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Fatty acid profile and stability of oil from the belly flaps of Nile perch (*Lates niloticus*)

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Abstract

Oil extracted from the belly flaps of Lake Victoria Nile perch (*Lates niloticus*) was evaluated for fatty acid composition, contents of vitamin A, β-carotene and α-tocopherol, and oxidative stability. The oil was found to contain substantial amount of palmitic, palmitoleic, stearic, oleic, docosapentaenoic and docosahexaenoic fatty acids (FAs) and had high vitamin A content (3.94 ± 0.02 to 5.90 ± 0.02 mg/100 g of oil). Docosahexaenoic acid ($10.45 \pm 0.38\%$), docosapentaenoic acid ($5.30 \pm 0.60\%$) and eicosapentaenoic acid ($3.63 \pm 0.05\%$) were the most dominant polyunsaturated fatty acids (PUFAs). Ratios of PUFAs to saturated FAs were in the range 0.68 ± 0.02 to 0.74 ± 0.03, while the ratio of total ω-3 FAs to total ω-6 FAs was 0.85 ± 0.02 to 0.95 ± 0.08. The oils showed exceptional resistance to accelerated oxidation at 65 °C probably because of its high content of β-carotene (2.93 ± 0.03 to 4.69 ± 0.01 mg/100 g of oil) and α-tocopherol (2.11 ± 0.03 to 11.4 ± 0.92 mg/100 g of oil). From the results, it can be concluded that Nile perch oil is a rich source of essential fatty acids and vitamin A.

Keywords: Nile perch; Lake Victoria; Belly oil; Fatty acid profile; Oxidative stability

1. Introduction

Polyunsaturated fatty acids (PUFAs) are known to confer health benefits, especially when the two predominant forms (ω-3 and ω-6 PUFAs) are consumed (Eristsland, 2000). Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are essential for the development of foetal brain (Hoffmann & Uauy, 1992). In addition, consumption of foods rich in ω-3 fatty acids has been correlated with reduction in coronary heart disease (Harper & Jacobson, 2001). Foods rich in polyunsaturated fatty acids are, in general, essential for normal growth and proper body function.

Fish oils have been shown to contain significant amounts of polyunsaturated fatty acids (Moffat & McGill, 1993). DHA and EPA are particularly found in oils of most fatty fish species. Levels of these essential fatty acids (EFAs) are known to vary largely with season and geographical location (Joseph, 1985). EFA content in fish is dependent on fish species and size (Huss, 1995), physiological demand (Oliveira, Agostinho, & Matsushita, 2003) and varies from tissue to tissue (Moffat & McGill, 1993). Fish liver and belly tissues are widely recognised for the high level of PUFAs and vitamin A in their fat (Moffat & McGill, 1993).

Fish oils, because of their high content of unsaturated fatty acids, are highly susceptible to oxidative deterioration reactions. Oxidation of these fatty acids leads to end products of pronounced off-flavour that affect the overall quality of oil (Eristsland, 2000). However, oils with high levels of lipid-soluble antioxidants have been associated with exceptional stability to oxidation. Studies on the oil of tecolote ray indicate a correlation between its natural antioxidant content and resistance to oxidation (Navarro-García, Bringas-Alvarado, Pacheco-Aguilar, & Ortega-García, 2004). Fish oils from fatty species have been shown to

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contain high levels of vitamin A, a natural antioxidant (Moffat & McGill, 1993). It is therefore desirable that oils meant for commercial purposes have high levels of natural antioxidants so as to delay oxidative processes.

Nile perch (*Lates niloticus*) is a freshwater fatty fish and the most dominant species from Lake Victoria. Byproducts from Nile perch processing account for nearly 50% of the total fish mass (MAAIF, 2006). Fish wastes, however, may be transformed into products of high nutritive value with significant health benefit. Fatty materials from Nile perch belly flaps are presently discarded or sold at very low cost (MAAIF, 2006). Therefore, the utilisation of belly tissue becomes essential for value addition. In this study, the fatty acid profile, contents of vitamin A, β-carotene and α-tocopherol, and oxidative stability of Nile perch belly oil were evaluated.

