Impact of modified industrial wet processing stages on the quantity and quality of Nile perch (*Lates niloticus*) oil extracted from the visceral organs

Authors: Otieno, D.,*Nyaboke, H[1]., Odoli, C[1]., Nyamweya, C[1] and Aura, C[1].

[1]Kenya Marine and Fisheries Research Institute

Corresponding author: *Denis Otieno.*
Chrisdennis445@gmail.com

Abstract

Global fish oil production is between 1 to 1.25 million tonnes and primarily exploiting fatty fish such as menhaden, herring, pilchards, anchovy, and sardine among others. The main producing countries include Japan, USA, Chile and Peru. Yet, fish oil from developing countries end up as bio waste since only the flesh is utilized. This paper discusses a simple approach for utilizing the fish bio waste to produce oil and how the oil quality and quantityis impacted by the extraction process. The visceral organs were collected from fish filleting factory and markets in Kisumu town and oil extracted by modified wet pressing method adopted from Blight and Dyer (1959). The effect of temperature and serial washing on the oil quality and quantity was assessed from extraction temperatures at 80^0C, 90^0C, 95^0C and 97^0C, while employing three serial washing using distilled water and pineapple juice. The quality of the oil produced was then determined based on the proportion ofomega -3, vitamin A and E, peroxide value, iodine value and free fatty acids. Results indicate that maximum extraction efficiency was achieved at 80^0C for 30 minutes where 156.05g of oil was extracted, when compared to 155.32g at 90ºC,155.24g at 95ºC,155.23g at 97ºC from a tissues of 171.25gin each case. The data was then analyzed using ANOVA at $P<0.05$ and post hock to determine significance. It was concluded that the temperature manipulation procedures as per this study was reliable to produce maximum yield and can be adopted by oil producing plants.

Key words: Fish oil, waste to resources

Introduction

Nile perch (*Lates niloticus*) is a freshwater fish found in central Africa's lakes and rivers (Turon et al., 2005). It was introduced in Lake Victoria in the 1950s 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. The Lake Victoria fisheries constitute an important source of protein for local communities and foreign exchange earnings via exports. Production of fish fillets generates waste consisting of the head, tail, skin and viscera. These wastes accounts for approximately50% of the total fish mass (Ogwok et al., 2008) which are not optimally utilized in Kenya and most third world countries. One of by-products development which has a good prospect in the market is fish oil that can be obtained by extraction from the visceral organs among other parts.Fish oil is commonly extracted throw wet processing procedures; this involves heat process that leads to the release of the oil from the tissues into the water. The oil is further separated based on Gravity and processed to consumable grade.This paper explores the conversion of the Nile perch bio waste to resources to get maximum benefits from the fish. The offal's which is often thrown away to waste or underutilized can be exploited for fish oils which is reach in Omega-3 fatty acids classified as "good fat." Because unlike other types of fat, they don't elevate blood pressure or clog your blood vessels. Instead, they clear the body of high cholesterol and bad fat. (Fotuhi et al., 2009; Kris-Etherton et al., 2002). This fatty acid family includes eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and alpha-linolenic acid (ALA) which are important component for the production of hormones, keeping your cardiovascular system and heart healthy (Chapkin et al., 2009) and are acquired by the body through diet.

Materials and methods

Study area

Freshly captured fish (*Lates niloticus*) was collected from Lake Victoria a fresh water lake and the second largest in the world after Lake Superior with surface area of 68,000 Km^2. It lies across the equator at 0° 30' N-30 O'S and 31° 40' E-34° 54' E at an altitude of 1135 metres above the sea level. The lake has a mean depth of 40 metres, maximum depth of 80 metres with a shoreline of 3,450 Km and is shared by three countries east African countries: Kenya 6%, Uganda 43% and Tanzania 51%. And a drainage basin of 258,700 Km2. (Figure 1)

