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Physico-chemical Characteristics of Refined Lake Victoria Nile Perch (*Lates niloticus*) Viscera Oil

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Abstract

Nile perch viscera oil was characterized in terms of its physico-chemical properties. The crude oil was refined by neutralization, deodorization and winterization to obtain low melting point (LMP) and the high melting point (HMP) fractions. 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). Crude was used as control. Data were analysed ($p \leq 0.05$) using GenStat software. The yield of winterized oil (LMP) was 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, while there were significant differences in the slip MP, SP, SV, IV and vitamin A (retinol) content. There were also significant differences in EPA and DHA contents. EPA and DHA for the LMP were highest, followed by crude and HMP having lowest. High vitamins A and E as well as omega – 3 polyunsaturated fatty acids (ω – 3 PUFA) contents and high SP make the crude and HMP fractions suitable for use in cooking. The high amount of LMP fraction has high content of ω – 3 PUFA, making it suitable for use in food, feed and nutraceutics.

***Keywords*:** Nile perch; viscera; wet rendering; refining; physico-chemical characteristics

1.0 Introduction

Nile perch (*Lates niloticus*) was introduced into Lake Victoria in about 1950s and 1960s (*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 over 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 (*Turon et al. 2005*). Nile perch is processed into fillets, mainly exported to Europe (*Turon et al. 2005*). Nile Perch 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*). Byproducts are nearly 50% of the total fish mass (*Ogwok et al. 2008*). Among the byproducts, the hide is tanned, the swim bladders are dried and exported for use in filtering beer and wine, as well as 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 (*Ogwok et al. 2008; Gbogouri et al, 2006*). 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. 2010; Turon et al. 2005*), roes (*Falch 2006*) and skins (*Sahena et al. 2010*) of fishes also possess high quality oil.

Crude oils require refining for human consumption (*Crexi et al. 2009*). Nile perch oil is relatively cheap fish oil normally sold off as a byproduct. Its high content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and low PUFA to SFA ratio, however, indicate a need to concentrate PUFA prior to human consumption. This would enable processing of high value products with increased EPA and DHA levels. 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 2011*). 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 SFAs. 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. Omega – 3 concentrates free of SFA and MUFA 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 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. These include adsorption chromatography, fractional or molecular distillation, enzymatic splitting, low-temperature crystallization, supercritical fluid extraction and urea complexing (*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 SFA (high melting point fraction) and ω – 3 PUFA (low melting point fraction), with the objective of preparing the oil's profile for appropriate industrial and consumer applications.

2.0 Materials and Methods

2.1 Materials

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). They were preserved by freezing in a deep freezer in the University's Pilot Plant prior to oil extraction.

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 obtained 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.

2.2 Methods

2.2.1 Processing methods

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 moisture exchange, 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^{o}C in covered pans. Oil recovery was done by sieving the cooked mass. The stick-water, aqueous layer and oil were separated by decanting in a separating funnel; the former two occupying the bottom and middle layers were drained off, leaving the oil in the funnel. The residue 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. 1* and *Fig. 2* Final packed product is illustrated in *Fig. 3*

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^{o}C for 3 hours with agitation, the soap-stock settling at the bottom of the vessel and the neutral oil obtained by filtration as described in literature [*Bockish 1998*] with muslin cloth.

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

Fig. 1: Fractionated Nile perch (*Lates niloticus*) viscera oil in different containers: Low (top) and high (bottom) melting point fractions

Fig. 2: Molten Nile perch (*Lates niloticus*) viscera oil. Crude (left) and high melting point (right) fractions

Fig. 3: Winterized Nile perch (*Lates niloticus*) viscera oil in amber colored 100ml ampoule (extreme left), clear 100ml (three middle) and clear 60ml ampoule (extreme right)

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 filtered as described in literature (*Gunstone and Norris 1983; Shahidi and Wanasundara 1998*). The filtration was done by use of muslin cloth folded into four layers.

2.2.2 Analytical methods and yield of the recovered oil

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* (*2009*) 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.

Density was determined by pycnometer method. Dry pycnometer was filled with sample at 20°C and the weight recorded. The pycnometer was then filled with water at 20°C and weighed. The sample weight was then compared with the weight of water to determine its density (*AOAC, 1998*):

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

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 (*AOAC 1998*).

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 (*AOAC 1998*).

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 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

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).

Vitamin A was determined as retinol by the method described by *Zahar and Smith* (*1990*) with 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 545.7ppm 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 serially diluted 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^{o}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^{o}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^{o}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.

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^{o}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.

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 (4ml) 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^{o}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^{o}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).

3.0 Results and Discussion

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.

3.2 Physico-chemical Properties

Table 1: 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.

Table 1 shows the density, melting point (MP) and smoke point (SP) of the Nile perch viscera oil fractions. There was no significant difference ($p>0.05$) 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 MP and SP of the three fractions of oil ($p\leq0.05$). The slip MP were 25 ± 8^{o}C, 33 ± 9^{o}C and 7 ± 5^{o}C for crude, HMP and LMP fractions respectively. The SP were 255 ± 3^{o}C, 278 ± 4^{o}C and 223 ± 3^{o}C for crude, HMP and LMP fractions respectively.

Table 2: 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^{a}	124.7 ± 4.1^{a}	5.21 ± 0.32^{a}	2.46 ± 0.10
High MP	190.2 ± 6.7^{b}	119.9 ± 1.9^{b}	5.16 ± 0.25^{b}	2.52 ± 0.09
Low MP	183.0 ± 5.2^{c}	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.

Table 2 shows the saponification value (SV), iodine value (IV) and contents of vitamins A and E for the crude, low MP and high MP oils. There were significant differences ($p\leq0.05$) in the SV, IV and vitamin A (retinol) content of the three fractions of oil. SV was lowest in LMP (183.0 mg KOH/g oil) and highest in HMP (190.2 mg KOH/g oil). IV was 119.9 ± 1.9 g 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. Vitamin E (tocopherol) values were not significantly different ($p>0.05$).

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*). 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 et al. 1993*). There is a variety of the saturated fatty acids in fish oil (*Mbatia 2011; Ogwok et al. 2008; Turon et al. 2005*), 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*), 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*) 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*). 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. Nutritive value of fish lipids and changes taking place in lipids during seafood storage and processing are significantly dependent on the fishing season. 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 1996*) 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 Omega – 3 PUFA Content

Table 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

There was significant difference ($p\leq$) 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*). 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 is 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; Crexi et al.*

2010; Jankowska et al. 2010; Sahena et al. 2010; Thammapat et al. 2010; Falch 2006; Haliloğlu et al. 2004; Navarro-García et al, 2004b; *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. However, the total PUFA as total of EPA and DHA was within the proposed range 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 et al. 2009*). 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 2011*). *Falch* (*2006*) 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 et al. 2011*). In fish PUFA are contained in phospholipids of cell membranes, where they are closely packed and surrounded with proteins (*Gladyshev et al. 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. In a study, European perch (*Perca fluviatilis*) displayed a high bioconversion efficiency of ω – 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 1996*). 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 2011*). High concentrations of n-3 PUFA have been realized with hydrolysis by *Candida cylindracea* lipase, of seal blubber oil and menhaden oil (*Wanasundara 1996*), while the concentration of DHA and EPA was doubled by using microbial lipases to hydrolyze Atlantic salmon (*Salmo salar* L.) viscera (*Sun et al. 2002*). This lipase (*Geotrichum Candidum)* has also been employed to concentrate EPA and 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, 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.

4.0 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.

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