes also possess high quality oil.

The bulk of the world's fish oil is extracted by the wet rendering method [*Sathivel, 2011; Rubio-Rodríguez et al., 2010; FAO, 1986*]. Other methods include enzyme extraction [*Sathivel et al., 2009*], ensilaging [*Crexi et al., 2010*], supercritical fluid extraction [*Sahena et al., 2010a; Sahena et al., 2010b*] and organic solvent methods [*Aryee and Simpson, 2009*]. The latter two methods are used largely for analytical purposes or as secondary stages to maximize yield. *Rubio-Rodríguez et al.* [*2010*] have reviewed the wet pressing, enzyme and supercritical fluid extraction processes. Dry rendering produces oil of low quality [*Sparre, 1965*], but is widely practiced by artisanal fish dealers [*Kabahenda and Hüsken, 2009*]. Acid ensilaging provides prospect for improving fish oil extraction [*Rubio-Rodríguez et al., 2010; Crexi et al., 2010*]. This study was designed to optimize the wet rendering method in terms of acid concentration and heating duration for oil yield and quality.

2.2 MATERIALS AND METHODS

2.2.1 Materials

2.2.1.1 Raw materials

Nile perch viscera were purchased from a fish filleting factory in Nairobi (W. E. Tilley Group) in late November 2011. They had been stored frozen in the factory for two days before collection. They were transported in cool box and stored in the University's Pilot Plant in a deep freezer operating at -18°C. The samples were analyzed and processed within ten days.

2.2.1.2 Chemicals for analysis

The reagents were of analytical grade, purchased from chemical stores in Nairobi. Petroleum ether 40 – 60°C (*Merck – RSA*) was used for extractable oil content. Citric acid (*BDH – England*) was used for crude oil extraction processes. Sodium hydroxide ampoule (*Rankem, India*), phenolphthalein indicator (*Loba Chemie, India*), ethanol (*Scharlan – Scharlab, Spain*) and diethyl ether (*Kobian Scientific*) were used for FFA determination. Chloroform (*Rankem*), glacial acetic acid (*Sigma Aldrich*, *Germany*), potassium iodide (*Panreac Quimica SAU*, *Spain*), sodium thiosulphate (*Rankem*) and starch indicator (*Merck – RSA*) were used for PV. Butanol (*Merck – RSA*) was used as solvent in determination of color intensity.

2.2.2 Methods

2.2.2.1 Oil extraction

Prior to oil extraction, the sample (*Fig. 2.1*) was minced to pass through 7.0 mm (0.276 inch) screen producing a mass shown in *Fig. 2.2*) then tumbled to achieve uniformity. The mass was then deep frozen, wrapped with polythene film to avoid gain or loss of water while awaiting oil extraction as long as there was no processing space. For each extraction, 1kg of sample was mixed with 1kg solution of citric acid with concentrations 0, 2, 4, 6, 8 and 10% (w/w). For each mix, the time of heating at 93°C varied in intervals of 5 minutes from 5 to 25 minutes. Each extraction was carried out in triplicate. The cooking was done in a covered pan. Oil was recovered by sieving the cooked mass through two layers of muslin cloth. When the mass had

settled in the separating funnel, it was partitioned into stick-water, aqueous layer and oil. The former two layers occupied the bottom and middle positions and were drained off, leaving the oil in the funnel. The residue in the muslin cloth was pressed using a plate and frame press to remove more adhering oil. This was also passed through the separating funnel to remove stick-water and emulsion. The oil from the two steps were combined and weighed to calculate yield as processed. Oil obtained from each process was calculated as percentage of total extractable oil content of the sample by the following equation:

$$\%\ \text{Oil yield} = \frac{\text{weight of recovered oil}}{\text{extractable oil content x weight of sample cooked}} \times 100$$

2.2.2.2 Analytical methods

The sample was first analyzed for moisture content by the air-oven method [*AOCS, 1998*]. Determination of the extractable oil content was done by modification of the Soxhlet method as described by *Egan et al.* [*1981*] for fatty fish sample to get the total oil. Petroleum ether 40 – 60°C was used as solvent. The determinations of moisture and extractable oil contents were each done in triplicates.

$$\%\ \text{Moisture content} = \frac{\text{weight loss}}{\text{sample weight}} \times 100$$

$$\%\ \text{Extractable Oil content} = \frac{\text{weight of solvent extracts}}{\text{original weight of sample before moisture determination}} \times 100$$

Determination of Free fatty acids (%FFA) was done volumetrically using aqueous sodium hydroxide standardized to 0.1M from ampoule and 1% phenolphthalein indicator in ethanol according to *AOCS* (*1998*) method Ca 5a-40. A neutral mixture (50 ml) of diethyl ether: ethanol (1:1) was used as a solvent. FFA values were reported as % oleic acid by weight. This was used to express the extent of hydrolysis in the oil.

Determination of peroxide value (PV) was done and expressed as mEq O_2/kg oil, according to *AOCS* (*1998*) method Cd 8b-90. Oil samples were dissolved in chloroform and mixed with glacial acetic acid and freshly prepared saturated potassium iodide solution.

Liberated iodine was titrated with sodium thiosulphate standardized to 0.01M solution using 1% starch indicator. This was used to represent the extent of oxidation in the oil.

Determination of color intensity was done by use of uv – visible absorbance spectrophotometry (*CECIL Elegant Instruments, CE 4400 – England*), employing standard methods [*Egan et al., 1981; AOCS, 1998*].

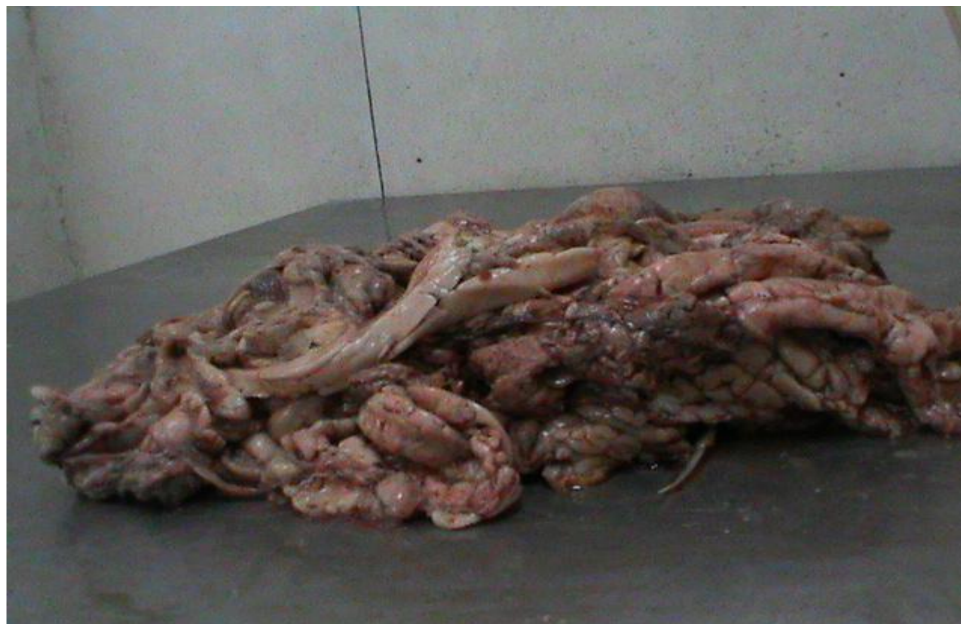

Fig. 2.1: Fresh sorted Nile perch viscera

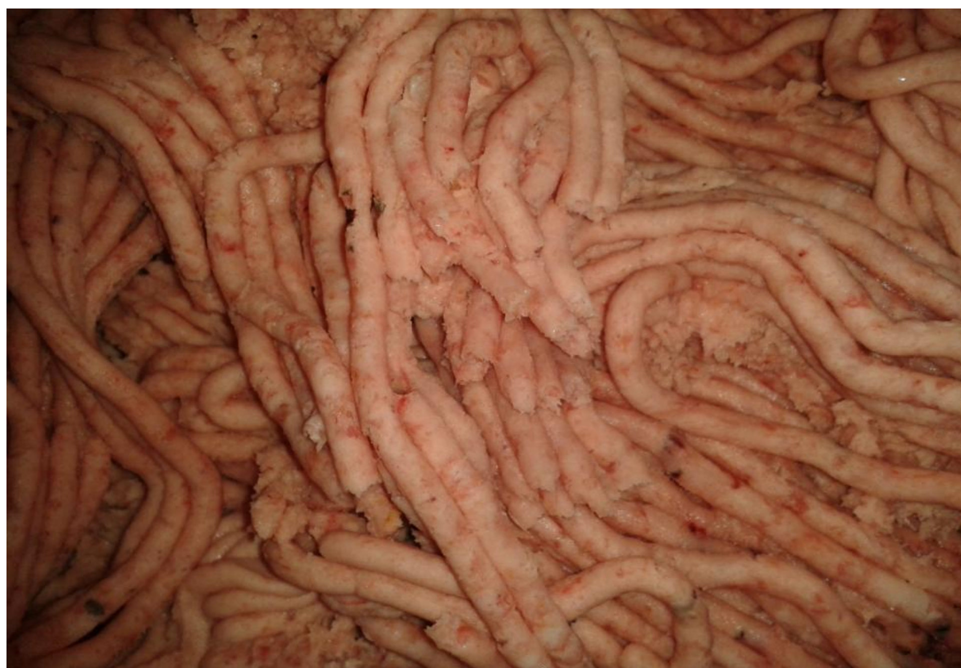

Fig. 2.2: Mince of the frozen Nile perch viscera

2.3 RESULTS AND DISCUSSION

2.3.1 Moisture and Extractable Oil Contents of Sample Viscera

Moisture content of the viscera was 24.3 ± 1.1% while the extractable oil content was 73.2 ± 1.6%. The extractable oil content was within the range of 74.1 ± 7.51% obtained by earlier work on Nile perch belly flaps [*Ogwok et al., 2008*], lower than the lipid content of the viscera of Asian catfish at 93.32% [*Thammapat, Raviyan and Siriamornpun, 2010*], but higher than 24.1 ± 6.1% reported for farmed Atlantic salmon (*Salmo salar* L.). The moisture content was, however, lower than 59.4 ± 0.6% reported for the viscera of farmed Atlantic salmon [*Sun et al., 2002*]. The extractable oil content is higher than the oil content reported for Nile perch adipose tissues (42.4%) and lungfish livers (39.6%) and 53.4% for adipose tissues [*Masa et al., 2011*]. it is also higher than 29.5 – 78.1% for liver and 0.7 – 7.2% for viscera of gadiform species [*Falch, 2006; Falch et al., 2006b*].

2.3.2 Oil Yields of the Sample Viscera

There were significant differences ($p \leq 0.05$) in yield, with interaction between acid concentration and heating time. There were high yields after 15 minutes heating, but with no significant difference at 0% and 2% acid concentrations. The oil yield after heating for 15 minutes were 75.8 ± 6.9 and 77.4 ± 1.0% for 0% and 2% acid concentrations respectively. These values are higher than 5.9 – 10.8% obtained for catfish viscera using various methods [*Sathivel et al., 2009*] and 14.5 – 19.8% from salmon by-products by use of enzymes [*Gbogouri et al., 2006*], but lower than 85% by fermentation ensilaging of freshwater fish (major Indian carp) viscera [*Rai et al., 2010*]. Approximately 85% recovery yield of crude oils in relation to carp viscera oil has been realized with fishmeal, ensilage and Bligh & Dyer methods [*Crexi, Souza-Soares and Pinto, 2009*]. *Mbatia et al.* [*2010a*] achieved 69.3% from salmon head through enzymatic hydrolysis. Generally, the conventional process leads to good results when using fish products or by-products with high-oil contents but is not so feasible when the oil content is low as the oil–water emulsion obtained after cooking is fairly stable and difficult to separate [*Rubio-*

Rodríguez et al., 2010]. At acid concentrations above 4%, the yield of oil decreased (*Fig. 2.3a and Fig. 2.3b*). This was probably due to formation of a larger and stable emulsified aqueous layer which resulted in larger oil loss.