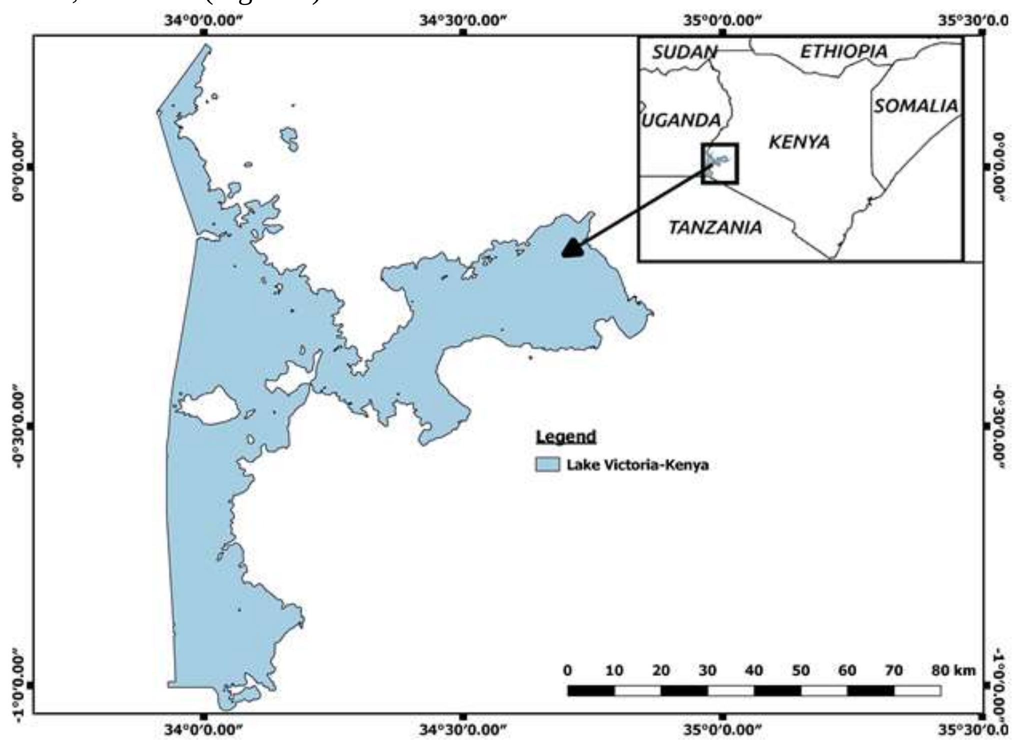

Figure 1: Map of Lake Victoria- Kenya showing points where the fish was obtained.

Sampling

The fish visceral organs were sourced from fish filleting factory and markets in Kisumu town where they were collected in the markets by hand and placed in the cool boxes. The samples were then transported to Kenya marine and fisheries Research institute Kisumu station for oil extraction.

Processing

Oil extraction was done bymodified wet rendering process(Blight and Dyer 1959). The visceral organs were put in heating pans and warm water at 60ºC was added at a ratio of 1:1 water to tissue weight. The cooking was done in triplicate at 80ºC to 97ºC for 15, 20 and 30 minutes in covered pans and cold water added 30 minutes after attaining maximum temperature. Oil recovery was done by separation of the debris from liquor through sieving the cooked mass into a separate bucket. The liquor consisted of water, varying amounts of oil and dry matter. The separation of the three fractions of the liquor, sludge and oil was based on their different specific gravities. The press liquor was left in the bucket and settled out in three layers with sludge at the bottom, water in between and oil at the top. The oil was then separated by decanting in a separating container; the former two layers occupying the bottom and middle were drained off, leaving the oil in the funnel. The residue was pressed mechanically to obtain more oil. This process ends up with three products which include the oil, the sludge and the stick water.The stick water which contains dissolved protein, minerals, suspended proteins, residual oil and vitamins (Maqsood et al., 2012) was enriched and tried in winning of piglets while the sludge was dried into cakes and tried as chicken feeds.

The oil polishing

The extracted oil appears mucky with few fine traces of tissue debris suspended within the oil Colum. This challenge therefore initiated the serial sieving and washing of the oil using warm water at between 80ºC to 97ºCto polish the oil towards removing undesirable volatile components and debris to obtain food grade oil with good quality. Warm water was bubbled in the oil in three serial washing and the resulting oil collected for quality assessment. The warm water ensured low viscosity for faster sedimentation of the debris during washing process. The final washing was then done using blended pineapple. Every serial washing was followed by sedimentation and sieving the oil with finer mesh sizes hence resulting to oil with no debris suspension.

Data analysis

Statistical analysis was conducted using R-data analysis software. Analysis of variance (ANOVA) was used to determine significant difference between the extraction temperatures and serial washing with $P< 0.05$. In places where there were significant differences, Tukey’s Multiple Range Test was used to separate the mean. Student’s T-tests were used in the analysis of two-group parameters. Statistical significance was set at $P< 0.05$.

Physico-chemical analyses

Free fatty acid

The FFA was determined according to AOAC2005 method,where 1g of the oil sample (to the nearest 0.005g) was mixed with 75 mL of 95% neutral ethyl alcohol and swirled. Phenolphthalein was added as indicator. The solution was titrated with 0.1 N Sodium hydroxide until pinkish colour was observed at end point. Percentage for FFA was expressed as oleic acid.

