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Storage Stability of Refined Oil From Lake Victoria Nile Perch (Lates niliticus) Viscera

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ABSTRACT

Nile perch (*Lates niloticus*) viscera oil was extracted by wet rendering method and refined by neutralization, deodorization and winterization. After winterization, the oil was decanted and separated by filtration into a low melting point fraction (LMPF) and a high melting point fraction (HMPF). The two fractions were used to carry out storage stability study. Each fraction was stored at room temperature (19 – 23°C) for a period of 20 weeks in transparent and amber colored glass containers. The oil fractions were subjected to quality tests to assess their storage stability. The effects of package color and storage duration were assessed by analyzing for the content of free fatty acids (FFA) as oleic acid and the peroxide value (PV) at the beginning of storage and at intervals of two weeks during storage. The resulting data were subjected to statistical analysis (p d" 0.05) using GenStat software, 13th edition. There was a general increase in FFA and PV, with significant differences between oil fractions and package color over storage time. For FFA, there was no interaction between the oil fraction and package color while for PV there was significant interaction between the oil fraction and package color. FFA increased from 0.26% to 0.59% for LMPF in clear package, 0.26 – 0.43% for LMPF in red package, 0.22 – 0.85% for HMPF in clear package, and 0.22 – 0.69% for HMPF in red package. PV increased from 0.50 mEq O_2/kg oil to 11.65 mEq O_2/kg oil for LMPF in clear package, from 0.5058 mEq O_2/kg oil to 10.58 mEq O_2/kg oil for LMPF in red package, from 1.01 mEq O_2/kg oil to 9.94 mEq O_2/kg oil for HMPF in clear package, after 20 weeks, and from 1.01 mEq O_2/kg oil – 6.86 mEq O_2/kg oil for HMPF in red package after 14 weeks. The levels of FFA and PV of LMPF in colored package remained within the CODEX limits of 0.3% and 5 mEq O_2/kg oil respectively for refined fish oils up to 18 weeks. The FFA of HMPF surpassed the limit after 6 and 10 weeks for transparent and colored packages respectively. The FFA of LMPF in transparent package surpassed the limit after 8 weeks. Only low melting point oil fraction in transparent pack had PV above limit at 18 weeks. Results showed that colored package is more suitable for both oil fractions studied. In terms of FFA and PV, HMPF and LMPF can retain good quality at ambient temperatures for 10 and 18 weeks respectively.

Key words: Fish oil; viscera; hydrolysis; oxidation.

INTRODUCTION

Nile perch (*Lates niloticus*) is found in fresh water lakes and rivers of central Africa[1]. Nile perch is processed into fillets, mainly exported to Europe[1]. Byproducts are nearly 50% of the total fish mass[2]. Leather products are made from hide while dried swim bladders are used for filtering beer and wine as well as for making soup stock[1]. Nile perch has been identified as a fatty freshwater fish, with the oil distributed in various tissues[1-4]. Among the tissues, viscera contain substantial quantities of oil[2 - 4].

Long-chain *ù*-3 PUFA, especially EPA and DHA known to have nutritional value and health benefits are found in fish oil[2, 5 - 7]. They have been reported to be essential for fetal brain development, normal growth and proper body function[2]. The bulk of the world's fish oil is extracted by the wet pressing method[8 - 10].

Lipid hydrolysis and oxidation increase during storage, with corresponding increases in free fatty acids and peroxide value of samples[11 - 14]. PUFA are very susceptible to oxidation[15]. The high content of antioxidants â-carotene and á-tocopherol in Nile perch oil makes it fairly stable to auto-oxidation[2]. In one study, the fatty acid profile of fish oil did not change over storage duration[16]. Oily tissues from Nile perch processing are currently underutilized among fishing communities in East Africa. Roes are sold and consumed locally without value addition yet they have substantial amount of oil. A lot of skins are also discarded or freely given away for animal feeding, despite containing high quality oil. The rural folk use the viscera oil to fry the local market fish and chips, domestic cooking, and lately to feed infants[17]. A possibility of the extracted oil being refined to obtain a value added product was explored, with the objective of establishing its storage quality of the refined oil.