2. Materials and methods

2.1. Sampling and sample preparation

Nile perch (*L. niloticus*) of three size categories (1–2, 10–30, and 40–80 kg) from Lake Victoria were collected from Ngege fish processing plant, Kampala, Uganda. Thirty fish samples from each of the different size categories were collected during September, 2006. Fatty materials from the belly cavity were obtained after eviscerating the fish. Collected fatty materials were weighed and separately packed in black polyethylene bags, placed in black plastic boxes and covered with ice.

2.2. Extraction of oil

Fatty material was defrosted at ambient temperature and then homogenised using a Waring blender for about 2 min. Crude oil was extracted by incubating the homogenate at 35 °C for 1 h. Suspended material was removed and anhydrous sodium sulphate (20 g/100 g of homogenate) added. Extracted oil was centrifuged, decanted, weighed and expressed as a percentage of the homogenate on a wet weight basis. Dry oil was stored at −20 °C in 30 ml brown (amber) vials and analysed within a week for peroxide value, iodine value, β-carotene, α-tocopherol, vitamin A and free fatty acids.

2.3. Determination of fatty acids profile

Fatty acids profile was determined quantitatively using gas chromatography (GC) (PerkinElmer, Norwalk, USA) in accordance with AOCS (1998) method Ce 1b-89. Boron trifluoride (BF_3) and methanol mixture (10%) was used for the preparation of fatty acid methyl esters (FAME). The mixture of oil (2 drops) and BF_3-MEOH (1 ml) was homogenised with a mixture of hexane (1 ml) and water (1 ml), to enhance the esterification process. Esterification was performed at 100 °C for 1 h. Analysis was performed using a gas chromatograph equipped with a fused silica capillary column (CP-Sil 88, 50 m × 0.22 mm; 0.2 μm film thickness; Chrompack, Middleburg, the Netherlands). The oven was heated from 150 to 225 °C at a rate of 5 °C/min and held at 225 °C for 10 min. The temperature of the injector and flame ionisation detector was maintained at 250 °C. Inlet pressure of the carrier gas (hydrogen) was held at 85 kPa. Percentages of peak areas obtained were divided by the relative molecular weight of respective fatty acid methyl esters to obtain moles percent of fatty acids.

2.4. Determination of vitamin A

Vitamin A content of oil was analysed by AACC method 86-06, using high-pressure liquid chromatography (PerkinElmer, Series 200 LC), equipped with a photometric detector and a reverse-phase column (C18, 10 μm; 4.6 × 250 mm) for the measurement of *trans*- and *cis*-isomers of retinol (AACC, 2004). Oil (3 g) was dissolved in a mixture of tetrahydrofuran:ethanol (50:50) and the absorbance assayed at 328 nm. The mobile phase was prepared by mixing 860 ml HPLC grade methanol (Sigma Chemical Co., St. Louis, MO) and 140 ml deionised water. A vitamin A working standard was prepared by dissolving 50 mg of USP-vitamin A acetate concentrate (Sigma) in 100 ml ethanol. The vitamin A standard was injected into the HPLC system with a mobile phase resolution of 1.5 for *cis*- and *trans*-isomers of retinol. Sample solutions were injected in triplicate and interspersed with standard solution injections for every nine samples to ensure accurate quantification. The vitamin A content of the sample was quantified by comparison of chromatographic peak heights for the standard and that corresponding to retinol in the sample extract.

2.5. Determination of carotene

The beta carotene content of the crude oil was determined using the method described by Simpson and Haard (1985). Oil (3 g) was dissolved in hexane and the absorbance of the mixture assayed at 468 nm using a UV–visible spectrophotometer (PerkinElmer, Lambda Bio 20). β-Carotene calibration curve was prepared by dissolving 15 mg β-carotene standard (Sigma Chemical Co.) into 100 ml hexane. Four working standards were prepared by transferring 0.20, 0.50, 1.00 and 2.00 ml from the stock solution to 50 ml flasks and topping up to volume with hexane.