FFA, as % oleic = (S-B) ×N× molar mass of oleic acid/W

S= Titration volume of sample

B = Titration volume of blank

W = weight of sample (g)

Molar mass of oleic acid= 28.2

Peroxide value

Determination of Peroxide value (PV) was done according to AOAC method (AOAC, 2005). A sample 5 g of oil was weighed to the nearest 0.05g into a 200 mL conical flask and mixed with 30 mL of glacial acetic acid and chloroform (3:1) and mixed thoroughly by swirling the flask. Saturated potassium iodide (0.5 mL) was then added and the mixture was left in the dark for 1 min with occasional swirling, followed with further addition of 30 mL distilled water. The mixture was titrated with 0.1 N sodium thiosulphate solution with 1 mL of 1.0% soluble starch as indicator until the blue colour disappeared. A blank sample titration was also carried out in the same manner but with no oil added. The peroxide value (milliequivalents peroxide/1000 g sample) was calculated as

PV = (a-b) ×N×1000/Weight (g) of sample

Where, a = Volume (mL) of 0.1 mol L-1 sodium thiosulphate consumed in the blank test;

b = Volume (mL) of 0.1 mol L-1 sodium thiosulphate consumed in the test;

N = normality of sodium thiosulphate solution per 1000 g of sample

Iodine value

Iodine value was determined as described by AOAC method (2002), Othman and Ngassapa (2010). A sample of 0.4 g was weighed into a conical flask and 20ml chloroform was added to dissolve the oil. 10ml from the solution was transferred into iodinated flask and 20ml iodine monochloride added and swirled for one minute, kept sealed and left in a dark room for 30 minutes. The blank was prepared by pipetting 20.0 mL of iodine monochloride into iodinated flask with 10ml chloroform stopped and swirled for one minute and left in a dark room for 30minutes. The test solution was retrieved and titrated with 0.1N sodium thiosulphate continuously till the yellow colour just disappeared,starchindicator was added and titrated further till the solution just turnscolourless. The same test was done with the blank and end points recorded. The iodine value was calculated as;

Iodine Value (IV) =127(a-b) ×N/10W

Where, a=Volume (mL) of 0.1 mol L-1 sodium thiosulphate consumed in the blank test; b = Volume (mL) of 0.5 mol L-1 sodium thiosulphate consumed in the test; N=Normality of sodium thiosulphate; W=Weight of sample.

Vitamin A

The vitamin A content was analyzed by method described by Zahar and Smith (1990) using high-pressure liquid chromatography (PerkinElmer) equipped with a photometric detector, the stock solution was preparedbyDissolvingretinyl acetate (Fluka – Sigma Aldrich, USA) in ethanol containing 0.1% ascorbic acid to form a concentration of 1mg/1ml and Stored in the dark at 4 °C from where the working solution was preparedonadaily basis. The sample was also prepared by dissolving 0.1ml oil in 10ml ethanol. The samples and the standards were injected into HPLC and the retinol concentration in samples was calculated using the standard curve.

Vitamin E

The vitamin E content of oil was determined by AOAC (1998) method 992.03 using high-pressure liquid chromatography (PerkinElmer) equipped with a UV detector to measure absorbance at 300nm.1g of the Oil 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 tomake working standards. The standard was injected into the HPLC to generate standard calibration curve. The peak area ratios of the tocopherols to the internal standard substance was Calculated based on the peak areas of each substance from the obtained chromatogram, and converted to weight ratios using the calibration curves constructed and result calculated as;

Vitamin E, % tocophenol= (Wx/Ws) ×Ms×100/S×1000

Wx/Ws=Weight ratio of a tocopherol to the internal standard substance, obtained from the calibration curve

Ms=weight of the internal standard substance contained in 1 ml of internal standard solution (mg)

S=Sample weight (g)

Fatty acid analysis

Omega-3 fatty acid was determined according to AOCS (1998) method Ce 1b-89. Stock solution was prepared by dissolving 5g of EPA methyl ester and 2.5g DHA methyl ester in 1l n-hexane and working standard by dilution the stock solution to desired concentrations. Samples solution was prepared for analysis by adding potassium hydroxide in the oil sample after centrifugation. The mixture was then warmed in a water bath at 60°C, shaken three times in the course of 20 minutes and 3 ml boron trifluoride methanol complex solution added and the mixture cooled. 2ml Saturated sodium chloride solution and 2ml n-hexane was added after filling the tube with nitrogen gas in a water bath for 5minutes. The content was then centrifuged at 4000 rpm for 10minutes and supernatant drawn to be use as sample solution. An aliquot of 1 µl supernatant was injected for GC-MS analysis. Calculation was done by dividing percentages of peak area obtained by the relative molecular weight of respective fatty acids methyl ester to obtain moles percent of fatty acids.

Oil yield

The extracted oil at different temperature and time was suspended in calibrated cylinders and the quantity of the oil extract recorded. This was achieved by allowing the oil to settle in the cylinders for one hour to allow time for draining of the stick water from the oil. After this period, the water was drained living the oil in the cylinder and the volume recorded. The oil was then evaporated in a hot plate at 110°C for ten minutes to evaporate any sticking water that may still be present. The reading was then recorded from the calibrated cylinders and value taken as the oil yield.

