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Optimization of acid ensilaging method for the extraction of oil from Lake Victoria nile perch' *lates niloticus* viscera

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Abstract

A study was carried out to optimize the wet acidic rendering for extracting oil from Nile perch viscera. Oil was extracted at 93oC by varying the acid concentration from 0 – 10% and heating time from 5 – 25 minutes. The oil yield, lipolytic, oxidative and color qualities were determined to compare the effect of acid and time using a factorial completely randomized design. Statistical analysis of data was carried out at p □ 0.05 using GenStat 13th edition software. There was significant difference in yield, with interaction between acid concentration and heating time, the yield being highest at 15 minutes and declining thereafter with increasing acid concentration. There was significant difference in free fatty acids (%FFA), with interaction between acid concentration and heating time, values increasing with acid concentration and heating time. Heating times of 5 and 15 minutes produced the lowest %FFA at 0% acid concentration, being significantly higher after 15 minutes. All the %FFA values were within permitted limits for crude fish oil, however. There was difference in peroxide value (PV) with interaction between acid concentration and heating time, decreasing with increasing concentrations. Heating for 15 and 25 minutes produced lower PV for 0% acid concentration, with no difference between them. But the PVs were within permitted limits for crude fish oil. There were differences in color intensity, with acid concentrations, heating time not producing a significant difference, and the interaction between acid concentration and heating time not significant. There was significant difference in color intensity between 0% and subsequent acid concentrations, increasing with acid concentration. Heating for 15 minutes without acid is therefore the most suitable combination for mass production of crude oil from Nile perch viscera.

Keywords: *viscera; rendering; oil yield; hydrolysis; oxidation; color intensity*

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Introduction

Nile perch (*Lates niloticus*) is a freshwater fish of central Africa's lakes and rivers. 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 (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. 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 eicsapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and have high nutritional value and health benefits (Ogwok *et al.,* 2008; Gbogouri *et al.,* 2006). 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, 2006; 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 (Rubio-Rodríguez *et al.,* 2010; FAO, 1986). Other methods include enzyme extraction (Sathivel *et al.,* 2009), ensilaging (Crexi *et al.,* 2010), supercritical fluid ext (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.

Materials and methods

Raw materials

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

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.

Processing methods

Prior to oil extraction, the sample (Figure 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 it 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:

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

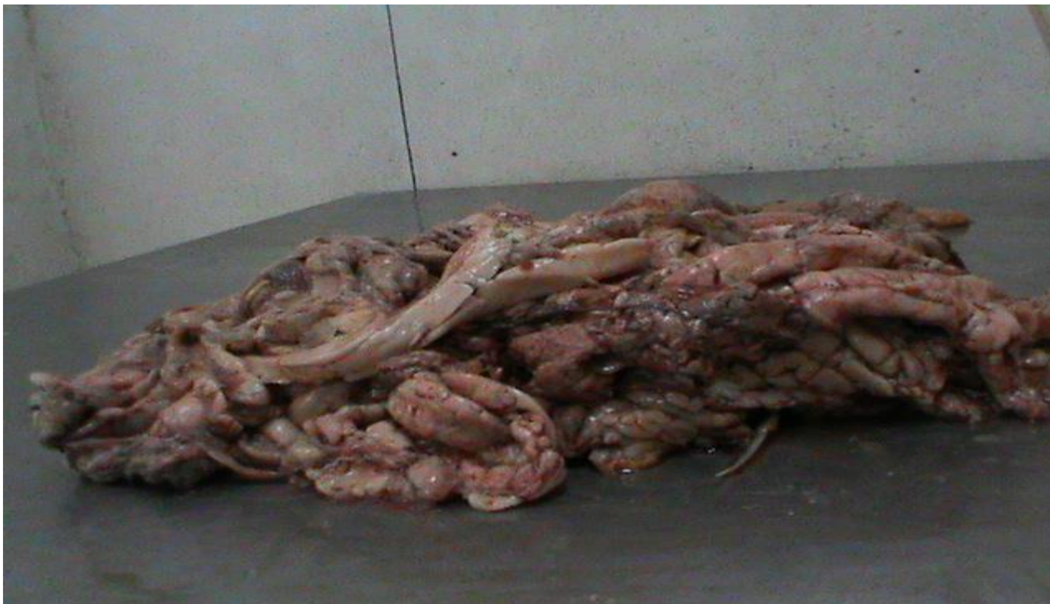


Figure 1: Fresh sorted Nile perch viscera

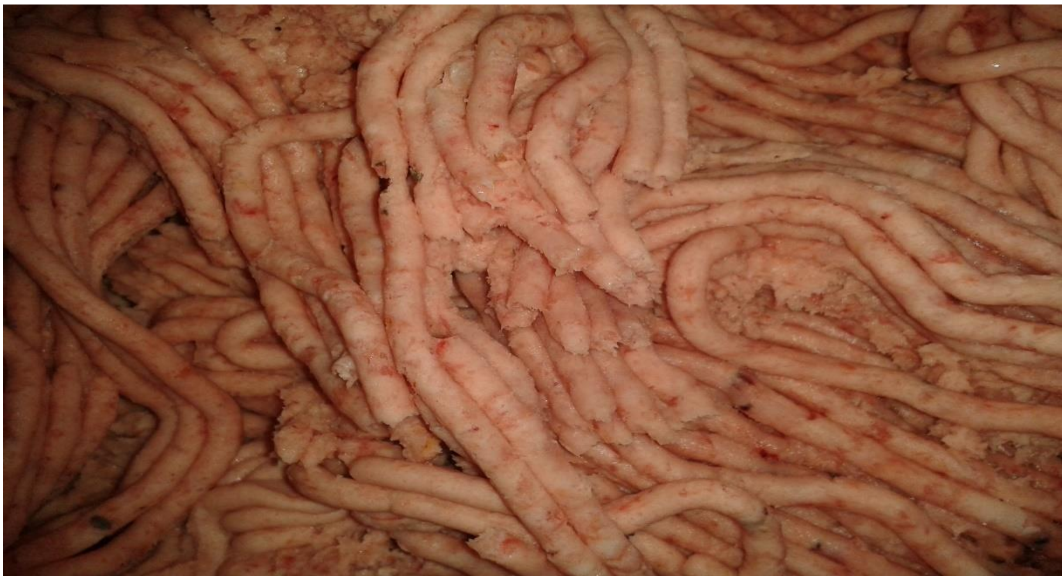


Figure 2: Mince of the frozen Nile perch viscera

Analytical methods

The extractable oil content was determined 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 sample was first analyzed for moisture content by the air-oven method (AOCS, 1998). The determinations of moisture and extractable oil contents were each done in triplicates.

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

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

Free fatty acids (%FFA) 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. This was used to express the extent of hydrolysis in the oil.

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. This was used to represent the extent of oxidation in the oil.

Color intensity was determined by use of uv – visible absorbance spectrophotometry (*CECIL Elegant Instruments, CE 4400 – England*), employing standard methods. The absorption spectrum of hemoglobin was used, taking measurements at 450nm where hemoglobin has highest absorbance (Egan *et al.*, 1981; AOCS, 1998). The color intensity was used as an indicator for the possible extraction of hemoglobin and related pigments into the oil during rendering processes.

Results

Moisture and Extractable Oil Contents

Moisture content of the viscera was 24.3 ± 1.1% while the extractable oil content was 73.2 ± 1.6%.

Oil Yields

There were significant differences in yield, with interaction between acid concentration and heating time (Figure 3). There were high yields after 15 minutes heating, but with no significant difference at 0% and 2% acid concentrations ($p>0.05$). 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.