MATERIALS AND METHODS

Raw materials

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

Chemicals for analysis

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

Processing methods

Prior to oil extraction, entire sample was minced in the pilot plant to pass through 7.0 mm (0.276 inch) screen, then tumbled to achieve uniformity. Oil extraction was done by wet rendering, heating sample in equal amount of water for 15 minutes. The cooking was done in triplicate at 93°C in covered pans. Oil recovery was done by sieving the cooked mass with two layers of muslin cloth. The stick-water, aqueous layer and oil were separated by draining through a separating funnel; the former two occupying the bottom and middle layers were drained off, leaving the oil in the funnel. The residue in the muslin cloth was pressed using a plate and frame press to obtain more oil. This was also passed through the separating funnel to remove stick-water and emulsion. The oil obtained was weighed then subjected to refining by neutralization, deodorization and winterization. The winterized oil (LMPF) and the residue (HMPF) were subjected to shelf-life study, a portion of each fraction kept in transparent glass and colored glass. The shelf-life study was done at room temperature as is the recommended standard for storage[18].

Analytical methods

The free fatty acids content of the oil was determined according to *AOCS* (*1998*) method Ca 5a-40 by using aqueous sodium hydroxide standardized to 0.1M from ampoule and 1% phenolphthalein indicator in ethanol[19]. The solvent used was a neutral mixture (50 ml) of diethyl ether: ethanol (1:1). FFA values were determined as % oleic acid by weight.

The peroxide value was determined according to *AOCS* (*1998*) method Cd 8b-90 and expressed as mEq O_2/kg oil[19]. Oil samples were first dissolved in chloroform and then mixed with glacial acetic acid and saturated potassium iodide solution. The solution was then titrated with 0.01 M sodium thiosulphate solution using 1% starch as indicator.

RESULTS AND DISCUSSION

Free fatty acid levels during storage

Variation of FFA levels during storage is shown in Figure 1. The FFA values for the different melting point fractions and package color were significantly different (pd"0.05). There was, however, no interaction between melting point fraction and package color. The results showed

a general increase in FFA during storage period, representing the trend observed earlier for crude Nile perch viscera oil under accelerated oven test[2]. The activity of residual lipases and phospholipases partly contribute to this trend[2, 20]. Much of the FFA was removed by the neutralization and deodorization refining steps. Deodorization also removed odoriferous compounds. Low hydrolytic activity is typical of the spawning season, particularly in males[21]. In this study, the combined effect of the refining processes reduced FFA to 23% in LMPF and 20% in HMPF. Deodorization can reduce FFA by up to 50% under optimum processing conditions[22]. The levels of FFA should generally be below 2% (as acid value) in medicinal cod liver oil. Levels of free fatty acids in crude oil may be reduced by alkali refinement to levels less than 0.05%[20]. There was significant difference in FFA values over time for low MP fraction stored in transparent package, the difference occurring after sixteen weeks. However, low MP fraction stored in colored package showed no significant difference in FFA values during storage ($p>0.05$). For high MP fraction stored in transparent package, there was significant difference from week eight, whereas for the high MP fraction stored in colored package there was significant difference from week twelve.

For the low melting point fraction in colored package, the values surpassed the maximum limit for refined fish oils, set by CODEX Alimentarius after 18 weeks. The FFA of high melting point fraction surpassed the limit after 6 and 10 weeks for transparent and colored packages respectively. The FFA of low melting point fraction in transparent package surpassed the limit after 8 weeks. The values were, however, below the maximum limit for nutraceutical grade fish oils in the United States, as set by the Council for Responsible Nutrition. The maximum limit for refined fish oils, set by CODEX Alimentarius is 0.3%, while the maximum limit for nutraceutical grade fish oils in the US is 1.5%[23].