Results and discussion

Physical appearance of the oil

The saturated oil Colum

The oil extracted from the viscera after serial washing had two layer of saturated and unsaturated Colum. The saturated layer is of higher density and settled at the bottom of the holding container. This layer is white to cloudy in colour and had more fishy smell as compared to the upper unsaturated layer. It remains in amorphous status at room temperatures but rapidly mix with the upper unsaturated layer when the oil is subjected to temperatures above 30°C. At temperatures above 40°C the saturated white Colum attains the golden yellow colour of the unsaturated Colum and uniform oil with no strata is formed. This uniformity is however broken a few minutes when temperatures reduce to room temperature or between 20°C-30C°.

The unsaturated oil Colum

This forms the upper layer which has less density as compared with the saturated layer. It is golden yellow and has less fishy smell. This layer also remains liquid at room temperature. However, when placed in deep freezers forms an amorphous solid rather than ice and return to liquid few minutes when retrieved from the freezer to room temperature. (Plate 1)

Plate 1: Saturated (a)and unsaturated (b)layer of fish oil from *Lates niloticus* of Lake Victoria

The picture (a1) demonstrates the saturated layer stored at 4ºC and (a2) the saturated Colum after immediately being retrieved from temperature of 35ºC. The oil at such temperatures starts to melt into a light yellow colour from the cream colour it has at room temperature. The other two b1 and b2 are pictures of unsaturated Colum after the third serial washing and final sedimentation.

Result showed that the oil yield had a positive liner relationship with extraction temperature and time where there was an increase in the oil yield from 15minutes to a maximum extraction yield at 30 minute for all the temperatures. Maximum extraction was however observed at 30minutes at 80ºC.The yield reduced with the increasing of extraction temperature progressively from 90ºC to 97ºC. This observation was in agreement with the research done by Wu and Peter 2008 where they concluded that decrease in yield percentage occurred at 90 °C was probably due to the oxidation process. They stated that there are physical factors that can affect the production of fish oil, one of which is processing temperature and that temperature can lead to oxidation thus resulting to fat breakdown. This experiment observed a sharp decrease in oil yield at 90 °C then a progressive decline, a factor that justifies that the inhibition of oil release (Yee, 2007).Proteins usually undergo denaturization at 90-100 °C resulting to dense structure which can cause inhibition of oil release (Ahren and Klibanow1985; Yee, 2007) thus explaining this results. Maximization of the yield was then achieved by temperature manipulation where cold water was added to the cooking tissues to reduce the temperature to 60ºC. These methods seem to work as the extraction process was facilitated. This may probably be due to the fact that the cold water could have disrupted the dense structure caused by denatured protein which inhibit oil release (Ahren and Klibanow 1985) and the reduction of temperature to below 80ºC which was observed to produce maximum yield. This therefore ment that the process was revamped and a liner positive production observed at the beginning reversed thus leading to more extracts. Mechanical pressing after this process was also observed to further assist to ensure maximum extraction of the oil.Manipulation as per this experiment worked best to improve the quantity of the oil produced and duration of exposure of the tissues gave different results as above. Series 3 which was exposed for 30 minutes gave the best results 91.46% followed by series 2 exposed for 20 minutes 91.23% and series 1 exposed for 15 minutes 89.98%. (Figure 2 and 3)