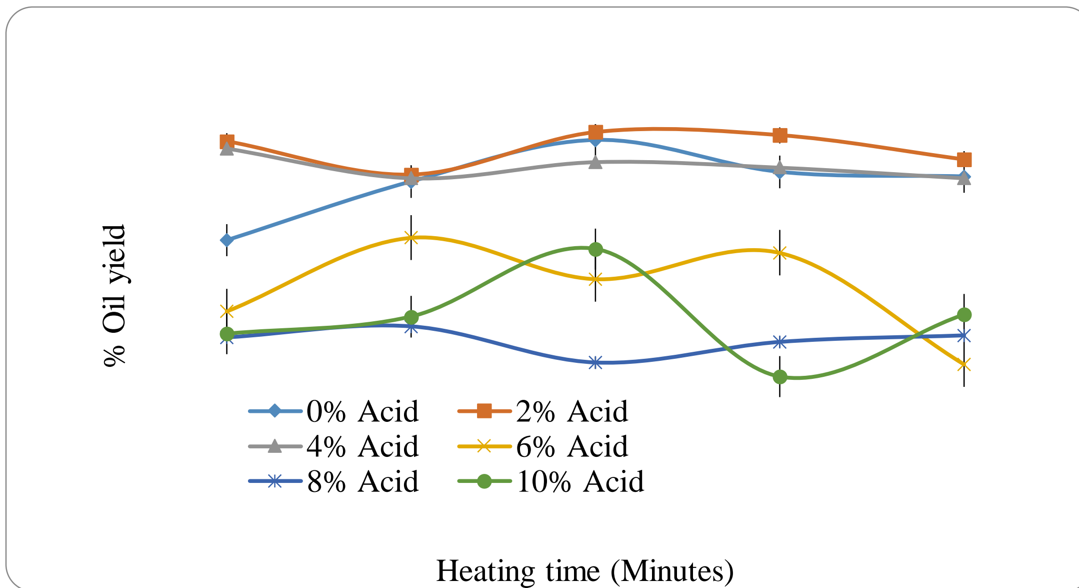
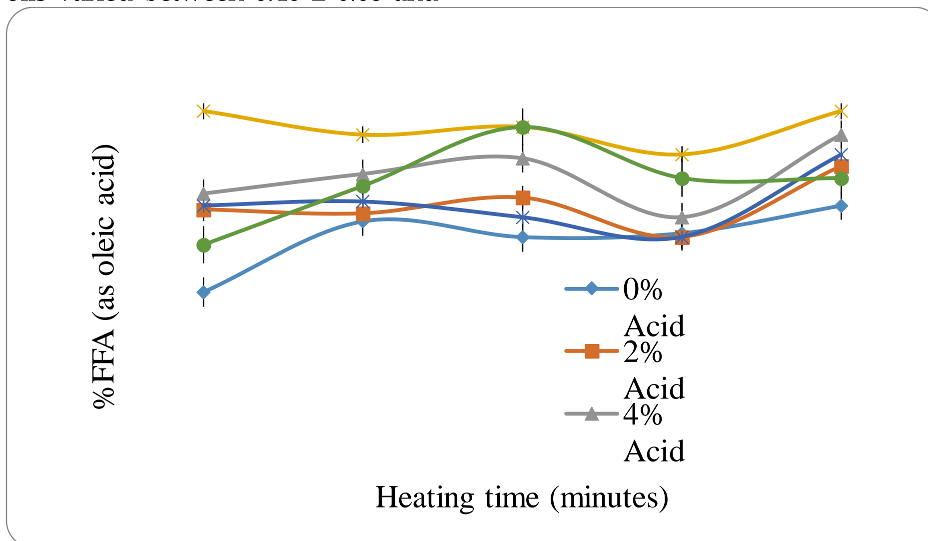


Figure 3. *Yield of Nile perch viscera oil at different heating times and acid concentrations*

Free Fatty Acids (%FFA)

There was significant difference ($p \leq 0.05$) in free fatty acids (%FFA), with interaction between acid concentration and heating time (Figure. 4). 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, the value for 15 minutes being significantly higher.

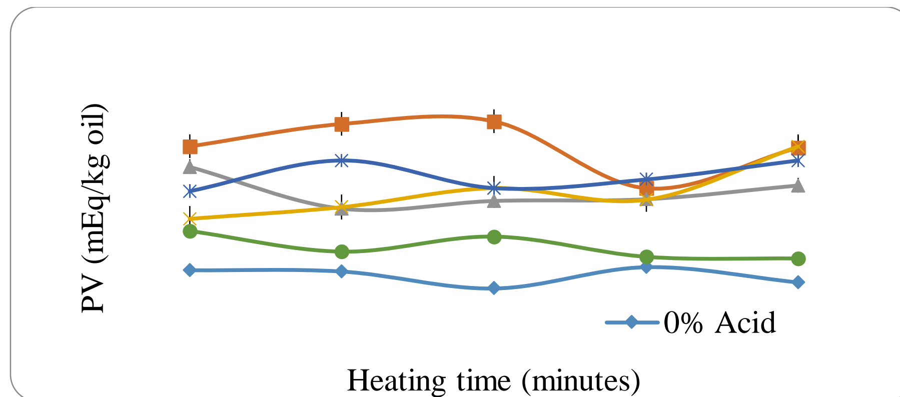


3.0
4.0

Figure 4. Free fatty acids of Nile perch viscera oil at different heating times and acid concentrations

Peroxide Value (PV)

There was significant difference in PV ($p \leq 0.05$) with interaction between acid concentration and heating time (Figure 5). 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 – 20 mEq/kg so far reported for crude fish oils.