2.6. Determination of tocopherol

The vitamin E content of oil was analysed by AACC (2004) method 86-06 using high-pressure liquid chromatography (PerkinElmer, Series 200 LC) equipped with a fluorescence detector and a reverse-phase column (C8, 10 μm; 4.6 × 250 mm) (AACC, 2004). Oil (2 g) was dissolved in a mixture of tetrahydrofuran:ethanol (50:50) and the emission measured at 330 nm. The mobile phase was prepared by mixing 940 ml HPLC grade methanol and 60 ml deion-

ised water. Vitamin E working standard was prepared by dissolving 50 mg of vitamin E acetate concentrate (Sigma) in 95% ethanol to make 100 ml. The vitamin E standard was injected onto an HPLC system with a mobile phase resolution of 1.5 for α- and β-tocopherol. Sample solutions were injected in triplicate and interspersed with standard solution injections for every nine samples.

2.7. Determination of free fatty acids (FFA)

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

2.8. Determination of peroxide value (PV)

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 (Sigma) and freshly prepared saturated potassium iodide solution. Liberated iodine was titrated with standard sodium thiosulphate (0.01 M) solution using starch indicator (1%).

2.9. Determination of the iodine value (IV)

The iodine value was determined according to the AOAC (1999) method no. 993.20, using carbon tetrachloride as solvent. Dissolved oil sample was mixed with Wijs' solution and freshly prepared potassium iodide (10%) solution. Liberated iodine was titrated with standard potassium thiosulphate (0.1 M) solution, using carbon tetrachloride as a blank and starch as an indicator.

2.10. Accelerated oxidation (Schaal oven test at 65 °C)

The schaal oven test was carried out according to the method of Wanasundara, Shahidi, and Amarowicz (1998). Extracted oil (about 20 g) was placed in an open amber glass bottle and placed in an oven operating at 65 °C. PV was determined and expressed as mEq O_2/kg oil according to AOCS (1998) method Cd 8b-90.

2.11. Statistical analysis

Data collected were analysed using General Linear Model (GLM) procedure of Genstat statistical programme (USN International Ltd., Hemel Hempstead, UK). Analysis of variance, was performed to compare level of oil yield, peroxide value, FFAs, carotene, tocopherol, vitamin A, and FA composition for the different fish size categories. Difference between means was tested for significance using the least significance difference (LSD) method of Montgomery (1996).

3. Results and discussion

3.1. Oil yield

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 ($2.00 \pm 0.79\%$) and lowest in the small category ($1.06 \pm 0.26\%$). The amount of extracted oil from the belly tissue was highest ($p < 0.05$) in the large fish category, while the small category had the lowest value ($74.1 \pm 7.51\%$ and $39.9 \pm 2.77\%$, respectively). Lipid deposition in Nile perch belly flap, therefore, seems to increase with increasing fish size.

3.2. Vitamin A content

The vitamin A content of Nile perch belly oil was in the range 3.94 ± 0.02 to 5.90 ± 0.02 mg/100 g of oil. The amount of vitamin A varied with fish size ($p < 0.05$) (Table 1). Oil from the large fish category had the highest amount of vitamin A followed by the medium, while the small category had the lowest (5.90 ± 0.02, 4.04 ± 0.01 and 3.94 ± 0.02 mg/100 g of crude oil, respectively). Increase in vitamin A content with fish size agrees with the observation that vitamin A bioaccumulation occurs with age (Huss, 1995). The vitamin A content of Nile perch oil was within the range (3–6 mg/100 g) recommended by CODEX STAN (1991). In addition, the content of vitamin A in oil from the large fish category was comparable to that reported for cod liver oil (5.40 mg/100 g) (UNUP, 2004).

3.3. β-Carotene and α-tocopherol content

Nile perch oil contained high contents of β-carotene (2.93 ± 0.03 to 4.69 ± 0.01 mg/100 g of oil) and α-tocopherol (2.11 ± 0.03 to 11.4 ± 0.92 mg/100 g of oil). The amount of carotenes in Nile perch belly oil increased while tocopherols decreased with increase in fish size (Table 1). Oil from the large size category had the highest amount of β-carotene (4.69 ± 0.01 mg/100 g) and lowest α-tocopherol (2.11 ± 0.03 mg/100 g of oil). In contrast, oil from the small fish category had the highest amount of α-tocopherol and lowest β-carotene (11.4 ± 0.92 and 2.93 ± 0.03 mg/100 g of oil, respectively). The increase in carotene with fish size is partly attributed to bioaccumulation (Huss, 1995).