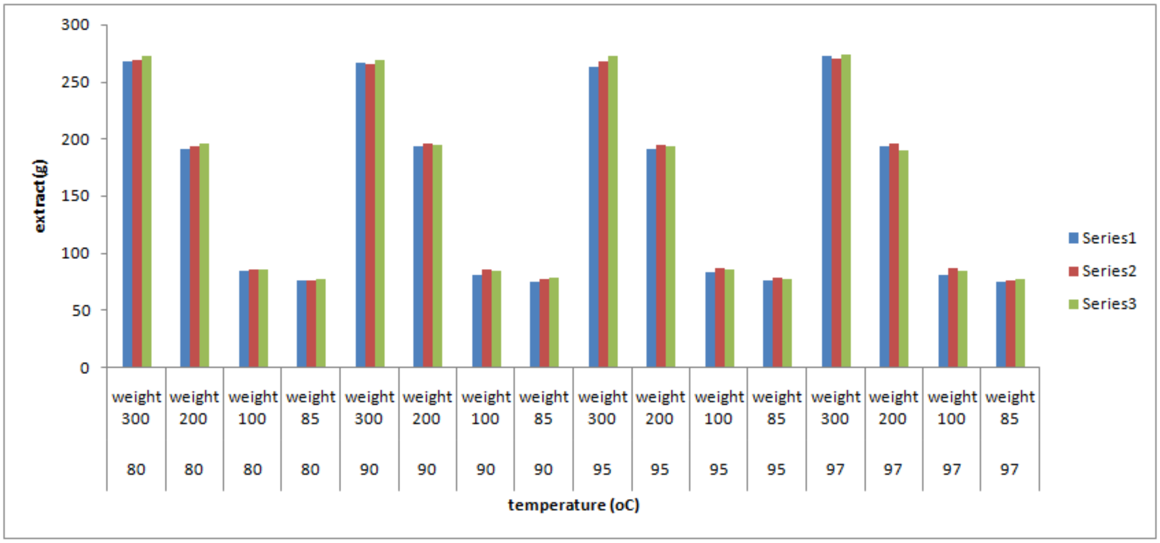

Figure 2;Bar graphs showing oil yield extracted at different temperature and time using varying mass of fatty tissues.

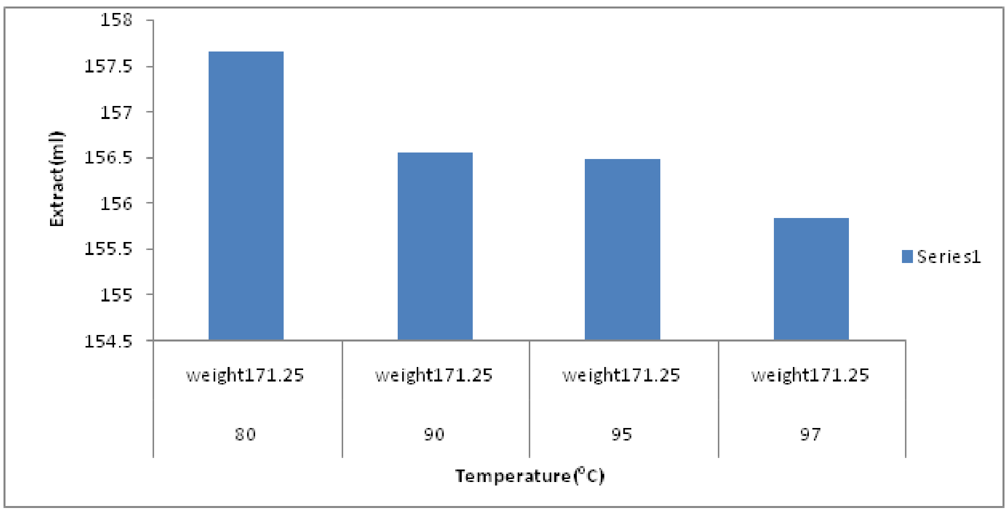

Figure 3: Showing results of oil yield extracted at different temperatures ranging from 80^{0}C-97^{0}C for 30 minutes.

Free Fatty Acids (FFA) is a measure of the extent of decomposition of lipase action which is usually accelerated by heat and light. Better quality oils have a low FFA value as the lipase content is low (Aryee and Simpson, 2009). Peroxide value of oil is used as a measurement of rancidity, which occurs by autoxidation (Othman and Ngassapa, 2010). It is defined as the milligram equivalents of peroxide oxygen combined in a kilogram of oil. Oils with low peroxide value have lower ability to go rancid. Peroxide value and free fatty acid was highest at 80°C extraction temperature and decreased with increase in temperature. Serial washing also affected the values. The lowest value was recorded at 95 °C after three serial washing. The extraction techniques deployed during this study significantly affected the peroxide values. Results shows that oil extracted at 97 °C, 95 °C and 90 °C were not significantly different. It means the peroxide value increased with the elevating of temperature, but the value decreased when extraction conducted at temperatures above 90 °C. This is probably due to less oxygen dissolved in the liquor since raising the temperature removes oxygen from the water which reacts with the free radicals to form peroxide compounds and denaturisation of endogenous enzymes that may cause autolysis during the extraction. The values at 80°C was due to early stages of oxidation as a result of instability and rapid decomposition of hydro peroxides (primary oxidation product) into secondary oxidation products since fish oil contain large amounts of polyunsaturated fatty acids(Zenebe et al., 1998).The reduction of the value during the third serial washing was probably due to the introduction of antioxidants and organic acid from the pinnacle juice which quenches the free radicals responsible for secondary oxidation and a fall in lipase activity due to acid denaturisation, resulting in lower %FFA hence reduced oxidation. (Figure 4)