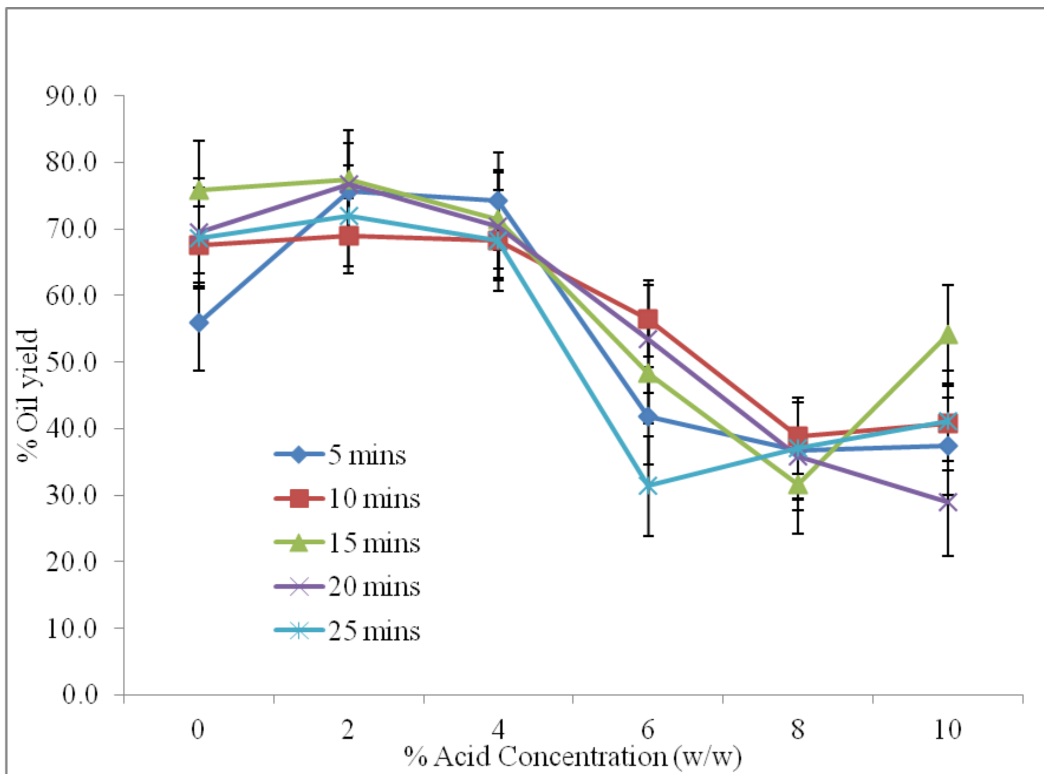

Fig. 2.3a: Yield of Nile perch viscera oil at different acid concentrations and time of heating

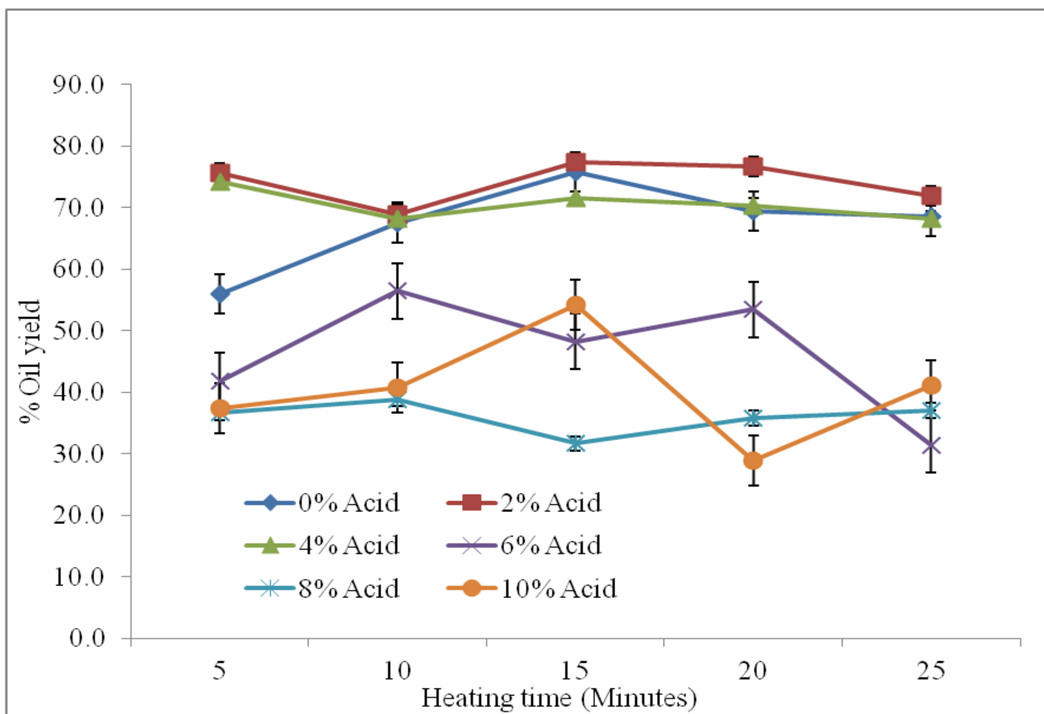

Fig. 2.3b: Yield of Nile perch viscera oil at different heating times and acid concentrations

The higher acid concentrations seem to have produced high amount of denatured proteins, probably forming protein–lipid complexes, which further reduce the free oil [*Daukšas et al., 2005*]. In salmon oil, the upper layer consisted mainly of neutral lipids while the heavy fraction (emulsion) was rich in polar lipids (55%), which due to their amphiphilic properties lead to strong interactions with peptides and proteins. Neutral lipids still accounted for 44% in the emulsion [*Gbogouri et al., 2006*]. The emulsion contains a high fat content also due to high contents of hydrophobic amino acids that retain more of the lipids [*Mbatia et al., 2010a*]. These observations are consistent with other reports on freshwater fish species such as trout and blue mackerel [*Gbogouri et al., 2006*].

2.3.3 Free Fatty Acids (%FFA) of Oil from Sample Viscera

There was significant difference in free fatty acids (%FFA), with interaction between acid concentration and heating time. The FFA of the oils varied between 0.46 ± 0.08 and 0.92 ± 0.08. Heating times of 5 and 15 minutes produced the lowest %FFA at 0% acid concentration, 0.60 ± 0.05% for 15 minutes being significantly higher than 0.46 ± 0.08% for 5 minutes. The values were lower than the limits of 1 – 7% reported for crude fish oils [*Ackman, 2005*], indicating superior quality of the extracted oil. The values are also below those obtained for crude carp viscera oil extracted by fishmeal (3.35 ± 0.02%) and acid ensilaging (6.63 ± 0.01%) [*Crexi et al., 2010*] as well as 54.3 – 60.5 for oil from freshwater fish viscera extracted by fermentation ensilaging [*Rai et al., 2010*]. The FFA generally increased with increase in acid concentration and heating time, with slight fall at acid concentrations above 6% (*Fig. 2.4a and Fig. 2.4b*). This was probably due to increased acid hydrolysis of the fish oil acylglycerols. Heating without acid corresponds to the conventional wet rendering (fishmeal) process. The FFA content of oil at 0% acid is probably due to hydrolysis by heat or the endogenous enzymes in the viscera at the beginning of the heating process [*Falch, 2006; McClements and Decker, 2008; Crexi et al., 2010*].

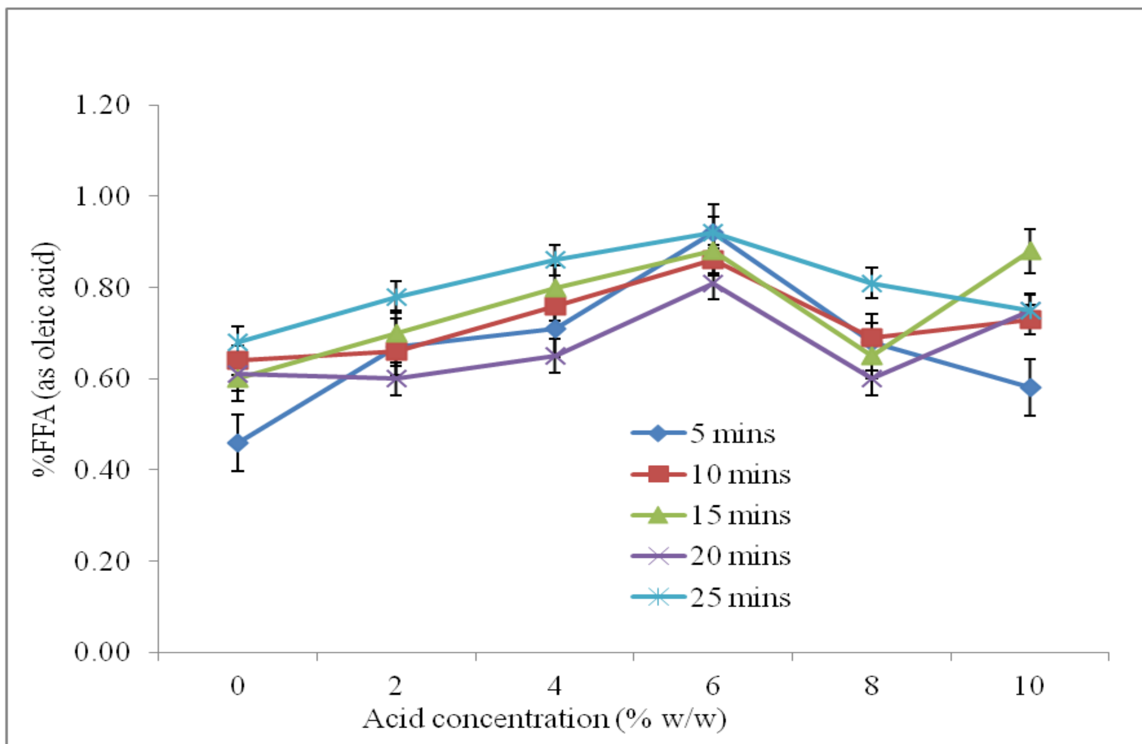

Fig. 2.4a: Free fatty acid of Nile perch viscera oil at different acid concentrations and duration of heating

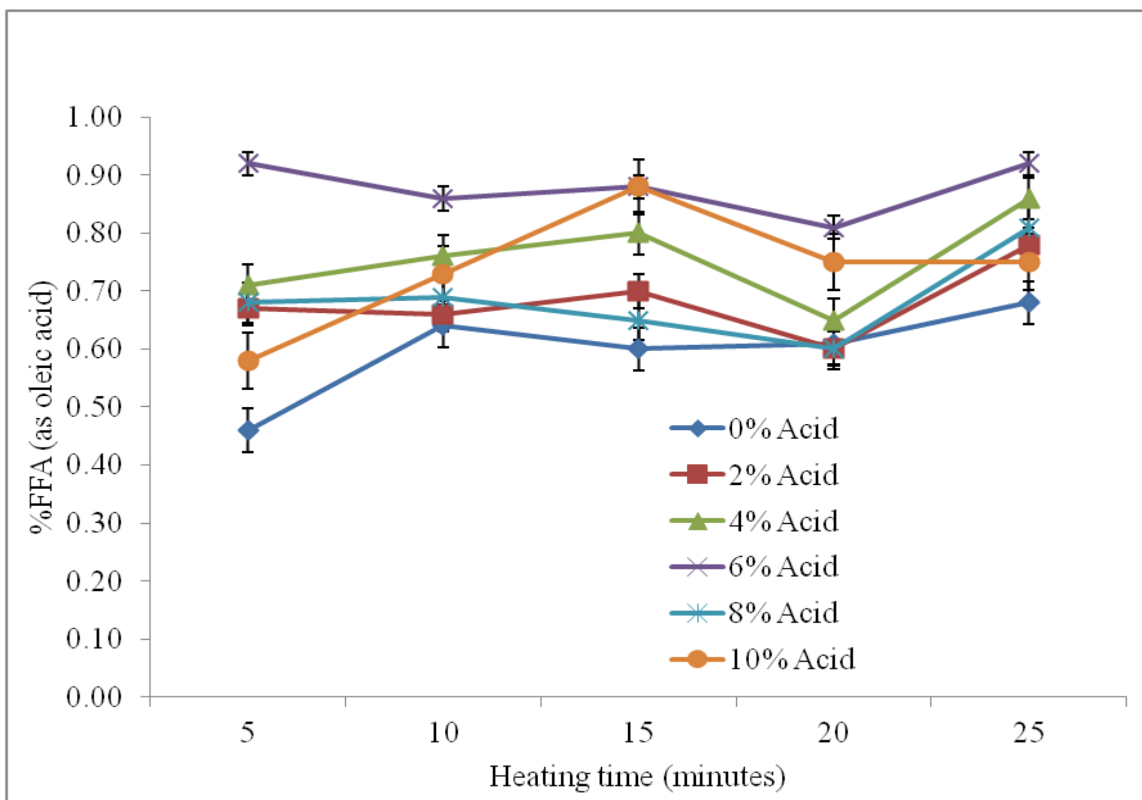

Fig. 2.4b: Free fatty acids of Nile perch viscera oil at different heating times and acid concentrations

Enzymatic hydrolysis of lipids has been reported in muscles of some fish such as the cod, skipjack, carp, sardine, and rainbow trout. During storage of silver carp for 8 days, changes in the lipid classes of the muscle were observed. FFA increased from 0 to 28%, polar lipids decreased from 89 to 60%, but triacylglycerol contents did not change. These phenomena were inhibited by heating the muscle. It was therefore suggested that hydrolysis of lipids had taken place with catalization by the enzyme phospholipase. Phospholipases in heated muscle were deactivated via denaturation by heat. The fatty acid composition of total lipid and triacylglycerol did not change throughout the storage. However, some changes of polar lipid fatty acid composition were observed [*Kaneniwa, 2011*]. *Zhou et al.* [*1995*] studied the FFA content of lipids during ensilage acidification of minced herring. In their study, the maximum %FFA was about 6%. Increase in acidity during ensilaging lowered the activity of lipases thereby lowering %FFA. This was also shown during fermentation ensilaging [*Rai et al., 2010*]. Studies have demonstrated seasonal variations in lipolytic activity in liver, viscera and cut-offs from Atlantic cod, higher activity being observed during the summer and spring compared to the winter catch [*Falch, 2006*]. Low hydrolytic activity is typical of the spawning season, particularly in males [*Kołakowska et al., 2003*]. Season, therefore, could have contributed to the low FFA values since the fish were landed during the breeding season.

2.3.4 Peroxide Value (PV) of Oil from Sample Viscera

There was significant difference in PV with interaction between acid concentration and heating time. Heating for 15 and 25 minutes produced low PV, with no significant difference between them at 0% acid concentration. All the peroxide values ranging between 1.63 and 2.48 for heating times of 10, 20 and 25 minutes were below the limits of 3 – 20mEq O_2/kg oil so far reported for crude fish oils. The values 2.83 to 5.51 for the other processes were still within the reported limits of 3 – 20mEq O_2/kg oil for crude fish oils [*Ackman, 2005*], and similar to those reported for carp viscera oil extracted by fishmeal and acid ensilaging [*Crexi et al., 2010*]. PV

increased at 2% acid concentration and fell thereafter (*Fig. 2.5a and Fig. 2.5b*) as acid concentration increased.