Table 1
Vitamin A, β-carotene and α-tocopherol contents of oil extracted from belly flaps of Nile perch of varying sizes (mg/100 g oil)

Fish size (kg)	Vitamin A	β-Carotene	α-Tocopherol
Small (1–2)	3.94 ± 0.02^a	2.93 ± 0.03^a	11.4 ± 0.92^a
Medium (10–30)	4.04 ± 0.01^b	3.35 ± 0.01^b	6.57 ± 0.63^b
Large (40–80)	5.90 ± 0.02^c	4.69 ± 0.01^c	2.11 ± 0.03^c

Values in columns followed by a different superscript are significantly different ($p < 0.05$). Values are averages of three replicates ($n = 30$) ± standard deviation.

The α-tocopherol content of the oils is related to diet, since fish cannot synthesise vitamin E (Moffat & McGill, 1993). The amount of carotene and tocopherol in Nile perch belly oil was however similar to values reported for the liver oil of ray *Rhinoptera steindechneri* (Navarro-García et al., 2004). Besides, the α-tocopherol content of Nile perch belly oil presented higher values than that reported for crude cod liver oil and seal blubber oil (Wanasundara et al., 1998).

3.4. Fatty acid composition

Nile perch oil was found to contain substantial amounts of palmitic (16:0), palmitoleic (16:1, *n*-7), stearic (18:0), oleic (18:1, *n*-9), docosapentaenoic (22:5, *n*-3) and docosahexaenoic (22:6, *n*-6) fatty acids (Table 2). Fractions of myristic (14:0), linoleic (18:2, *n*-6), linolenic (18:3, *n*-3) and eicosapentaenoic (20:5, *n*-3) acids were also found in levels exceeding 1% of the total fatty acids. Docosahexaenoic acid (DHA) (10.5 ± 0.38%), docosapentaenoic acid (5.30 ± 0.60%) and eicosapentaenoic acid (EPA) (3.63 ± 0.05%) were predominant among the polyunsaturated fatty acids (PUFAs). The total polyunsaturated FA content (22.8 ± 0.20%) of Nile perch belly oil was apparently higher than most reported levels (6.8–29.5%) in tropical freshwater fish (Zenebe, Ahlghren, Gustaffson, & Boberg, 1998). The total PUFA composition in Nile perch was found to be close to values for Atlantic herring (Aidos, Lourenço, Van Der Padt, Luten, & Boom, 2002) and mackerel (Foegending & Lanier, 1996). DHA, which is known to confer benefits to human health, was higher than in marine catfish (Osman, Jaswir, Khaza'ai, & Hashim, 2007) and standard menhaden oil (FDA, 1997). Oleic acid was the most dominant monounsaturated fatty acid (MUFA), accounting for 63.5% total MUFA. Total MUFA content (33.1 ± 0.24%) was generally higher than values reported for freshwater fish by Zenebe et al. (1998) and Osman, Jaswir, Khaza'ai, and Hashim (2007). The contents of palmitic (22.4 ± 0.11%) and stearic acids (7.57 ± 0.08%), the most abundant saturated fatty acids (SFAs), in Nile perch belly oil, were similar to reported values for tilapia fish, *Oreochromis niloticus*, from Ethiopian lakes (Zenebe et al., 1998).

The proportion of individual fatty acids in Nile perch belly oil varied significantly ($p < 0.05$) with fish size, except for stearic acid (18:0) (Table 2). The total contents of saturated fatty acids and monounsaturated fatty acids increased significantly ($p < 0.05$) with increase in fish size. The total SFA values were 32.9 ± 0.08% for small, 33.7 ± 0.05% for medium and 32.8 ± 0.05% for large fish categories. The total MUFA values were, 31.2 ± 0.22, 34.2 ± 0.30 and 33.8 ± 0.06% for small, medium and large fish categories, respectively. On the other hand, PUFA values decreased significantly ($p < 0.05$) with increase in fish size. The small fish category possessed the highest total PUFA value (24.2 ± 0.36%) while the medium fish category showed the lowest (21.7 ± 0.21%). Varied amount of FAs in the various fish size categories can partly be attributed to nutrient requirement. Studies by Watanabe, Kitajima, and Fujita (1983) demonstrated that ω-3 PUFAs were required in substantial amount, depending on the EFA requirement. In young fish, PUFAs are required for normal growth and development. In this regard, the fish synthesises these fatty acids *de novo*, in response to physiological demand, making them available in the fish tissue (Moffat & McGill, 1993). Meanwhile, variation in fatty acid composition in Nile perch of different