5.0
6.0 *Figure 5. Peroxide value of Nile perch viscera oil at different heating times and acid concentrations*

Color Intensity

There was significant difference in color intensity of the oils, with acid concentrations (Figure 6). 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 ($p \leq 0.05$), with no significant difference between 15 and 20 minutes of heating ($p>0.05$).

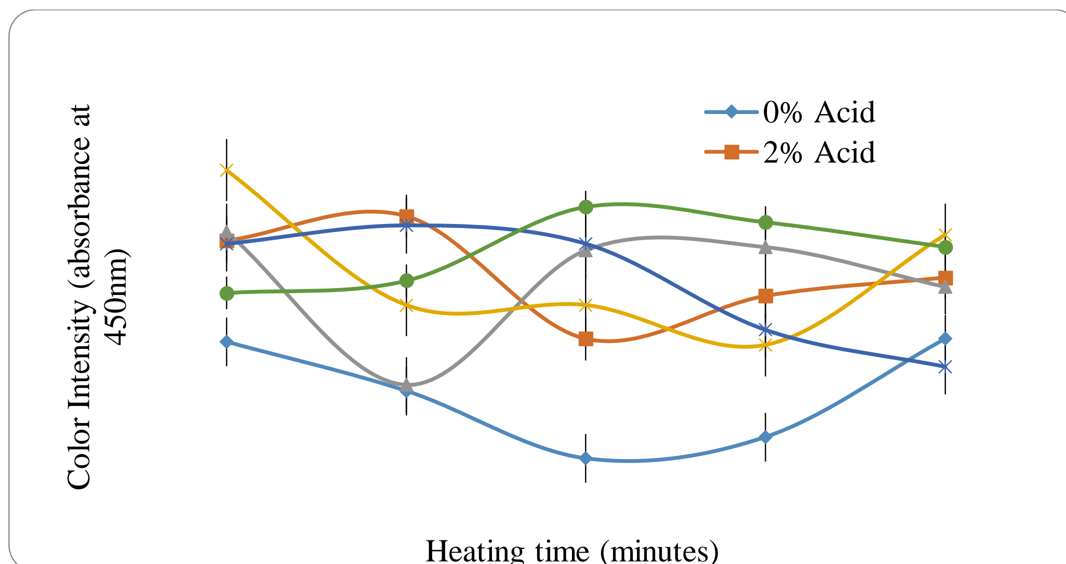


Figure 6. Color intensity of Nile perch viscera oil at different heating times and acid concentrations

Discussion

Moisture and Extractable Oil Contents

The extractable oil content was within the range obtained by earlier work on Nile perch belly flaps (Ogwok *et al.*, 2008), lower than the lipid content of the viscera of Asian catfish (Thammapat *et al.*, 2010), but higher than that reported for farmed Atlantic salmon (*Salmo salar* L.). The moisture content was, however, lower than that 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, lungfish livers and adipose tissues (Masa *et al.*, 2011), liver and viscera of gadiform species (Falch, 2006).

6.1 Oil Yields

These values are higher than those obtained for catfish viscera using various methods (Sathivel *et al.*, 2009) and from salmon by-products by use of enzymes (Gbogouri *et al.*, 2006), but lower than 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 et al*.,, 2009). Mbatia *et al.,* (2010) 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. This was probably due to formation of a larger and stable emulsified aqueous layer which resulted in larger oil loss.

The higher acid concentrations seem to have produced high number 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.,* 2010). These observations are consistent with other reports on freshwater fish species (Gbogouri *et al.,* 2006).

Free Fatty Acids (%FFA)

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 and acid ensilaging (Crexi *et al.,* 2010) and 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%. 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).

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 catalysis 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.

Peroxide Value (PV)

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/kg 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/kg 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 as acid concentration increased.

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 et al., 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 led to very rapid generation of an active species, ferryl radical, which could initiate lipid peroxidation (Thiansilakul *et al.,* 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.

Color Intensity

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 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).

Conclusion

The study showed that for optimal yield and quality, 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. Heating beyond 15 minutes does not result in significant improvement in oil yield.

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