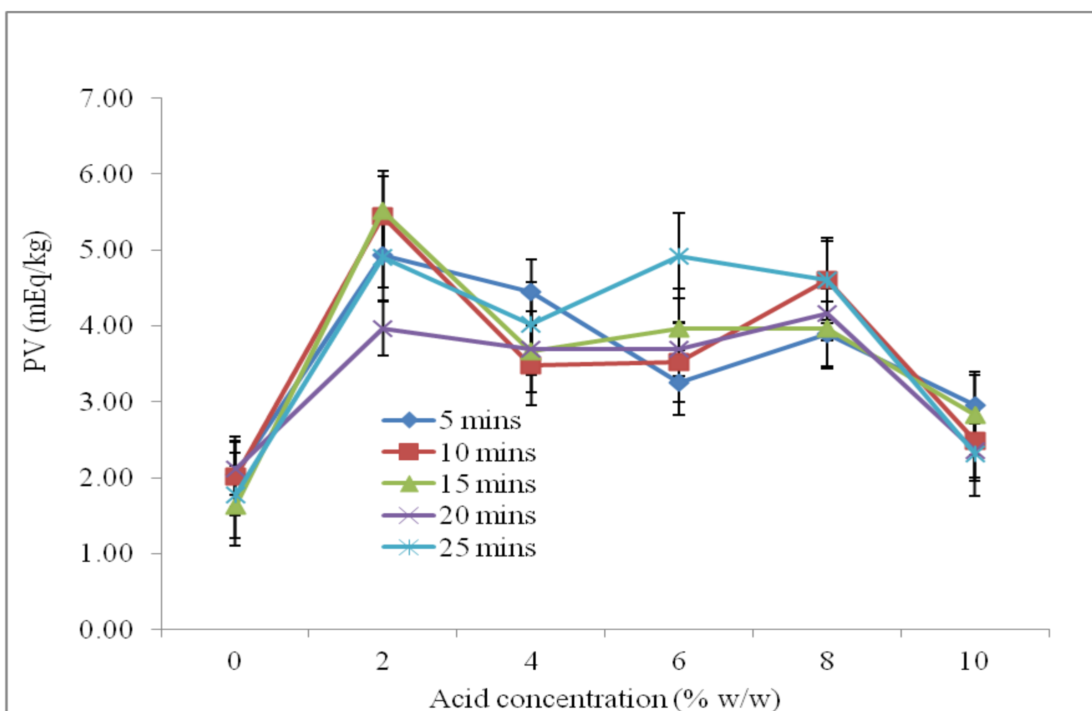

Fig. 2.5a: Peroxide value of Nile perch viscera oil at different acid concentrations and time of heating

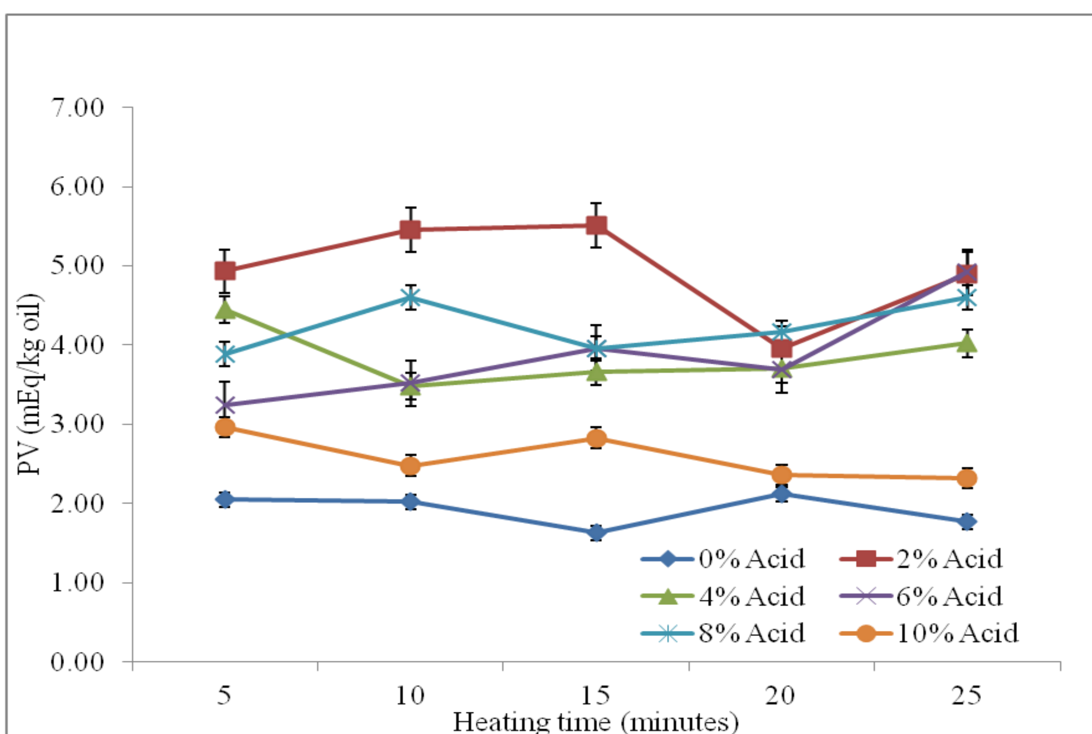

Fig. 2.5b: Peroxide value of Nile perch viscera oil at different heating times and acid concentrations

Free fatty acids are more susceptible to oxidation compared to those acylated to glycerol [*McClements and Decker, 2008; Falch, 2006*]. Heating at 85°C for 10 minutes has been used for complete inactivation of lipases. In addition to preventing taste deterioration of lipids, inactivation of lipases is also believed to reduce the lipid oxidation [*Falch, 2006*]. In fish PUFA are contained in phospholipids of cell membranes, where they are closely packed and surrounded with proteins. Thereby, their susceptibility to degradation by heating may differ from that of pure PUFA. As found for many fish species, their content of EPA and DHA did not decrease under various ways of cooking compared to raw fish. Canned fish appeared to be more valuable products concerning the PUFA content [*Gladyshev, Sushchik and Makhutova, 2013*].

Studies on herring have suggested presence of an enzyme which catalyses oxidation of the oil [*Tsuchiya, 1961*]. Acid hydrolysis of fish tissues results in production of hematin compounds [*Crexi et al., 2010*]. Hematin compounds enhance oxidation, particularly of linoleic acid and fish liver oils [*Opara et al., 2007; McClements and Decker, 2008*]. A study of sea bass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus* x *O. niloticus*) during iced storage has shown that autoxidation of myoglobin could be associated with enhanced lipid oxidation. Autoxidation of oxymyoglobin results in the formation of metmyoglobin and superoxide, which rapidly dismutate to hydrogen peroxide and oxygen. The interaction of hydrogen peroxide with metmyoglobin lead to very rapid generation of an active species, ferryl radical, which could initiate lipid peroxidation [*Thiansilakul, Benjakul, and Richards, 2010*]. Another study on Asian sea bass (*Lates calcarifer*) muscle during iced storage showed that the initiation and propagation steps of lipid oxidation were more pronounced in the un-bled samples when compared with the bled samples [*Maqsood and Benjakul, 2011*]. Heat-processing denatures iron-containing proteins, particularly myoglobin, causing release of iron into the catalytic pool. The iron is then set free and brought into closer proximity with the oxidation substrate, resulting in a higher oxidation rate. This has been demonstrated by tuna head oil extracted by different heating time regimes [*Chantachum et al., 2000*].

The generally low oxidation in the present study could be attributed to the presence of carotenoids and tocopherols in Nile perch oil [*Ogwok et al., 2008*]. Carotenoids and tocopherols are natural anti-oxidants. Citric acid also exerts anti-oxidant activities [*McClements and Decker, 2008*]. Therefore, as acidity increased, there was probably a fall in lipase activity due to acid denaturation, resulting in lower %FFA hence reduced oxidation. The anti-oxidant components probably further enhanced the stability to oxidation of the oil during the processes.

2.3.5 Color Intensity of Oil from Sample Viscera

There was significant difference in color intensity of the oils, with acid concentrations. Heating time did not produce a significant difference, and there was no interaction between acid concentration and heating time. There was significant difference between 0% and higher acid concentrations, with no significant difference between 15 and 20 minutes of heating. The oil extracted without acid was yellow-orange, characteristic of fish oil as a result of the deposition of vitamin A and carotenoids [*Ogwok et al., 2008*]. Oil pigmentation generally increased during ensilaging, as acid concentration was increased (*Fig. 2.6a and Fig. 2.6b*) and is caused by the release through acid hydrolysis of the product of hemoglobin, hem. In ensilage oil an increase in free fatty acids (FFA) results in formation of lipid–protein complexes, consequently increasing color intensity [*Crexi et al., 2010*].

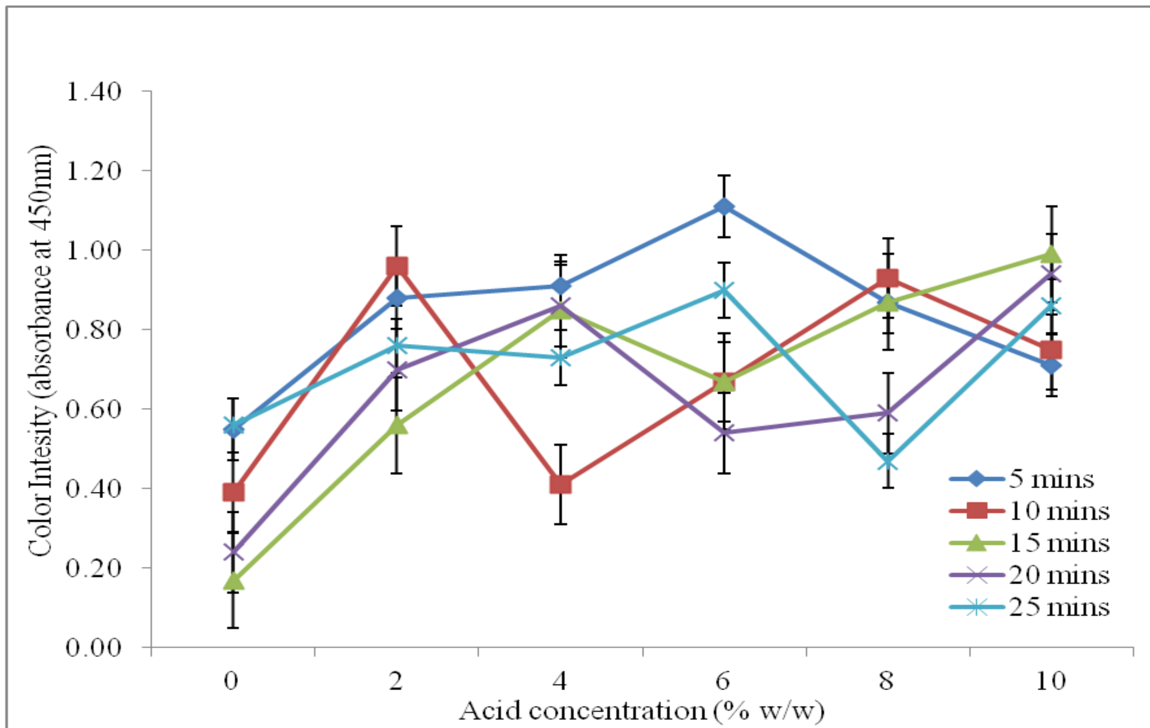

Fig. 2.6a: Color intensity of Nile perch viscera oil at different acid concentrations and time of heating

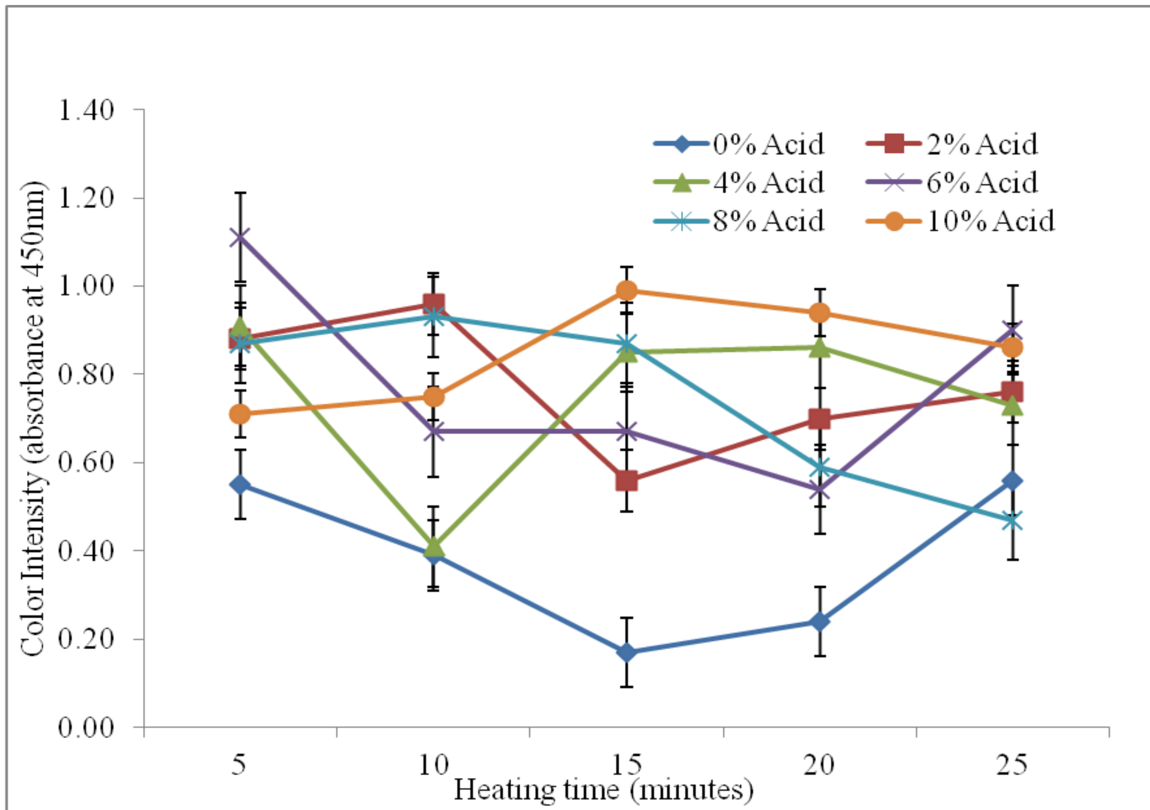

Fig. 2.6b: Color intensity of Nile perch viscera oil at different heating times and acid concentrations

2.4 CONCLUSION

The study showed that 0% acid concentration and 15 minutes heating is the most suitable combination for mass production. Although the use of acid leads to a slight increase in oil yield, there is no significant improvement in oil quality in terms of %FFA and PV, but with potential of color damage. Extraction of crude Nile perch viscera oil should be done for 15 minutes in 2% citric acid solution. If the crude oil is to be refined however, acid treatment would not be necessary.