Table 2
Fatty acid profile of oil extracted from the belly flaps of Nile perch of varying sizes

Fatty acids	Fish size (kg)			Mean
	Small (1–2)	Medium (10–30)	Large (40–80)	
	Concentration (mol%)	Concentration (mol%)	Concentration (mol%)	Concentration (mol%)
Myristic (14:0)	3.00 ± 0.02^{a}	3.35 ± 0.03^{b}	3.15 ± 0.01^{c}	3.17 ± 0.03
Palmitic (16:0)	21.60 ± 0.02^{a}	22.95 ± 0.01^{b}	22.55 ± 0.03^{c}	22.37 ± 0.11
Palmitoleic (16:1, *n*-7)	10.45 ± 0.06^{a}	12.85 ± 0.19^{b}	12.90 ± 0.00^{c}	12.07 ± 0.21
Stearic (18:0)	8.15 ± 0.01^{a}	7.45 ± 0.06^{b}	7.10 ± 0.00^{c}	7.57 ± 0.08
Oleic (18:1, *n*-9)	20.70 ± 0.06^{a}	21.35 ± 0.44^{b}	20.90 ± 0.07^{c}	20.98 ± 0.05
Linoleic (18:2, *n*-6)	2.60 ± 0.10^{a}	2.00 ± 0.02^{b}	1.95 ± 0.01^{c}	2.18 ± 0.05
Linolenic (18:3, *n*-3)	2.45 ± 0.10^{a}	1.70 ± 0.02^{b}	1.80 ± 0.00^{c}	1.98 ± 0.06
EPA (20:5, *n*-3)	3.40 ± 0.15^{ab}	3.50 ± 0.00^{bc}	4.00 ± 0.00^{cd}	3.63 ± 0.05
Docosapentaenoic (22:5, *n*-3)	5.30 ± 0.60^{ab}	5.35 ± 0.06^{b}	5.10 ± 0.07^{a}	5.25 ± 0.05
DHA (22:6, *n*-6)	10.45 ± 0.38^{a}	9.15 ± 0.08^{b}	9.50 ± 0.05^{c}	9.70 ± 0.10
Others[A]	11.90 ± 0.67^{a}	10.35 ± 0.25^{b}	11.05 ± 0.57^{c}	11.10 ± 0.23
Total saturated fatty acids	32.75 ± 0.08^{a}	33.75 ± 0.05^{b}	32.80 ± 0.05^{c}	33.10 ± 0.08
Total monounsaturated fatty acids	31.15 ± 0.22^{a}	34.20 ± 0.30^{b}	33.80 ± 0.06^{c}	33.05 ± 0.24
Total polyunsaturated fatty acids	24.20 ± 0.36^{a}	21.70 ± 0.21^{b}	22.35 ± 0.15^{c}	22.75 ± 0.20
Total ω-3 fatty acids	11.15 ± 0.10^{a}	10.55 ± 0.08^{b}	10.90 ± 0.06^{c}	10.87 ± 0.05
Total ω-6 fatty acids	13.05 ± 0.27^{a}	11.15 ± 0.10^{b}	11.45 ± 0.08^{c}	11.88 ± 0.15
Polyunsaturated FA/saturated FA	0.74 ± 0.03^{a}	0.64 ± 0.02^{b}	0.68 ± 0.02^{c}	0.69 ± 0.01
ω-3 fatty acids/ω-6 fatty acids	0.85 ± 0.02^{a}	0.95 ± 0.08^{b}	0.95 ± 0.06^{b}	0.92 ± 0.01

Values in rows followed by a different superscript are significantly different ($p < 0.05$). Values are averages of three replicates ($n = 30$) ± standard deviation. FA, fatty acid.