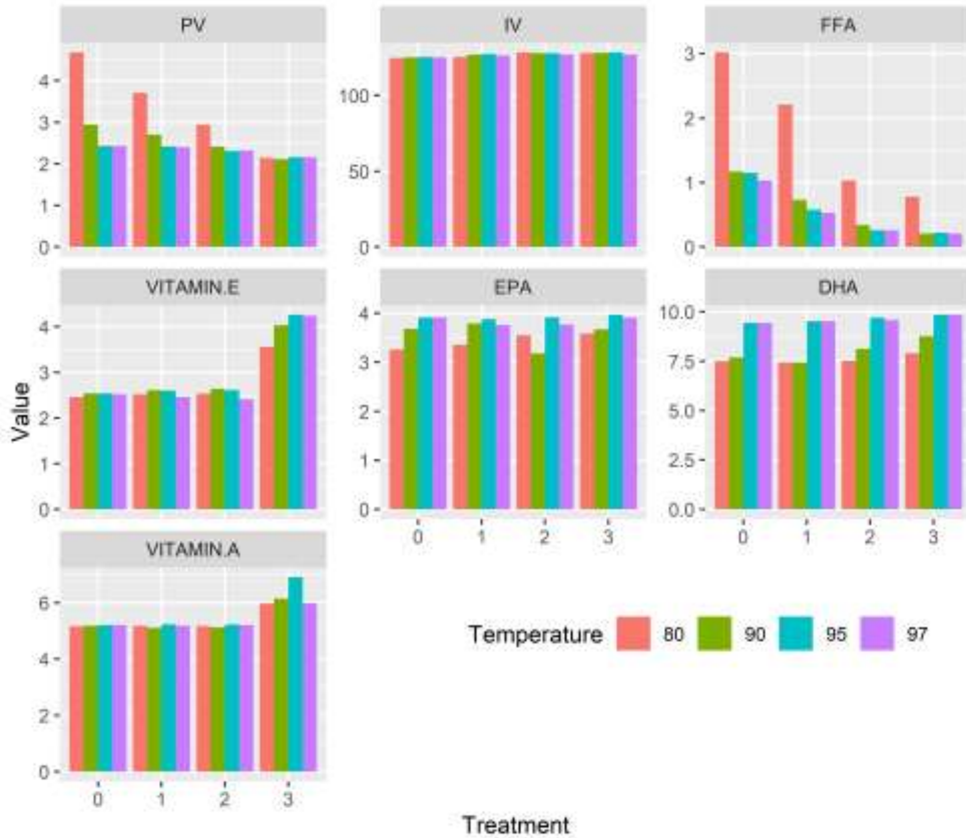

Figure 4: Bar graphs showing how the interaction between serial washing and extraction temperature effects the concentrations of the parameters.

Iodine value is a measure of the degree of unsaturation in a fat or oil (Thitiphan and Waranya, 2015) and it is defined as the number of iodine absorbed by 100 g of the oil. Oils and fats with low iodine value have greater oxidative storage stability.Extraction temperature and serial washing did not affect the iodine value significantly. The highest value was recorded at 95°C and the lowest at 80°C. Results also indicate a reverse relationship between peroxide value, free fatty acid and iodine value. This constant and relatively stable variation during the extraction and polishing of the oil indicates that iodine value is affected by the fish species more that the set conditions during this study. The relationship observed between PV, FFA and IV suggests a possible interaction between the concentrations of these parameters. Peroxide values is a factor of rancidity due to oxidation which results to secondary compound that may affect the unsaturation of the oil as a secondary factor, this is also true for lipase action in free fatty acids hence explaining the reverse relationship between these parameters.

Vitamin E is a powerful antioxidant which are fat soluble vitamins hence our body can store them in the liver for future use. Adequate vitamin E intake is thought to be protective against cardiovascular disease, cataracts, cancer, dementia and the oxidative stress associated with diabetesVitamin A content in the oil was relatively high in Nile perch oil at 5.19±0.03. Both Vitamins A and E were not significantly affected with the extraction temperature but serial washing did, especially the third washing. The highest values were recorded at 95°C for three serial washing. Diet contributes significantly to variability in vitamin A content since fish do not synthesize vitamin A (Lovern et al., 1933). Lake Victoria contains higher fish biomass and is therefore likely to provide an adequate and a wide spectrum of food for Nile perch (Yongo et al., 2005) hence the high value of vitamin A recorded in this study. Vitamin E belongs to a group of fat soluble vitamins which include vitamin A, D, E and K(Ross et al., 1993) the third serial washing hence impacted these values through adsorbed during this final washing routine.