CHAPTER 3

3.0 PHYSICO-CHEMICAL CHARACTERISTICS OF REFINED NILE PERCH (*Lates niloticus*) VISCERA OIL

ABSTRACT

A study was carried out to characterize Nile perch viscera oil to determine its physico-chemical properties. Nile perch viscera were obtained from local fish filleting plant and oil extracted from them at 93°C by wet rendering process without acid and heating for 15 minutes, earlier established as the optimum conditions of extraction. The crude oil was refined by neutralization, deodorization and winterization to obtain low melting point (LMP) and the high melting point (HMP) oil fractions for analysis. The oil fractions were characterized by analyzing for omega – 3 fatty acids (eicosapentaenoic acid – EPA and docosahexaenoic – DHA), vitamin A, vitamin E, iodine value (IV), saponification value (SV), density, melting point (MP), and smoke point (SP). The crude fish oil was used as control. Data was subjected to statistical analysis ($p \leq 0.05$) using GenStat 13th edition software. The results showed that the yield of winterized oil (LMP fraction) was 45.6 ± 0.5% of deodorized oil, 39.8 ± 0.5% of crude oil and 19.6 ± 0.5% of raw material weight. There were no significant differences in density and vitamin E (tocopherol) content of the three fractions of oil, density ranging between 916.6 and 920.5g/l while vitamin E was 2.46 – 2.54 mg/100g oil. There were significant differences in the slip melting points, smoke points, saponification value (SV), iodine value (IV) and vitamin A (retinol) content of the three fractions of oil. MP was 7 – 33°C, SP was 223 – 278°C, SV was 183.0 – 190.2mg KOH/g oil, IV was 119.9 – 138.7g iodine/100g oil while vitamin A was 5.16 – 5.91mg/100g oil. There were significant differences in EPA and DHA contents of the crude, LMP and the HMP fractions of the oil. EPA and DHA values for the LMP fraction (7.02 ± 0.58 and 33.76 ± 4.04% w/w total fatty acids) being highest, followed by crude (2.76 ± 0.31 and 9.5 ± 0.16% w/w total fatty acids) and HMP having lowest (1.2 ± 0.39 and 4.07 ± 0.54% w/w total

fatty acids). High vitamins A and E as well as omega – 3 polyunsaturated fatty acids (PUFA) contents and high smoke points make the crude and HMP fractions suitable for use in cooking. The high amount of LMP fraction has high content of omega – 3 PUFA, making it suitable for use in food, feed and nutraceutics.

3.1 INTRODUCTION

Nile perch (*Lates niloticus*) was introduced into Lake Victoria in about 1950s and 1960s [*Njiru et al., 2008; Turon et al., 2005*] and has since become the dominant fish species, accounting for about two thirds of the lake's population by early 1990s [*Turon et al., 2005; Ogwok et al., 2008*].. It is the backbone of the East African fisheries, contributing above 60% of the total landings. Over 90% of Nile perch landed is exported, creating employment opportunities in the harvesting, processing and marketing sectors of the industry [*Njiru et al., 2008; Mkumbo et al., 2007; Turon et al., 2005*]. Nile perch is processed into fillets, mainly exported to Europe [*Turon et al., 2005*]. Nile Perch (*Lates niloticus*) products [*CCFO, 2013*] represent over 90 percent of Uganda's fishery exports, where consumers demand it for the presence of key omega-3-fatty acids [*MAAIF, 2011; MAAIF, 2010*]. Byproducts are nearly 50% of the total fish mass [*Ogwok et al., 2008*]. Among the byproducts, the hide is tanned and used mainly for making shoes, belts and purses, the swim bladders are dried and exported to England for filtering beer and wine and to Orient for making soup stock [*Turon et al., 2005*]. In fish processing, viscera are considered wastes and often discarded [*Sun et al., 2002*]. Fatty materials from Nile perch belly flaps are presently discarded or sold cheaply [*Ogwok et al., 2008*].

Long-chain ω-3 PUFA, especially EPA and DHA present in fish oils possess nutritional value and health benefits [*Del Nobile et al., 2009; Ogwok et al., 2008; Gbogouri et al., 2006; Aidos et al., 2002*]. They are essential for fetal brain development. Foods rich in PUFA are essential for normal growth and proper body function [*Ogwok et al., 2008*]. The essential fatty acids content depends on fish species and size, physiological demand and varies from tissue to

tissue. Fish liver and belly tissues are widely recognized for high PUFA and vitamin A contents in their oil [*Ogwok et al., 2008*]. Other than the liver, belly oil is mainly found in pyloric ceaca and messenteria [*Jaquot, 1961*]. Heads [*Sahena et al., 2010b; Turon et al., 2005*], roes [*Bekhit et al., 2009; Falch et al, 2006c; Remme et al., 2005*] and skins [*Sahena et al., 2010a; Aryee and Simpson, 2009*] of other fishes also possess high quality oil.

Crude oils require refinement for human consumption [*Crexi, Souza-Soares and Pinto, 2009*]. Nile perch oil is relatively inexpensive fish oil that is normally sold off as a byproduct [*Mbatia et al., 2011*]. Its high content of saturated fatty acids (SFA), monounsaturated fatty acids and low PUFA to SFA ratio, however, indicates a need to concentrate PUFA prior to human consumption [*Mbatia et al., 2010b*]. This would enable processing of high value products with EPA and DHA increased to favorable levels [*Mbatia et al., 2011*]. Consumption of Nile perch PUFA concentrates may be more effective than the crude oil itself because the concentrates would contain less SFA thus allowing daily intake of total lipids to remain low [*Mbatia et al., 2010b*]. The dosage of cod liver oil required to achieve the desired biological effects carries the risk of vitamin A and D overdose and subsequent toxic effects as well as an increase in the intake of cholesterol and other saturated fatty acids. Therefore, concentrated forms of ω – 3 PUFA from marine oils have been developed. Marine oils could be concentrated, as such, or as modified triacylglycerols (TAG) and as free fatty acids or their alkyl esters. Studies have concluded that omega – 3 concentrates devoid of saturated and monounsaturated fatty acids are much better than marine oils themselves since they keep the daily intake of total lipids as low as possible. Therefore, concentrated forms of ω – 3 PUFA are preferred items for pharmaceutical applications as well as possible enrichment of foods. Methods for concentration of ω – 3 PUFA are numerous, but only few are suitable for large-scale production. The available methods include adsorption chromatography, fractional or molecular distillation, enzymatic splitting, low-temperature crystallization, supercritical fluid extraction and urea complexation [*Shahidi and Wanasundara, 1998*]. It is therefore necessary to separate crude Nile perch oil and determine the

physico-chemical characteristics of the different fractions obtained: concentrates of saturated fatty acids (high melting point fraction) and omega – 3 PUFA (low melting point fraction), with the objective of preparing the oil's profile for appropriate industrial and consumer applications.

3.2 MATERIALS AND METHODS

3.2.1 Materials

3.2.1.1 Raw materials

Viscera were obtained from Nile perch of processing size, purchased from a fish filleting factory in Nairobi (W. E. Tilley Group). The amount, size, age and species landed are regulated by the relevant government department responsible for fisheries [*Laws of Kenya, 2012*]. They were preserved by freezing in a deep freezer in the University's Pilot Plant prior to oil extraction.

3.2.1.2 Chemicals for analysis

All reagents were of analytical grade. Sodium hydroxide ampoule caustic soda, chloroform and sodium thiosulphate were sourced from Rankem, India. Phenolphthalein indicator was acquired from Loba Chemie, India. Ethanol was ordered from Scharlan – Scharlab, Spain and diethyl ether from Kobian Scientific. Potassium hydroxide ampoules (*Volucon*) were procured from May & Baker, England, while hydrochloric acid ampoules were from Sigma Aldrich, Germany. Starch and iodine bromide (*Merck – Germany*) as well as potassium iodide (*Panreac Quimica SAU, Spain*) were also used in titrations.

3.2.2 Methods

3.2.2.1 Processing methods

3.2.2.1.1 Preliminary sample processing

Prior to oil extraction, entire sample (viscera) was minced in the pilot plant using 7.0 mm (0.276 inch) screen, then tumbled to achieve uniformity. The mass was then deep frozen, wrapped with polythene film to avoid gain or loss of water, since it was not possible to extract oil the same day. Oil extraction was done by wet rendering, heating sample in equal amount of water for 15 minutes. The cooking was done in triplicate at 93°C in covered pans. Oil recovery

was done by sieving the cooked mass with two layers of muslin cloth. The stick-water, aqueous layer and oil were separated by decanting for 10 – 15 minutes in a separating funnel; the former two occupying the bottom and middle layers were drained off, leaving the oil in the funnel. The residue in the muslin cloth was pressed using a plate and frame press to obtain more oil. This was also passed through the separating funnel to remove stick-water and emulsion. The oil obtained was weighed and sample drawn for preliminary quality analysis of free fatty acids before refining. The oil was then refined by neutralization, deodorization and winterization. Winterized oil (LMP fraction) and the residue (HMP fraction) were subjected to physico-chemical tests. Crude Nile perch viscera oil was also analyzed. The separated oil fractions are illustrated in fig. 3.1 and fig. 3.2.

3.2.2.1.2 Neutralization

Neutralization was done to remove free fatty acids from the oil employing the hot dry method. Amount of caustic soda was calculated as: %NaOH = %FFA x 0.142 [*Gunstone and Norris, 1983*]. Oil with the calculated caustic soda pellets was heated at 80°C for 3 hours with agitation, the soap-stock settling at the bottom of the vessel and the neutral oil obtained by filtration using muslin cloth as described by *Hamilton and Bhati, 1980 and Bockish, 1998*].

3.2.2.1.3 Deodorization

Deodorization was done by heating the oil of approximately 4kg for 8 hours at 110°C in a vacuum oven at 200mmHg. This was based on the method used by *Wanasundara* [*1996*], in which a laboratory scale vacuum steam distillation apparatus was used with temperature at 100 ± 5°C; pressure at 0.01 – 0.03mmHg for 4 hours for 2.3kg of oil.

3.2.2.1.4 Winterization

Dry fractionation was accomplished by means of winterization. The oil was cooled to 13°C then further to 8°C over period of 6 days without addition of chemicals then decanted and

filtered as described in literature [*Hamilton and Bhati, 1980; Gunstone and Norris, 1983; Rajah, 1996*]. The filtration was done by use of cloth.

3.2.2.2 Analytical methods

3.2.2.2.1 Determination of free fatty acids

Oil obtained from rendering was subjected to preliminary hydrolysis test by determining free fatty acids (%FFA) prior to refining. This was determined volumetrically using aqueous sodium hydroxide standardized to 0.1M from ampoule and 1% phenolphthalein indicator in ethanol according to *AOCS* (*1998*) method Ca 5a-40. A neutral mixture (50 ml) of diethyl ether: ethanol (1:1) was used as a solvent. FFA values were reported as % oleic acid by weight. Yield was also calculated for each process, as percentage of the raw material used.

Fig. 3.1: Fractionated Nile perch (*Lates niloticus*) viscera oil in different containers: LMP (top) and HMP (bottom) fractions

Fig. 3.2: Molten Nile perch (*Lates niloticus*) viscera oil. Crude (left) and high melting point (right) fractions

3.2.2.2.2 Determination of density

Density of oil fractions was determined by pycnometer method. Dry pycnometer was filled with sample at 20°C and the weight taken. The pycnometer was then filled with water at 20°C. The sample weight was then compared with the weight of water to determine its density [*Bradley Jr., 2010*].

$$\text{Density} = \frac{\text{weight of sample filled pycnometer} - \text{weight of empty pycnometer}}{\text{weight of water filled pycnometer} - \text{weight of empty pycnometer}} \times 1000 \text{ g/l}$$

3.2.2.2.3 Determination of melting point

Melting point was determined as slip melting point, performed using the open tube method. It measures the temperature at which a column of fat moves in an open capillary tube when heated. Oil was kept frozen in an open capillary tube over 16 hours to stabilize then placed in warm water bath with thermometer attached to read the temperature [*O'Keefe and Pike, 2010*].

3.2.2.2.4 Determination of smoke point

Smoke point was determined by filling a cup with oil and heated in a well lit container. The smoke point was recorded as the temperature at which a thin, continuous stream of bluish smoke was given off by the sample oil [*O'Keefe and Pike, 2010*].

3.2.2.2.5 Determination of saponification value (SV)

Saponification value (SV) is the amount of alkali needed to saponify a given quantity of fat or oil, expressed as mg potassium hydroxide (KOH) per 1g sample. It was determined using *AOCS* (*2009*) method Cd 3a-94. Excess alcoholic KOH was added to the sample, and then heated to saponify. The unreacted KOH was back-titrated with standardized hydrochloric acid using phenolphthalein indicator. The calculated amount of reacted KOH was used to determine the saponification value (SV).