[A] Individual fatty acids <0.50% total fatty acids: 4:0, 6:0, 8:0, 10:0, 12:0, 14:1, 17:0, 20:0, 20:1, 20:3, 20:4, *n*-6, 22:0, 22:1, *n*-9 and 24:0.

sizes could also be related to their varied dietary sources (Moffat & McGill, 1993). Young Nile perch feed abundantly on prawns (*Caridina*), fish fry and small gastropods and bivalves. Adult Nile perch, however, prey predominantly on haplochromine (*Rastrineobola*) (Acere, 1993). The exceptionally high level of palmitic acid in Nile perch oil is, however, not influenced by diet since it is a major metabolite in fish (Ackman & Eaton, 1966).

Amounts of fatty acids in Nile perch belly oil were in the order SFA > MUFA > PUFA for all fish size categories. The ratios of PUFA to MUFA were found to decrease significantly ($p < 0.05$) with increase in fish size category. However, values for all fish size categories (0.68 ± 0.02 to 0.74 ± 0.03) were above the recommended minimum PUFA:MUFA ratio of 0.50 (Gurr, 1984). PUFA to SFA ratios in Nile perch oil were higher than that for marine catfish (Osman, Jaswir, Khaza'ai, & Hashim, 2007) but similar to that for crude herring oil (Aidos et al., 2002). The PUFA to SFA ratios in this study were above that of standard menhaden oil (0.58) recommended for PUFA supplements (FDA, 1997). Ratios exceeding 0.50 have been shown to lower blood cholesterol (Gurr, 1984). Consumption of Nile perch oil is therefore likely to stabilise or even lower the level of blood cholesterol, given its high PUFA to SFA ratios. On the other hand, Nile perch belly oil had considerable levels of ω-3 ($10.9 \pm 0.05\%$) and ω-6 fatty acids ($11.9 \pm 0.15\%$). Values of (total ω-3)/(total ω-6) fatty acids varied from 0.85 ± 0.02 to 0.95 ± 0.08. Nutrient intake estimates demonstrated by Simoupoulos (1989) show that ratios of ω-3 to ω-6 fatty acids close to 1:1 have significant health benefits. Based on this fact, consumption of Nile perch oil can be considered important for human health.

3.5. Accelerated oxidation and hydrolysis

Oils from the various fish categories showed significant ($p < 0.05$) variation in peroxide value (PV) and free fatty acids (FFA) under accelerated storage in an oven at 65 °C (Figs. 1 and 2, respectively). Initial PV (3.99 ± 0.45 to 7.93 ± 1.25 mEq O_2/kg oil) and FFA (0.26–1.12% oleic acid by weight) values were all within the range considered acceptable for non-refined oil (CODEX STAN, 1991). Oxidative and hydrolytic changes, measured by PV and FFA, respectively, increased significantly ($p < 0.05$) with storage period. Crude oil from the large fish category demonstrated high stability with lowest PV (248 ± 0.24 mEq O_2/kg oil) and FFA ($2.21 \pm 0.01\%$ oleic acid by weight) after 120 h of Schaal storage (65 °C). Oil from the small fish category had the highest PV (393 ± 0.34 mEq O_2/kg oil) and FFA ($3.58 \pm 0.05\%$ oleic acid by weight) value.

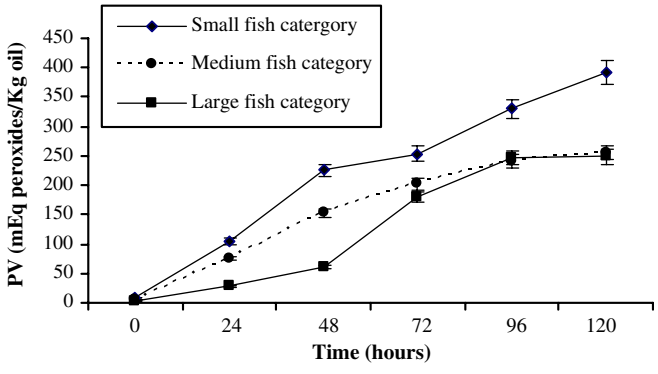

Fig. 1. Peroxide value of Nile perch belly oil under accelerated storage conditions (65 °C).