Fish oil is rich in polyunsaturated fatty acids mainly EPA, DPA and DHA which is known to treat many disorders such as cardiovascular problems and brain development in children (Kidd,2007). To maintain the cardiovascular health in normal adults, most recommendations fall in the range 0.25 to 0.5 g per day of EPA and DHA. EPA plus DHA in most fish species range between 11% in herring to 27% in anchovy (Berbert et al., 2005; Moffa et al., 1993) hence considering the lower end of the recommendations would be met by 2.5 g/day or 1 g/day herring and anchovy oil respectively. The highest DHA and EPA contents were recorded at 95ºC while the lowest at 80ºC. This could suggest that both FFA and PV affect their concentration as a result of oxidative rancidity since the concentration of FFA and PV showed a reverse relation with EPA and DHA. There was however a reduction in the concentration of both the DHA and EPA at temperature beyond 95ºC, this could be attributed to the double bonds possessed by both EPA and DHA being susceptible to temperatures hydrolysis. (Bligh and Dyer 1959; Yee, 2007) .Results indicates that serial washing did not affect the omega -3 significantly the highest value for both EPA and DHA was recoded at 95ºC after three serial washing. (Figure 5)

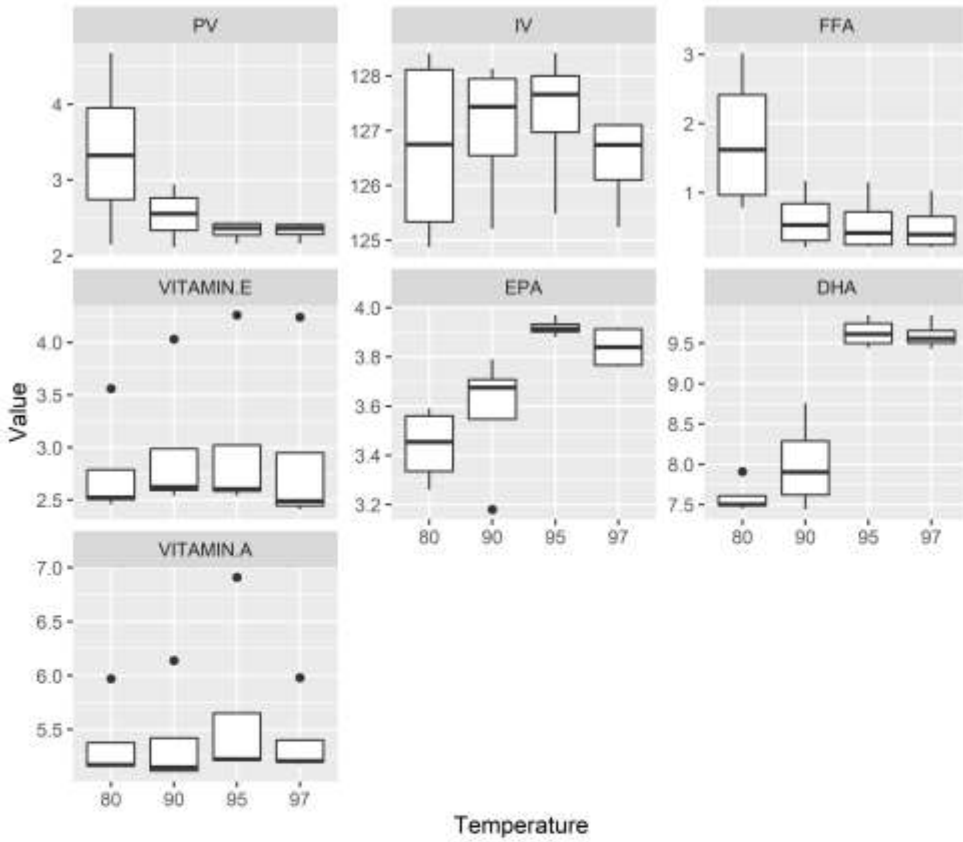

Figure 5: Box plot showing how extraction temperature effects the concentrations of the parameters.

Conclusion and Recommendation

Maximum oil yield from Nile perch visceral can be achieved at 80°C for 30 minutes using the modified wet processing as discussed in this paper while best quality standards can be achieved at 95°C after thee serial washing.

Fish bio waste can potentially be utilized to create wealth, job opportunity for youth and women, help in environmental sanitization and sustainable food for a large population.

Fish oil from Nile perch of Lake Victoria can potentially replace the existing omega-3 supplements in the market with a reliable quality competence.

Recommendation

Fish oil from Nile perch offal's should be extracted at 80°C for 30 minutes and to achieve the best quality standards the oil should be washed three times with warm water at 95°C

Thereis potential of fish oil extraction using simple resources available locally with little investment required, Owing to the fact that there is very little utilization of these products especially in Kenya and third world counties, further research need to be carried out on the use of fish oil in other fields such as health, agriculture and industrial uses in order to promote its exploitation on a large scale.

The authorities involved should disseminate the knowledge to the fisheries sectors to educate the fishermen and the sector on the importance of the fatty tissues which are thrown away as waste to enhance the income of those involve and protect the environment hygiene.