$SV = \frac{(S - B) \times N \times 56.1}{W}$; B = blank titre, S = sample titre, N = normality of HCl, W = sample weight

3.2.2.2.6 Determination of iodine value (IV)

Iodine value (IV) was determined by Hanus method, using chloroform as solvent. Dissolved oil sample was mixed with iodine bromide solution and freshly prepared 10% potassium iodide solution. Liberated iodine was titrated with standard 0.1M sodium thiosulphate solution, using chloroform as a blank and starch as the indicator. The iodine value (IV) was calculated as follows: $Iodine\ Value = \frac{1.269(a-b)}{w}$; a = the titre (ml) for blank; b = the titre (ml) for sample; w = the weight (g) of sample (*AOAC, 1998*, official method 920.158).

3.2.2.2.7 Determination of vitamin A

The vitamin A was determined as retinol by the method described by *Zahar and Smith* [*1990*] with slight modification on sample weight. Standard retinol was prepared by dissolving 50mg of retinyl acetate (*Fluka – Sigma Aldrich, USA*) in 50ml ethanol containing 0.1% ascorbic acid to give 1mg/ml, i.e. 1000µg/ml. This is equivalent to 545.7 ppm of retinol. The retinol standard solutions were subjected to same treatment as samples but with the following modifications: 1ml of standard solution was taken and 0.1ml peanut oil added before saponification, to protect retinol from oxidation. After centrifugation, 10ml of the upper hexane phase was withdrawn, evaporated to dryness and residue re-dissolved in 10ml of methanol. This gave 54.6ppm which was taken as stock solution. This stock was diluted accordingly to make working standard solutions which were injected into the high performance liquid chromatography (HPLC) to give corresponding peak areas. Using the different concentrations and their peak areas, a standard curve was generated. To a 50ml glass stoppered centrifuge tube, 4g of oil samples were added followed by 5ml of absolute ethanol containing 0.1% (w/v) ascorbic acid followed by 2ml of 50% potassium hydroxide (KOH). Tubes were stoppered, agitated carefully and placed in a water bath at 80°C for 20min. During this period, tubes were agitated periodically to ensure complete digestion of the oil. After saponification the tubes were cooled with running water and then placed in an ice water bath. Hexane (20ml) containing 0.01%

butylated hydroxytoluene (BHT) was added. Tubes were again stoppered and mixed vigorously with a vortex for 1 min, allowed to stand for 2 min, and again vortexed for 1 min. Cold water (15ml) at 1°C were added to each tube then inverted ten times. Centrifugation was at 1000 x g for 10min. The upper, organic layer was accurately pipetted (10ml) into a tube and the solvent evaporated under vacuum at 40°C using a rotary evaporator. The residue was immediately re-dissolved in 1ml of methanol. The samples and the standards were injected into HPLC (*Shimadzu LC 10A*), equipped with a photo-diode array detector (*Waters 2996*) and a reverse-phase column (*Discovery HS C18, 10µm; 4.6 x 250mm*). Using the standard curve, concentrations of retinol in samples were calculated.

3.2.2.2.8 Determination of vitamin E

The vitamin E content of oil was analyzed by *AOAC* (*1998*) method 992.03 using high-pressure liquid chromatography (*Shimadzu VP 10A*) equipped with a uv-diode array detector (*Waters 2996*) and a normal phase column (*Silica 60*). Oil (1g) was dissolved in n–hexane and made to 25ml, and then filtered using 0.45µl syringe filters. Sample aliquots of 20µl were injected into the HPLC. The mobile phase was a mixture of 98ml hexane and 2ml isopropanol. Vitamin E working standard was prepared by dissolving 100mg of α-tocopherol acetate concentrate (*Fluka – Sigma Aldrich, USA*) in 100ml hexane to make 1000ppm. The stock solution was diluted to make working standards of 1–100ppm. Oven temperature was 25°C and the flow rate was 1ml/min. The standard was injected into the HPLC to generate standard calibration curve. Standard and sample solutions were injected in triplicate.

3.2.2.2.9 Determination of omega – 3 fatty acids

Omega-3 fatty acids were determined quantitatively by modified *Bligh and Dyer* [*1959*] method. Methanolic HCl solution was used for the preparation of fatty acid methyl esters (FAME). The mixture of oil (10mg) and Methanolic HCl solution (4 ml) was heated under reflux for 1 hour to enhance esterification, and then cooled with tap water. The solution with extracted

methyl esters was transferred into a separating funnel and 4ml of hexane added. After vigorous shaking and letting to stand, the hexane layer was collected and the aqueous layer returned for extraction once more. The hexane fractions were combined then filtered using defatted cotton wool and anhydrous sodium sulphate to remove water. The filtrate was concentrated using nitrogen gas to about 0.5-1ml. Analysis was performed using a gas chromatography (*Shimadzu GC 14B*) equipped with a fused silica capillary column (*Supelco Omegawax 530*, 30m x 0.53mm internal diameter, 0.5µm film thickness) operating at 180°C. The carrier gas was nitrogen with flow rate of 2ml/min; sample injection in splitless mode. The temperature of the injector and flame ionization detector was maintained at 220°C. Air-hydrogen flame ionization detector was operated at pressures of 50kPa for air and 60kPa for hydrogen. Peak areas obtained were used to compare with those obtained for standard fatty acids (*Sigma Aldrich – USA*: *Supelco FAME mix*), to calculate the percentage of individual omega-3 fatty acids (EPA and DHA).

3.3 RESULTS AND DISCUSSION

3.3.1 Preliminary Analyses

The free fatty acids (%FFA) value of the extracted oil was 1.17 ± 0.04 (as oleic acid). The recovery of oil from each refining stage was as follows: crude extraction (49.2 ± 0.9% of raw material weight), neutralized oil (87.4 ± 0.5% of crude and 43.1 ± 0.5% overall), deodorized oil (99.8 ± 0.1% of neutralized oil, 87.3 ± 0.1% of crude and 43.0 ± 0.4% of raw material weight), winterized low melting point oil fraction (45.6 ± 0.5% of deodorized; 39.8 ± 0.5% of crude and 19.6 ± 0.5% of raw material weight). Recoveries of 78 and 63% of seal blubber and cod liver oils respectively have been realized after deodorization from their source materials [*Wanasundara, 1996*]. The %FFA (as oleic acid) of the high MP and low MP fractions immediately after winterization were 0.22 ± 0.08 and 0.26 ± 0.04 respectively.

3.3.2 Physico-chemical Properties

There was no significant difference in density of the three fractions of oil i.e. crude (918.6 ± 0.4g/l), HMP (920.5 ± 1.1g/l) and LMP (916.6 ± 0.7g/l) fractions. There were

significant differences in the slip melting points and smoke points of the three fractions of oil. The melting points were 25 ± 8^oC, 33 ± 9^oC and 7 ± 5^oC for crude, HMP and LMP fractions respectively. The smoke points were 255 ± 3^oC, 278 ± 4^oC and 223 ± 3^oC for crude, HMP and LMP fractions respectively There were significant differences in the saponification value (SV), iodine value (IV) and vitamin A (retinol) content of the three fractions of oil. SV varied from 183.0 ± 5.2mg KOH/g oil for LMP to 186.4 ± 4.8 mg KOH/g oil for crude and 190.2 ± 6.7mg KOH/g oil for HMP. IV was 119.9 ± 1.9g iodine/100g oil for HMP, 124.7 ± 4.1g iodine/100g oil for crude and 138.7 ± 6.5g iodine/100g oil for LMP. Retinol on the other hand varied from 5.16 ± 0.25mg/100g oil for HMP to 5.21 ± 0.32mg/100g oil for crude and 5.91 ± 0.11mg/100g oil for LMP fraction. The vitamin E (tocopherol) contents (2.46 ± 0.10, 2.52 ± 0.09 and 2.54 ± 0.06mg/100g oil) of the crude, HMP and LMP fractions respectively showed significant differences (*Table 3.1 and Table 3.2*).

Table 3.1: Chemical properties and vitamin content of Nile perch viscera oil fractions

Sample	Saponification Value (mg KOH/g oil)	Iodine Value (g iodine/100g oil)	Vitamin (mg/100g oil)	
			Vitamin A	Vitamin E
Crude	186.4 ± 4.8	124.7 ± 4.1^a	5.21 ± 0.32^a	2.46 ± 0.10
High MP	190.2 ± 6.7	119.9 ± 1.9^b	5.16 ± 0.25^b	2.52 ± 0.09
Low MP	183.0 ± 5.2	138.7 ± 6.5^c	5.91 ± 0.11^c	2.54 ± 0.06

Values in columns followed by a different superscript are significantly different ($p \leq 0.05$). The values are averages of three replicates ± standard deviation

The high melting fraction is solid at room temperature and contains largely the saturated fatty acids while the low melting fraction is liquid due to high content of unsaturated fatty acids in the triacylglycerols. Most of the saturated fatty acids have short carbon chain lengths while the unsaturated fatty acids possess long carbon chains [*McClements and Decker, 2008; Nawar, 1985*]. The lower saponification value of low melting than those of crude and high melting point

fractions may confirm this since the higher the SV, the lower the mean chain length of the component fatty acids of an acylglycerol [*Haas, 2005*]. The SV of HMP and crude fractions are close to those reported for crude liver oil of ray species *Dasyiatis brevis* and *Gymnura marmorata*, while for the LMP, SV is lower. The IV of all fractions are below those reported for crude liver oil of ray species *Dasyiatis brevis* and *Gymnura marmorata* [*Navarro-García et al., 2004b*]. The IV of crude Nile perch oil has been reported to be 106 – 142g iodine/100g oil depending on the tissue [*Kiiza, Taylor and Barlow, 1993*].

Table 3.2: Physical properties of Nile perch viscera oil fractions

Sample	Density (g/l)	Melting point (^{o}C)	Smoke point (^{o}C)
Crude	918.6 ± 0.4	25 ± 8^{a}	255 ± 3^{a}
High MP	920.5 ± 1.1	33 ± 9^{b}	278 ± 4^{b}
Low MP	916.6 ± 0.7	7 ± 5^{c}	223 ± 3^{c}

Values in columns followed by a different superscript are significantly different ($p \leq 0.05$). The values are averages of three replicates ± standard deviation.

There is a variety of the saturated fatty acids in fish oil [*Mbatia et al., 2010; Ogwok et al., 2008; Turon et al., 2005; Aidos et al., 2002; Aidos, 2002*], explaining the wide range of the melting points of the crude and the high melting point fractions. This has been observed in menhaden oil also. It is attributed to the presence of impurities such as phospholipids, ketones and other materials in the unrefined fish oil. Those impurities melt uncharacteristically compared to pure fatty acids, the fatty acids having their own melting points. The melting points of fish oil are sharper after each purification step that removes impurities [*Yin and Sathivel, 2010*]. The melting point of fatty acids changes considerably with the type and degree of unsaturation and thus separation of mixtures of saturated and unsaturated fatty acids may become possible [*Haraldsson, 1984*]. At low temperatures, long chain saturated fatty acids which have higher

melting points crystallize out and PUFA remain in the liquid form [*Shahidi and Wanasundara, 1998*]. The low melting fraction is a concentration of the unsaturated fatty acids, giving a uniform composition [*Bockish, 1998; Gunstone and Norris, 1983; Hamilton and Bhati, 1980*], hence the melting point is within a narrow range. The saturated fatty acids are also more compact and are separated alongside the triacylglycerols with long high molecular weight fatty acids (waxes). These contribute to the higher densities [*Bockish, 1998; Hamilton and Bhati, 1980*] of the high melting and crude fractions of the oil, the magnitude decreasing with the higher degree of unsaturation. The densities are below that of bulk unrefined menhaden oil at room temperature [*Yin and Sathivel, 2010*] but higher than for crude liver oils of rays *Dasyiatis brevis* and *Gymnura marmorata* [*Navarro-García et al., 2004b*]. Saturated fatty acids are more stable to heat [*McClements and Decker, 2008; Nawar, 1985; Dugan Jnr, 1976*]. This explains why the smoke points of crude oil and the higher melting fractions are higher compared to that of the low melting point fraction. The smoke points for crude and the high melting point fractions are within the ranges recommended for frying oils [*Gupta, 2005*].

Only mature fish are permitted for processing in Kenya [*Laws of Kenya, 2012*]. Nutritive value of fish lipids and changes taking place in lipids during seafood storage and processing are significantly dependent on the fishing season [*Kołakowska et al., 2003*]. Vitamin A bioaccumulation occurs with age [*Ogwok et al., 2008*] and this must have resulted in its deposition in the tissues (including viscera) of the processed fish. The vitamin A content of all fractions of Nile perch viscera oil (5.21 – 5.91mg/100g) is within the recommended range of 3 – 6mg/100g [*CODEX STAN, 1991*] and comparable to 5.40mg/100g reported for cod liver oil [*Ogwok et al., 2008*]. This could be due to the inclusion of liver in the viscera of Nile perch, since liver oils have high Vitamin A [*Ackman, 2005*]. The amount of tocopherol in the refined Nile perch viscera oil was similar to values reported for the liver oil of ray, *Rhinoptera steindechneri* [*Navarro-García, et al., 2004a*] and Nile perch belly oil [*Ogwok et al., 2008*]. The values are however higher than that reported for crude cod liver and seal blubber oils

[*Wanasundara, Shahidi and Amarowicz, 1998*] and lower than those for Horse mackerel from Southern Adriatic Coast of Italy in different seasons [*Orban et al., 2011*]. The α – tocopherols content is related to diet since fish cannot synthesize vitamin E [*Ogwok et al., 2008*].