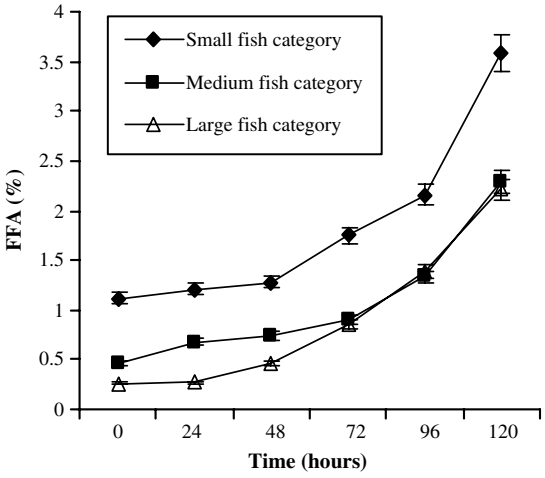

Fig. 2. Free fatty acids of Nile perch belly oil under accelerated storage conditions (65 °C).

Oxidative resistance of Nile perch oils stored at 65 °C increased significantly ($p < 0.05$) with increase in fish size category (Fig. 1). Fish oils from the different fish categories demonstrated high resistance to oxidation except for the small category. PVs of Nile perch oil were similar to values reported for refined cod liver oil and seal blubber oil under similar Schaal storage conditions of 65 °C (Wanasundara et al., 1998). Oil from large fish showed exceptional stability to oxidation, due to its lower level of unsaturation (iodine value of 26.8 ± 4.07), and possibly its high content of β-carotene (4.69 ± 0.01 mg/100 g oil, respectively). On the other hand, oil from the small fish showed least stability under accelerated oxidation conditions (Fig. 1). This could be a result of higher proportion of unsaturated fatty acids (29.2 ± 2.37 mEq O_2/kg oil) and lower β-carotene content (2.90 ± 0.03 mg/100 g oil) in the fat. Notably, although the tocopherol content of oil from the small fish size category (11.4 ± 0.92 mg/100 g oil) was higher than that of the large size category (2.11 ± 0.03 mg/100 g oil), its oxidative stability was lower ($p < 0.05$). β-carotene and α-tocopherol are known to have different antioxidant activity during lipid peroxidation. Studies have further shown that β-carotene is a more effective antioxidant than α-tocopherol in highly unsaturated membranes, particularly those with high ω-3 PUFAs (Palloza, Luberto, & Bartoli, 1995). Consequently, Nile perch belly oil from the large fish size category with high carotene content and low tocopherols showed high resistance to oxidative stress. Generally, fish oils rich in both β-carotene

and α-tocopherol are known to possess high resistance to oxidative stress (Navarro-García et al., 2004). These natural antioxidant vitamins have free radical quenching ability, capable of slowing down or terminating the onset of oxidative deterioration processes (Wanasundara et al., 1998).

Free fatty acids in Nile perch oil subjected to the accelerated oven test at 65 °C increased significantly ($p < 0.05$) with the storage period (Fig. 2). Formation of FFAs in Nile perch oil can partly be attributed to the activity of residual lipases and phospholipases. The FFAs in oils from the different fish size categories were within acceptable limits for non-refined oils (CODEX STAN, 1991; Young, 1982). 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 (Abou-Gharbia, Shehata, Youssef, & Shahidi, 1996). Based on this, unrefined Nile perch oil can generally be considered stable for at least five months of storage at ambient temperature, based on hydrolytic changes.

4. Conclusion

Based on its high content of vitamin A and essential fatty acids, particularly docosahexaenoic acid, docosapentaenoic acid and eicosapentaenoic acid, it can be concluded that Nile perch is beneficial to human health. A high content of natural antioxidants in the belly oil provides protection from oxidative degradation processes, prolonging its shelf-life. Nile perch is a potential raw material for commercial production of fish oil, given the high belly material weight and oil yield.

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