There is need to undertake experimental extractions of oil from other fish species both marine and fresh water and carry out laboratory analysis of the quality of the omega-3 fatty acids present in tropical fish oil and compare it with what is available in the market as oil supplements which are majorly imported from temperate regions.

REFFERENCES

Ahren, T.J., Klibanow, A.M., 1985. The mechanism of irreversible enzyme inactivation at 100°C.J Science, 228: 1280-1284.

AOAC,2005. Official Method of Analysis. Virginia (US): Association of Analytical Chemist, Inc.

AOCS,1998. Official Methods and Recommended Practices of the American OilChemists' Society. 5th ed. Champaign, IL: AOCS.

Lovern, J. A., Edisbury, J. R. and Morton, R. A., 1933.Variations in vitamin A contentof fish-liver oils, with particular reference to seasonal fluctuations in thepotency of halibut-liver oil. J. Biochem. 27(5): 1461–1469.

Ogwok, P., Muyonga, J. H.andSserunjogi, M. L., 2008. Fatty acid profile and stabil-ity of oil from the belly flaps of Nile perch (Lates niloticus). Food Chem.108:103–109.

Othman, O. C. and Ngassapa,F. N.,2010. Physicochemical characteristics of some imported edible vegetable oils and fat marketed in Dares Salaam. Tanzania Journal of Natural and Applied Sciences, 1(2): 138 – 147.

Thitiphan, C. and Waranya,W., 2015. Effect of microwave pretreatment on extraction yield and quality of catfish oil in Northern Thailand. MATEC Web of Conf. 1–5. Available at http://www.matec-conferences.org.Accessed 30 September, 2016.

Turon, F., Rwabwogo, B., Baréa, B., Pina, M. and Graille, J., 2005. Fatty acidcomposition of oil extracted from Nile perch (Lates niloticus) head. J. FoodCompos. Anal. 18(7): 717–722.

Wu, T.H., Peter, J.B., 2008. Salmon by-product storage and oil extraction.J Food Chem 111(08):868-871.

Yee, T.H., 2007. Influence of extraction temperature and time on yield and quality of oil recovered from Tilapia (Oreochromis niloticus) by product [Thesis]. Malaysia: University Sains Malaysia.

Yongo, E., Keizire, B. B. and Mbilinyi, H. G., 2005.Socio-economic impact of fishtrade. In: The State of the Fisheries Resources of Lake Victoria and TheirManagement. Jinja, Uganda: Lake Victoria Fisheries Organisation.

Chapkin, R. S., Kim, W., Lupton, J. and McMurray, D.N., 2009.Dietary docosahexaenoic and eicosapentaenoic acid: emerging mediators of inflammation. Prost.,Leuk., and Essent. *Fatty Acids*81:187-191.

Njiru, M., Ojuok, J., Getabu, A., Jembe, T., Owili, M. and Ngugi, C., 2008, Increasing dominance of Nile tilapia, Oreochromis niloticus (L) in Lake Victoria, Kenya: Consequences for the Nile perch Lates niloticus (L) fishery, Aquatic Ecosystem Health & Management, 11(1):42-49.

Bligh, E. G., Dyer,W. J., 1959.A rapid method of total lipid extraction and purification,*Can. J. Biochem. Physiol.* 37: 911-917.

Berbert, A. A., Kondo, C. R., Almendra, C. L., Matsuo, T. and Dichi., 2005. Supplementation of fish oil and olive oil in patients with rheumatoid arthritis.*Nutrition.*21:131-136.

Kidd, P. M., 2007.Omega-3 DHAand EPA for cognition, behavior, and mood: clinical findings and structural-functional synergies with cell membrane phospholipids.*Alt. Med. Rev.* 12(3):207-227.

Fotuhi, M., Mohassel, P. and Yaffe, K., 2009.Fish consumption, long-chain omega-3 fatty acids and risk of cognitive decline or Alzheimer disease: a complex association.NatClinPractNeurol. 5(3):140-152

Maqsood, S., Benjakul,S. andAfaf,K.,2012. Extraction, processing, and stabilization of health-promoting fish oils. Recent Patents on Food, Nutrition and Agriculture, 4(2): 1–7.

Kris-Etherton, P.M., Harris, W.S., Appel, L.J.,2002.Fish consumption, fish oil, omega-3 fatty acids and cardiovasculardisease.Circulation.2002;106:27472757.doi:10.1161/01.CIR.0000038493.65177.

Moffat, C. F. and McGill, A. S., 1993. Variability of the composition of fish oils:Significance for the diet. Proc. Nutr. Soc. 52: 441–456.

Ross, A, C. andTernus, M, E.,1993 Vitamin A as a hormone: recent advances in understanding the actions of retinol, retinoic acid, and beta carotene. J Am Diet Assoc. 1993;93:1285–90.