3.3.3 Omega – 3 Fatty Acids

There was significant difference in eicosapentaenoic acid (EPA; C20:5, n – 3) and docosahexaenoic acid (DHA; C22:6, n – 3) contents of the crude, low melting point and the high melting point fractions of the oil (*Table 3.3*).

Table 3.3: Essential fatty acids content of Nile perch viscera oil fractions

Sample	Omega - 3 Fatty Acids (% w/w of total fatty acids)	
	EPA	DHA
Crude	2.76 ± 0.31^{a}	7.5 ± 0.16^{a}
High MP	2.23 ± 0.69^{b}	4.24 ± 1.24^{b}
Low MP	7.02 ± 0.54^{c}	38.16 ± 4.04^{c}

Values in columns followed by a different superscript are significantly different ($p \leq 0.05$). The values are averages of three replicates ± standard deviation

LMP fraction had 7.02 ± 0.58% w/w of total fatty acids and 33.76 ± 4.04% w/w of total fatty acids of EPA and DHA respectively being highest, followed by crude (2.76 ± 0.31 and 9.5 ± 0.16% w/w of total fatty acids) and lowest being HMP (1.2 ± 0.39 and 4.07 ± 0.54). DHA content of winterized Nile perch viscera oil was higher than those reported for crude oils obtained from various tissues of different fish species, ranging from 0.87% for catfish viscera to 13.01% for sardine head [*Fiori et al., 2012; Guil-Guerrero et al., 2011; Masa et al., 2011; Crexi et al., 2010; Jankowska et al., 2010; Sahena et al., 2010a; Thammapat et al., 2010; Zhong et al., 2007; Falch, 2006; Haliloğlu et al., 2004; Navarro-García et al., 2004b*; *Aidos, 2002; Sun et al., 2002*]. EPA content on the other hand varied from those reported for the crude oil from the different species and tissues; in most of the cases it was far lower for example 0.28% in catfish

viscera and 10.95% in sardine head. The total PUFA as total of EPA and DHA was, however, within the proposed range of 40 – 60% for concentrated fish oils [*CCFO, 2013*].

Omega-3 FAs have been found to be higher in Nile perch landed during the wet season than during the dry season. Belly flaps of Nile perch landed during the wet season have been shown to possess higher amounts of omega-3 FA, coinciding with spawning. EFAs are needed in substantial amounts for gonad development. DHA and EPA are obtained through dietary sources or may be synthesized to meet the immediate nutrient requirement. DHA synthesis increases during reproduction, as it is a major component of cell membrane structure. Moreover, MUFAs are utilized in preference to DHA and EPA during reproduction for metabolic energy. This conserves the DHA and EPA reserves making their proportions relatively high in fish lipids during spawning. Nile perch spawn during the two long wet seasons of March to June and October to December [*Ogwok et al., 2009*]. The lipid metabolism in Japanese catfish is greatly influenced by spawning and season [*Shirai et al., 2001*]. The fatty acid profiles of horse mackerel and bogue have exhibited a seasonal fluctuation too. They possess high proportions of polyunsaturated fatty acids (PUFA) in spring and winter than summer [*Orban et al., 2011*]. In the Brazilian Amazonia area, relatively high amounts of DHA were found in the muscular tissues of two freshwater fish species *Hypophthalmus sp*. and tucunaré (*Cichla sp.*) during the wet season than the dry season [*Inhamuns, Franco and Batista, 2009; Inhamuns and Franco, 2008*]. Feeding period and seasons significantly influence the fatty acid composition in the muscle of carp as DHA is the major PUFA in summer and winter, whereas linoleic acid is the major PUFA in spring and autumn [*Guler et al., 2008*]. Although the viscera of gadiform species have been shown to contain lower levels of PUFA during the winter catch [*Falch, 2006*], a decrease of environmental temperature usually leads to an increased proportion of unsaturated fatty acids that are important in maintaining membrane fluidity [*Mbatia et al., 2010a*]. *Falch et al.,* [*2006b*] found no seasonal differences in the levels of EPA in visceral oil from gadiform species, while the levels of DHA in summer were higher than in autumn. Spawning herring has been shown to

contain significantly higher PUFA in the organ tissues particularly in the milt and ovary, the greatest proportion being DHA. Because DHA is an important component of membrane structural lipids, the relative percentage of this HUFA increases during the gonad development stage. High polyunsaturated fatty acids (HUFA) are also the major source of metabolic energy for reproduction. There is selective catabolism of EPA, relative to DHA, in fatty acid oxidation which produces energy for gonadogenesis [*Huynh et al., 2007*]. This can explain the high content of DHA of the present sample, since the viscera were obtained from the November fish, landed during the spawning wet season.

Furthermore, age impacts significantly on the variation of fatty acid dietary requirements in fish [*Namulawa, Mbabazi and Kwetegyeka, 2011*]. In fish PUFA are contained in phospholipids of cell membranes, where they are closely packed and surrounded with proteins [*Gladyshev, Sushchik and Makhutova, 2013*]. DHA is the major component of phosphoglycerols of cellular biomembranes in fish. Bioconversion of $\omega - 3$ fatty acids in fish leads to their transformation into their long-chain derivatives with a higher degree of unsaturation. In a study, European perch (*Perca fluviatilis*) displayed a high bioconversion efficiency of $\omega - 3$ fatty acids, which resulted in a higher concentration of DHA in the analyzed tissues [*Jankowska et al., 2010*]. DPA is an important intermediate in the conversion of EPA to DHA [*Wanasundara and Shahidi, 1998*]. A time-course fatty acid analysis showed that DPA and EPA are interconvertable in the cells. One study involving human feeding trials showed that EPA increased the DPA levels in plasma and platelet polar lipids. The media from DPA-incubated endothelial cells contained small amounts of DHA suggesting that DPA was converted to DHA and then released into the media [*Kaur et al., 2011*].

The winterization step is used to concentrate PUFAs [*Crexi et al., 2010*]. Hydrolysis of Nile perch (*Lates niloticus*) viscera oil with lipase from *Candida rugosa* gave a good enrichment of combined DHA and EPA in the glyceride fraction. Lipase from *Thermomyces lanuginosus* enriched DHA to 32 mol% [*Mbatia et al., 2010b*]. High concentrations of n-3 PUFA have been

realized with hydrolysis by *Candida cylindracea* lipase, of seal blubber oil and menhaden oil [*Wanasundara and Shahidi, 1998*], while the concentration of DHA and EPA was doubled by using microbial lipases to hydrolyze Atlantic salmon (*Salmo salar* L.) viscera [*Sun, Pigott, and Herwig, 2002*]. This lipase (*Geotrichum Candidum)* has also been employed to concentrate EPA together with DHA in tuna oil up to 50% levels with high recoveries of EPA and DHA [*Halldorsson et al., 2004*]. Physical methods, such as solvent fractionation and winterization, have been shown to increase *ω*-3 PUFA content up to 40% in fish oil [*Hou, 2002*]. This has been confirmed by the values obtained for the low melting point fraction of the oil after winterization. The DHA and EPA contents in the high melting point fraction are most probably due to the occluded liquid phase and the phospholipids. Phosphoacylglycerols have high density and naturally high content of PUFA [*Gbogouri et al., 2006*]. These must have settled out with the high density triacylglycerols that posses higher contents of saturated fatty acids. The occluded liquid phase is part of the refining losses.

3.4 CONCLUSION

Based on the physico-chemical properties, including vitamin and ω – 3 PUFA contents, the low melting point fraction of the Nile perch (*Lates niloticus*) viscera oil is suitable as concentrated fish oil for human consumption as alternative to imported cod liver and salmon oils. The high melting point fraction is suitable for cooking due to its high smoke point. The neutralized, deodorized crude can also be used for cooking. Both would be nutritious cooking oils/fat due to the presence of some amount of vitamins A and E as well as omega – 3 PUFA.

CHAPTER 4

4.0 STORAGE STABILITY OF REFINED NILE PERCH (*Lates niloticus*) VISCERA OIL

ABSTRACT

Oil was extracted from Nile perch (*Lates niloticus*) viscera by wet rendering method and refined by neutralization, deodorization and winterization. After winterization, the oil was decanted and filtered to produce a filtrate (low melting point fraction – LMP) and the residue (high melting point fraction – HMP). The two fractions were used to carry out storage stability study. Each fraction was stored at room temperature (19 – 23^oC) for a period of 20 weeks in transparent and amber colored glass containers. The oil fractions were subjected to quality tests to assess their storage stability. They were analyzed for percent free fatty acids (FFA) as oleic acid and peroxide value (PV) at the beginning of storage and at intervals of two weeks during storage, to assess the effect of package color and storage duration. The resulting data were subjected to statistical analysis ($p \leq 0.05$) using GenStat software, 13th edition. Results showed a general increase in FFA and PV, with significant differences between oil fractions and package color over storage time. For FFA, there was no interaction between the oil fraction and package color while for PV there was significant interaction between the oil fraction and package color. FFA increased from 0.26% to 0.59% for LMP fraction in clear package, 0.26 – 0.43% for LMP in red package, 0.22 – 0.85% for HMP in clear package, and 0.22 – 0.69% for HMP in red package. PV increased from 0.50mEq O_2/kg oil to 11.65mEq O_2/kg oil for LMP fraction in clear package, 0.50 – 10.58mEq O_2/kg oil for LMP in red package, 1.01 – 9.94mEq O_2/kg oil for HMP in clear package, after 20 weeks; and 1.01 – 6.86mEq O_2/kg oil for HMP in red package after 14 weeks. The levels of FFA and PV of LMP fraction in colored package remained within the CODEX limits of 0.3% and 5mEq O_2/kg oil respectively for refined fish oils up to 18 weeks. The FFA of HMP fraction surpassed the limit after 6 and 10 weeks for transparent and colored packages respectively. The FFA of LMP fraction in transparent package surpassed the limit after

8 weeks. Only low melting point oil fraction in transparent pack had PV above limit at 18 weeks. Results showed that colored package is more suitable for both oil fractions studied. In terms of FFA and PV, HMP and LMP fractions can retain good quality at ambient temperatures for 10 and 18 weeks respectively.

4.1 INTRODUCTION

Nile perch (*Lates niloticus*) is a freshwater fish of central Africa's lakes and rivers [*Turon et al., 2005*]. Nile perch is processed into fillets, mainly exported to Europe [*Turon et al., 2005*]. Byproducts are nearly 50% of the total fish mass [*Ogwok et al., 2008*]. Shoes, belts and purses are made from hide; dried swim bladders are sent to England for filtering beer and wine and to Orient for making soup stock [*Turon et al., 2005*]. Nile perch has been identified as a fatty freshwater fish, with the oil distributed in various tissues [*Turon et al., 2005; Ogwok et al., 2008; Ogwok et al., 2009; Masa et al., 2011*]. Among the tissues, viscera contain substantial quantities of oil [*Ogwok et al., 2008; Ogwok et al., 2009; Masa et al., 2011*].

Fish oil is a source of long-chain ω-3 PUFA, especially EPA and DHA with nutritional value and health benefits [*Del Nobile et al., 2009; Ogwok et al., 2008; Gbogouri et al., 2006; Aidos et al., 2002*]. DHA and EPA are essential for fetal brain development. Foods rich in PUFA are essential for normal growth and proper body function [*Ogwok et al., 2008*]. The bulk of the world's fish oil is extracted by the wet pressing method [*Sathivel, 2011; Rubio-Rodríguez et al., 2010; FAO, 1986*].

Lipid hydrolysis and oxidation increase during storage, with corresponding increases in free fatty acids and peroxide value of samples [*Namulema, Muyonga and Kaaya, 1999; Aubourg et al., 2005a; Boran et al., 2006; Thiansinakul et al., 2010*]. PUFA are very susceptible to oxidation [*McClements and Decker, 2008*]. Nile perch oil has been shown to possess high content of antioxidants β-carotene and α-tocopherol which make it fairly stable to deteriorative oxidative processes [*Ogwok et al., 2008*]. In one study, the fatty acid profile of fish oil did not

change over storage duration [*de Castro et al., 2007*]. Oily tissues from Nile perch processing are currently underutilized among fishing communities in East Africa. Roes are sold and consumed locally without value addition yet they have substantial amount of oil. A lot of skins are also discarded or freely given away for animal feeding, despite containing high quality oil. The rural folk use the viscera oil to fry the local market fish and chips, domestic cooking, and lately to feed infants [*Kabahenda and Hüsken, 2009*]. A possibility of the extracted oil being refined to obtain a value added product was explored, with the objective of establishing its storage quality of the refined oil.

4.2 MATERIALS AND METHODS

4.2.1 Materials

4.2.1.1 Raw materials

Viscera were obtained from Nile perch of processing size, purchased from a fish filleting factory in Nairobi (W. E. Tilley Group) in late November 2011 and early May 2013. They were frozen in the factory before collection. They were transported in cool box to the deep freezer (-18°C) in the University's Pilot Plant. The samples were kept frozen until oil extraction. The November 2011 consignment was processed in April 2012 and analyzed between May and August 2012. The May 2013 consignment was processed in May 2013 and analyzed between May and August 2013.

4.2.1.2 Chemicals for analysis

All reagents were of analytical grade. Sodium hydroxide ampoule (*Rankem, India*), phenolphthalein indicator (*Loba Chemie, India*), ethanol (*Scharlan – Scharlab, Spain*) and diethyl ether (*Kobian Scientific*) were used for FFA. Chloroform (*Rankem*), glacial acetic acid (*Sigma Aldrich*, *Germany*), potassium iodide (*Panreac Quimica SAU*, *Spain*), sodium thiosulphate (*Rankem*) and starch indicator (*Merck – RSA*) were used for PV.

4.2.2 Methods

4.2.2.1 Processing methods

Prior to oil extraction, entire sample was minced in the pilot plant to pass through 7.0 mm (0.276 inch) screen, then tumbled to achieve uniformity. Oil extraction was done by wet rendering, heating sample in equal amount of water for 15 minutes. The cooking was done in triplicate at 93°C in covered pans. Oil recovery was done by sieving the cooked mass with two layers of muslin cloth. The stick-water, aqueous layer and oil were separated by draining through a separating funnel; the former two occupying the bottom and middle layers were drained off, leaving the oil in the funnel. The residue in the muslin cloth was pressed using a plate and frame press to obtain more oil. This was also passed through the separating funnel to remove stick-water and emulsion. The oil obtained was weighed then subjected to refining by neutralization, deodorization and winterization. The winterized oil (low melting point fraction) and the residue (high melting point fraction) were subjected to shelf-life study, a portion of each fraction kept in transparent glass and colored glass. The shelf-life study was done at room temperature as is the recommended standard for storage [*Codex Alimentarius Commission, 1987*].

4.2.2.2 Analytical methods

The free fatty acids content of the oil was determined volumetrically using aqueous sodium hydroxide standardized to 0.1M from ampoule and 1% phenolphthalein indicator in ethanol according to *AOCS* (*1998*) method Ca 5a-40. A neutral mixture (50 ml) of diethyl ether: ethanol (1:1) was used as a solvent. FFA values were reported as % oleic acid by weight.

The peroxide value was determined and expressed as mEq O_2/kg oil, according to *AOCS* (*1998*) method Cd 8b-90. Oil samples were dissolved in chloroform and mixed with glacial acetic acid and freshly prepared saturated potassium iodide solution. Liberated iodine was titrated with sodium thiosulphate standardized to 0.01M solution using 1% starch indicator.

4.3 RESULTS AND DISCUSSION

4.3.1 Changes in Free Fatty Acids during Storage

There was significant difference in the FFA values for the different melting point fractions and package color. There was, however, no interaction between melting point fraction and package color. The results showed a general increase in FFA during storage period (*Fig. 4.1*), representing the trend observed earlier for crude Nile perch viscera oil under accelerated oven test [*Ogwok et al., 2008*]. This can partly be attributed to the activity of residual lipases and phospholipases [*Falch, 2006; Ogwok et al., 2008*]. Much of the FFA was removed by the refining steps. Neutralization removed most of the FFA, and during the deodorization, vaporization of odoriferous compounds and free fatty acids took place. Low hydrolytic activity is typical of the spawning season, particularly in males [*Kołakowska et al., 2003*]. In this study, the combine effect of the refining processes reduced FFA to 23% in low melting point fraction and 20% in high melting point fraction. Under appropriate processing conditions, FFA can be reduced by up to 50% during deodorization [*Crexi et al, 2010*]. The levels of FFA should generally be below 2% (as acid value) in medicinal cod liver oil. Levels of free fatty acids in crude oil may be reduced by alkali refinement to levels less than 0.05% [*Falch, 2006*]. There was significant difference in FFA values over time for low MP fraction stored in transparent package, the difference occurring after sixteen weeks. On the other hand, there was no significant difference for low MP fraction stored in colored package. For high MP fraction stored in transparent package, there was significant difference from week eight, whereas for the high MP fraction stored in colored package there was significant difference from week twelve.

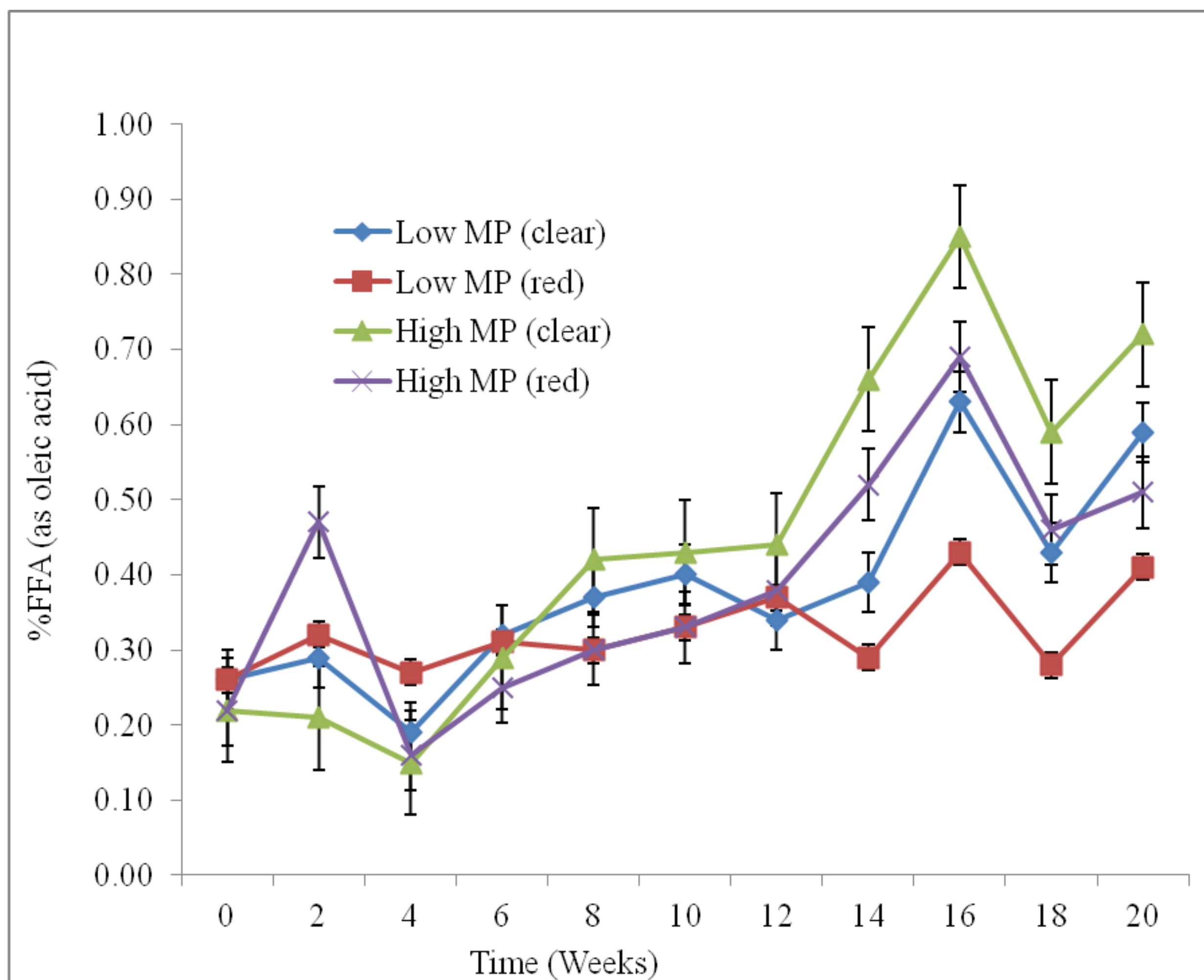

Fig. 4.1: Changes in free fatty acids during storage of refined Nile perch viscera oil

For the low melting point fraction in colored package, the values surpassed the maximum limit for refined fish oils, set by CODEX Alimentarius after 18 weeks. The FFA of high melting point fraction surpassed the limit after 6 and 10 weeks for transparent and colored packages respectively. The FFA of low melting point fraction in transparent package surpassed the limit after 8 weeks. The values were, however, below the maximum limit for nutraceutical grade fish oils in the United States, as set by the Council for Responsible Nutrition. The maximum limit for refined fish oils, set by CODEX Alimentarius is 0.3%, while the maximum limit for nutraceutical grade fish oils in the US is 1.5% [*Ackman, 2005*]. Based on this, low melting point fraction of refined Nile perch viscera oil can be considered to maintain nutraceutical grade status for at least 18 weeks of storage at ambient temperature, based on hydrolytic changes.

4.3.2 Changes in Peroxide Value during Storage

There were significant differences in the PV for the different melting point fractions and package color. There was interaction between melting point fraction and package color as well. PV increased during storage, significant difference occurring for all conditions in the second week, but the values remaining within the limits for refined fish oils for eighteen weeks. High MP fraction stored in colored package, however, remained below the maximum limit throughout the storage period of twenty weeks (*Fig. 4.2*). It has been suggested that decreased oxidative stability of oils during processing is due to the loss of natural antioxidants and synergists such as tocopherols and phospholipids [*Wanasundara, 1996*], but the high MP fraction retained much of the phospholipids left after alkali refining. The values closely represent the trend observed earlier for crude Nile perch viscera oil under accelerated oven test [*Ogwok et al., 2008*] and those reported for refined cod liver and seal blubber oils under similar Schaal storage conditions of 65°C [*Wanasundara, Shahidi and Amarowicz, 1998*]. The results concur with studies on the oils from other fishes stored at +4°C and -18°C [*Boran, Karaçam and Boran, 2006*]. The maximum limit for refined fish oils, set by CODEX Alimentarius is 5mEq O_2/kg oil [*Ackman, 2005*]. Deodorization might have increased the oxidative stability of the oil because pro-oxidants such as residual moisture, monoacylglycerols and free fatty acids in the oil were removed [*Wanasundara, 1996*]. Winterization has been shown to significantly reduce secondary oxidation products of lipids arising from heating processes such as deodorization, thereby improving oxidation stability [*Crexi et al., 2010*].

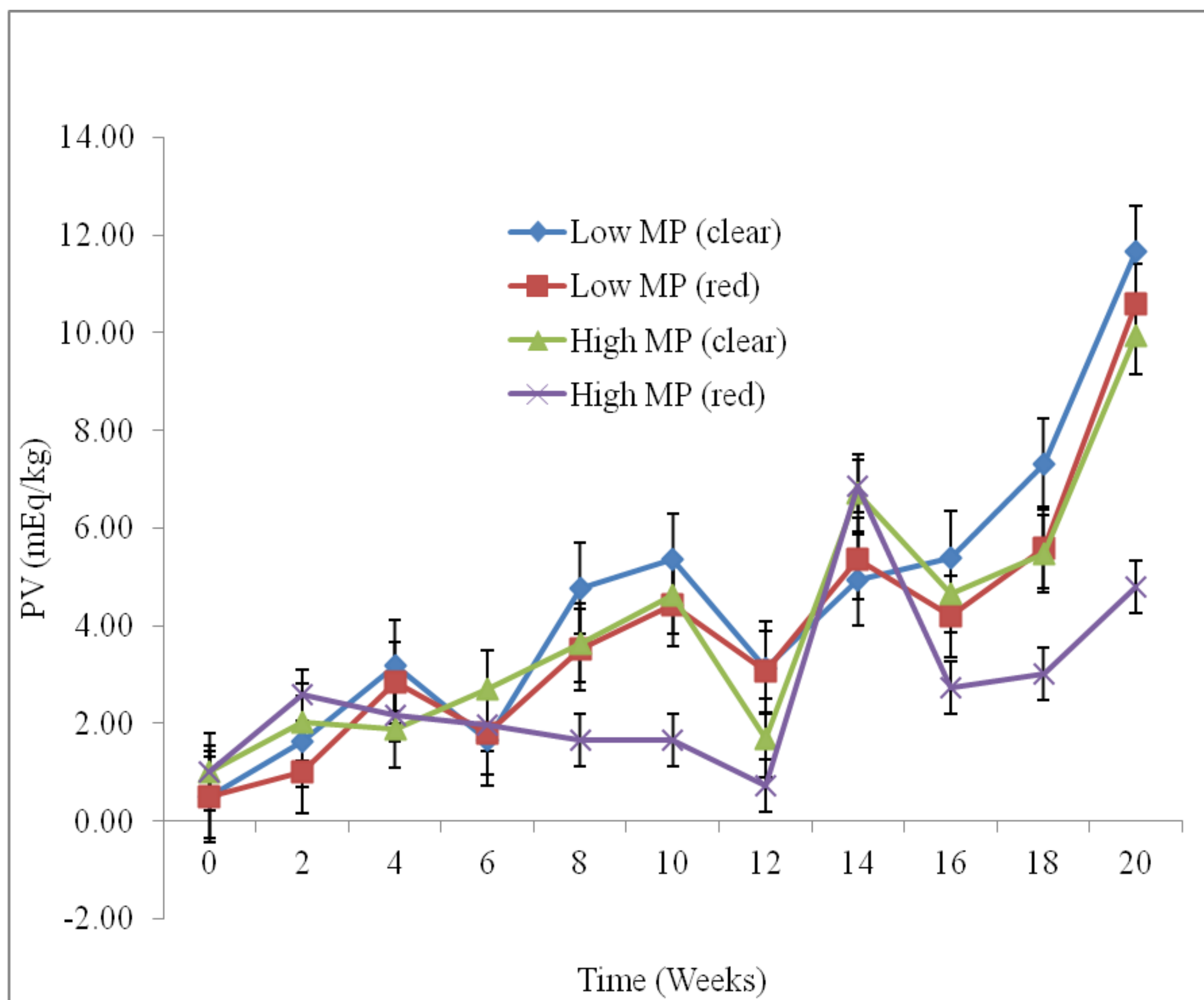

Fig. 4.2: Changes in peroxide value during storage of refined Nile perch viscera oil in different packages

The enhanced exceptional oxidative stability of the oil during the refining processes and storage must have been due to its high content of antioxidants β-carotene and α-tocopherol [*Ogwok et al., 2008*]. Generally, fish oils rich in both β-carotene and α-tocopherol are known to possess high resistance to oxidative stress [*Navarro-García et al., 2004a*]. These natural antioxidant vitamins have free radical quenching ability, capable of slowing down or terminating the onset of oxidative deterioration processes [*Wanasundara, Shahidi and Amarowicz, 1998*]. There is, however, possibility of improving the oil stability by means of oxygen scavengers and antioxidants as has been used on rainbow trout fillets [*Mexis, Chouliara and Kontominas, 2009*].

4.4 CONCLUSION

Results showed that amber colored package is the more suitable for both low and high melting point oil fractions studied. In terms of FFA and PV, high melting point and low melting point fractions can retain good quality at ambient temperatures for 10 and 18 weeks respectively. HMP and LMP Nile perch oil fractions should therefore be packed in colored containers and used within 10 and 18 weeks respectively in any commercial adventure.

CHAPTER 5

5.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 GENERAL DISCUSSION

During extraction optimization, low oil yields were observed at high acid concentrations. This was probably due to formation of a larger and stable aqueous layer which resulted in larger oil loss. The higher acid concentrations also seem to have produced high amount of denatured proteins, probably forming protein–lipid complexes, which further reduce extraction of oil [*Daukšas, Falch, Šližytė and Rustad, 2005*]. The FFA generally increased as acid concentration increased, with slight fall at higher acid concentrations. This was probably due to hydrolysis of the oil acylglycerols mediated by the citric acid. Zero (0%) acid corresponds to the conventional wet rendering (fishmeal) process. The FFA content of oil at 0% acid is probably due to hydrolysis by endogenous enzymes in the viscera prior to the heating process [*Falch, 2006; McClements and Decker, 2008; Crexi et al., 2010*]. *Zhou et al.* [*1995*] studied the FFA content of lipids during ensilage acidification of minced herring and found the maximum %FFA to be about 6%. Increased acidity during ensilaging lowered the activity of lipases thereby lowering %FFA. This has also been shown during fermentation ensilaging [*Rai et al., 2010*]. PV generally increased as acid concentration increased, with slight fall at higher acid concentrations. Acid hydrolysis of fish tissues results in production of hematin compounds [*Crexi et al., 2010*]. Hematin compounds enhance oxidation [*Opara et al., 2007; McClements and Decker, 2008*]. Autoxidation of myoglobin could be associated with enhanced lipid oxidation. Autoxidation of oxymyoglobin results in the formation of metmyoglobin and superoxide, which rapidly dismutate to hydrogen peroxide and oxygen. The interaction of hydrogen peroxide with metmyoglobin has been shown to very rapidly lead to generation of an active species, ferryl radical, which could initiate lipid peroxidation [*Thiansilakul, Benjakul and Richards, 2010*]. The initiation and propagation of lipid oxidation have been shown to be more pronounced in the un-bled than bled fish muscles [*Maqsood and Benjakul, 2011*]. The generally low oxidation could

be attributed to the presence of carotenoids and tocopherols in Nile perch oil [*Ogwok et al., 2008*]. Carotenoids and tocopherols are natural anti-oxidants. Citric acid also exerts anti-oxidant activities [*McClements and Decker, 2008*]. Therefore, as acidity increased, there must have been a fall in lipase activity, resulting in lower %FFA hence reduced oxidation. The anti-oxidant components must have further enhanced the oxidative stability of the oil during the processes.

Refining produced varied physico-chemical properties for the different fractions. The high melting fraction contains saturated fatty acids most of which saturated fatty acids while the low melting fraction contains high content of unsaturated fatty acids in the triacylglycerols. Most of the unsaturated fatty acids possess long carbon chains [*McClements and Decker, 2008; Nawar, 1985*]. The lower saponification value of low melting than those of crude and high melting point fractions may confirm this since the higher the SV, the lower the mean chain length of the component fatty acids of an acylglycerol [*Haas, 2005*]. There is a variety of the saturated fatty acids in fish oil [*Mbatia et al., 2010; Ogwok et al., 2008; Turon et al., 2005; Aidos et al., 2002; Aidos, 2002*], explaining the wide range of the melting points of the crude and the high melting point fractions. It is attributed to the presence of impurities such as phospholipids, ketones and other materials in the unrefined fish oil. The melting points of fish oil are sharper after each purification step that removes impurities [*Yin and Sathivel, 2010*]. At low temperatures, high melting point long chain saturated fatty acids crystallize out and PUFA remain in the liquid form [*Shahidi and Wanasundara, 1998*]. The saturated fatty acids are more compact and are separated alongside the triacylglycerols with long high molecular weight fatty acids (waxes). These contribute to the higher densities [*Bockish, 1998; Hamilton and Bhati, 1980*] of the high melting point and crude fractions, the magnitude decreasing with the higher degree of unsaturation. Saturated fatty acids are more heat stable [*McClements and Decker, 2008; Nawar, 1985; Dugan Jnr, 1976*]. This explains why the smoke points of crude and the high melting point fractions are higher than that of the low melting point fraction. The smoke

points for crude and high melting point fractions are within the ranges recommended for frying oils [*Gupta, 2005*].

Vitamin A bioaccumulation occurs with age [*Ogwok et al., 2008*] and this must have resulted in its deposition in the tissues (including viscera) of the processed fish. The vitamin A content of all fractions of Nile perch viscera oil () are comparable to 5.40mg/100g reported for cod liver oil [*Ogwok et al., 2008*]. This could be due to the inclusion of liver in the viscera of Nile perch, since liver oils have high Vitamin A [*Ackman, 2005*]. The amount of tocopherol in the refined Nile perch viscera oil was similar to values reported for the liver oil of ray, *Rhinoptera steindechneri* [*Navarro-García, et al., 2004a*] and Nile perch belly oil; its content is related to diet since fish cannot synthesize vitamin E [*Ogwok et al., 2008*].

There was significant difference in eicosapentaenoic acid (EPA; C20:5, n – 3) and docosahexaenoic acid (DHA; C22:6, n – 3) contents of the crude, low melting point and the high melting point fractions of the oil. The total PUFA as total of EPA and DHA was within the proposed range for concentrated fish oils [*CCFO, 2013*]. Omega-3 FA have been found to be higher in belly flaps of Nile perch landed during the wet season, coinciding with spawning, than during the dry season [*Ogwok et al., 2009*]. EFAs are needed in substantial amounts for gonad development. DHA synthesis increases while MUFA are metabolic for energy in preference to DHA and EPA during reproduction [*Huynh et al., 2007*]. This conserves the DHA and EPA reserves making their proportions relatively high in fish lipids during spawning. Although the viscera of gadiform species contain lower levels of PUFA during the winter catch [*Falch, 2006*], a decrease of environmental temperature usually leads to an increased proportion of unsaturated fatty acids that are important in maintaining membrane fluidity [*Mbatia et al., 2010a*]. Nile perch spawn during the two long wet seasons of March to June and October to December [*Ogwok et al., 2009*]. This can explain the high content of DHA of the present sample, since the viscera were obtained from the November fish, landed during the spawning wet season. Furthermore, age impacts significantly on the variation of fatty acid dietary requirements in fish [*Namulawa,*

Mbabazi and Kwetegyeka, 2011]. In fish PUFA are contained in phospholipids of cell membranes, where they are closely packed and surrounded with proteins [*Gladyshev, Sushchik and Makhutova, 2013*]. DHA is the major component of phosphoglycerols of cellular biomembranes in fish. Bioconversion of ω – 3 fatty acids in fish leads to their transformation into their long-chain derivatives with a higher degree of unsaturation [*Kaur et al., 2011; Jankowska et al., 2010*]. DPA is an important intermediate in the conversion of EPA to DHA [*Kaur et al., 2011; Wanasundara and Shahidi, 1998*]. The winterization step is used to concentrate PUFAs [*Crexi et al., 2010*]. Physical methods, such as solvent fractionation and winterization, have been shown to increase *ω*-3 PUFA content up to 40% in fish oil [*Hou, 2002*]. This has been confirmed by the values obtained for the low melting point fraction of the oil after winterization. The DHA and EPA contents in the high melting point fraction are most probably due to the occluded liquid phase and the phospholipids. Phosphoacylglycerols have high density and naturally high content of PUFA [*Gbogouri et al., 2006*]. These must have settled out with the high density triacylglycerols that posses higher contents of saturated fatty acids. The occluded liquid phase is part of the refining losses

During shelf-life study on refined Nile perch viscera oil, the values of FFA and PV remained within the limits for refined fish oils for eighteen weeks. High MP fraction stored in red glass, however, remained below the maximum limit throughout the storage period of twenty weeks. The enhanced exceptional oxidative stability of the oil was perhaps due to its high content of antioxidants β-carotene and α-tocopherol [*Ogwok et al., 2008*]. Generally, fish oils rich in both β-carotene and α-tocopherol are known to possess high resistance to oxidative stress [*Navarro-García et al., 2004a*]. These natural antioxidant vitamins have free radical quenching ability, capable of slowing down or terminating the onset of oxidative deterioration processes [*Wanasundara, Shahidi and Amarowicz, 1998*].

5.2 CONCLUSIONS

Based on the oil content, the viscera of Nile perch are good raw materials for production of fish oils. On the basis of oil yield and quality, 0% acid and 15 minutes heating is the most suitable combination for mass production of refined Nile perch oil. Nile perch (*Lates niloticus*) viscera lipids possess high levels of the nutritious ω – 3 fatty acids and are therefore a valuable resource as ingredient in food, feed and healthcare products. The low melting point fraction of the Nile perch (*Lates niloticus*) viscera oil is suitable as concentrated fish oil for use as a nutraceutic. The high melting point fraction is suitable for use as a cooking fat due to its high smoke point. The neutralized, deodorized crude can also be used as cooking oil. Both would be nutritious cooking fat due to the presence of vitamins A and E as well as omega – 3 PUFA. Based on hydrolytic and oxidative stability measured by FFA and PV respectively, refined (winterized) Nile perch viscera oil can generally be considered to maintain nutraceutical grade status for at least eighteen weeks.

5.3 RECOMMENDATIONS

Due to high lipid content, physico-chemical characteristics as well as the nutritional value, Nile perch (*Lates niloticus*) viscera lipids could be a valuable resource as ingredient in food, feed and healthcare products. It is, however, necessary to further explore the use of acid to remove contaminants in Nile perch viscera oil rather than for improving yield or quality. Further studies also need to be carried out on packaging conditions to establish the full shelf-life of refined Nile perch viscera oil. It is hoped that this knowledge will be a valuable contribution to reaching the goal of maximizing the value of fish processing. Nile perch viscera should be used as raw material for fish oil production on industrial scale. This, apart from producing high quality edible oil through value addition, will also contribute to alleviating the challenge of waste disposal in fish processing industry. Refined Nile perch viscera oil will as well be an alternative to imported fish oils such as cod liver oil and salmon oil.

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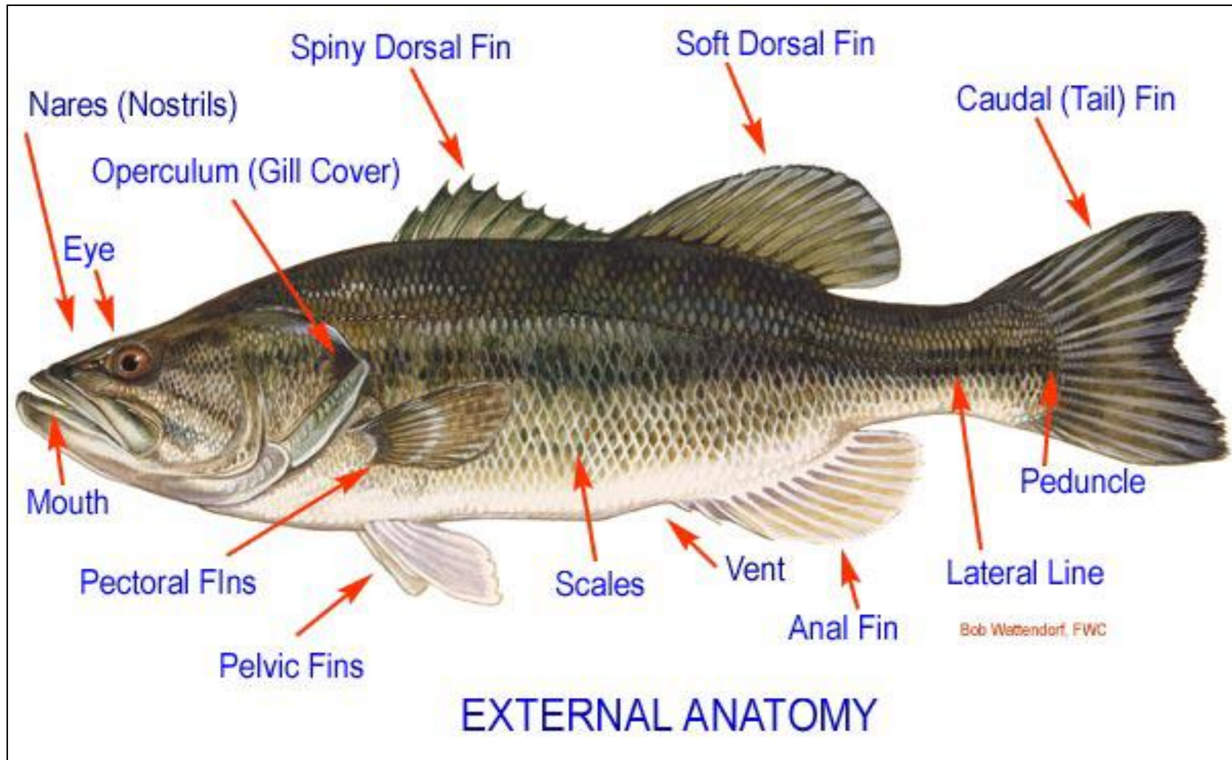
APPENDICES:

Appendix I: External fish anatomy (*Source*: Fish & Wildlife Conservation Commission)



Appendix II: Internal fish anatomy (*Source*: Fish & Wildlife Conservation Commission)