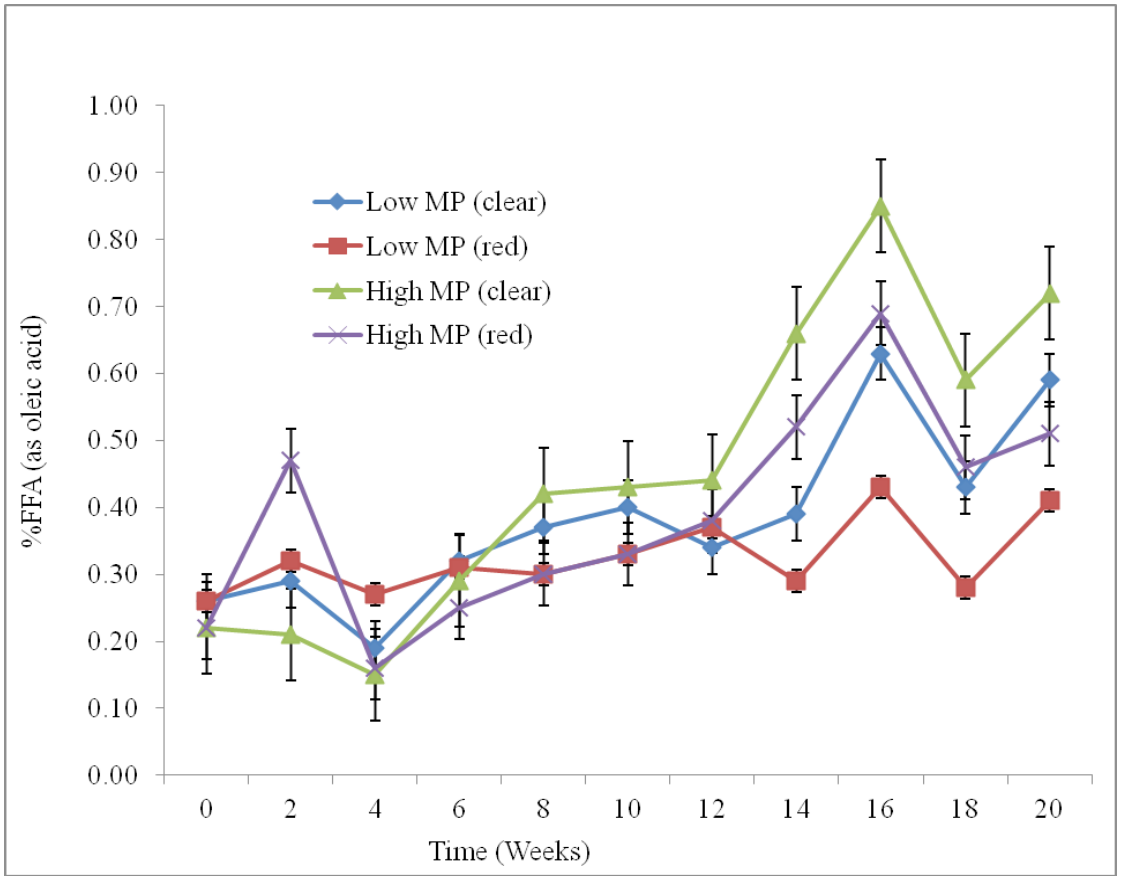

Fig. 1: Changes in free fatty acids during storage of refined Nile perch viscera oil

Based on this, low melting point fraction of refined Nile perch viscera oil can maintain nutraceutical grade status for at least 18 weeks of storage.

Changes in peroxide value during storage

There were significant differences in the PV for the different melting point fractions and package color. There was interaction between melting point fraction and package color as well. PV increased during storage, significant difference occurring for all conditions in the second week, but the values remaining within the limits for refined fish oils for eighteen weeks. High MP fraction stored in colored package, however, remained below the maximum limit throughout the storage period of twenty weeks (Fig. 2).

The high MP fraction retained much of the phospholipids left after alkali refining, which contributes to its oxidative stability[24]. The values closely represent the trend observed earlier for crude Nile perch viscera oil under accelerated oven test[2] and for refined cod liver and seal blubber oils stored at 65°C[24 - 25]. The results concur with studies on the oils from other fishes stored at +4°C and -18°C[13]. The maximum limit for refined fish oils, set by CODEX Alimentarius is 5mEq O_2/kg oil[23]. Removal of pro-oxidants such as residual moisture, monoacylglycerols and free fatty acids during deodorization might have increased the oxidative stability of the oil[24]. Winterization has been shown to significantly reduce secondary oxidation products of lipids arising from heating processes such as deodorization, thereby improving oxidation stability[22].

The enhanced exceptional oxidative stability of the oil during the refining processes and storage must have been due to the high levels of â-carotene and á-tocopherol[2, 26]. These vitamins slow down

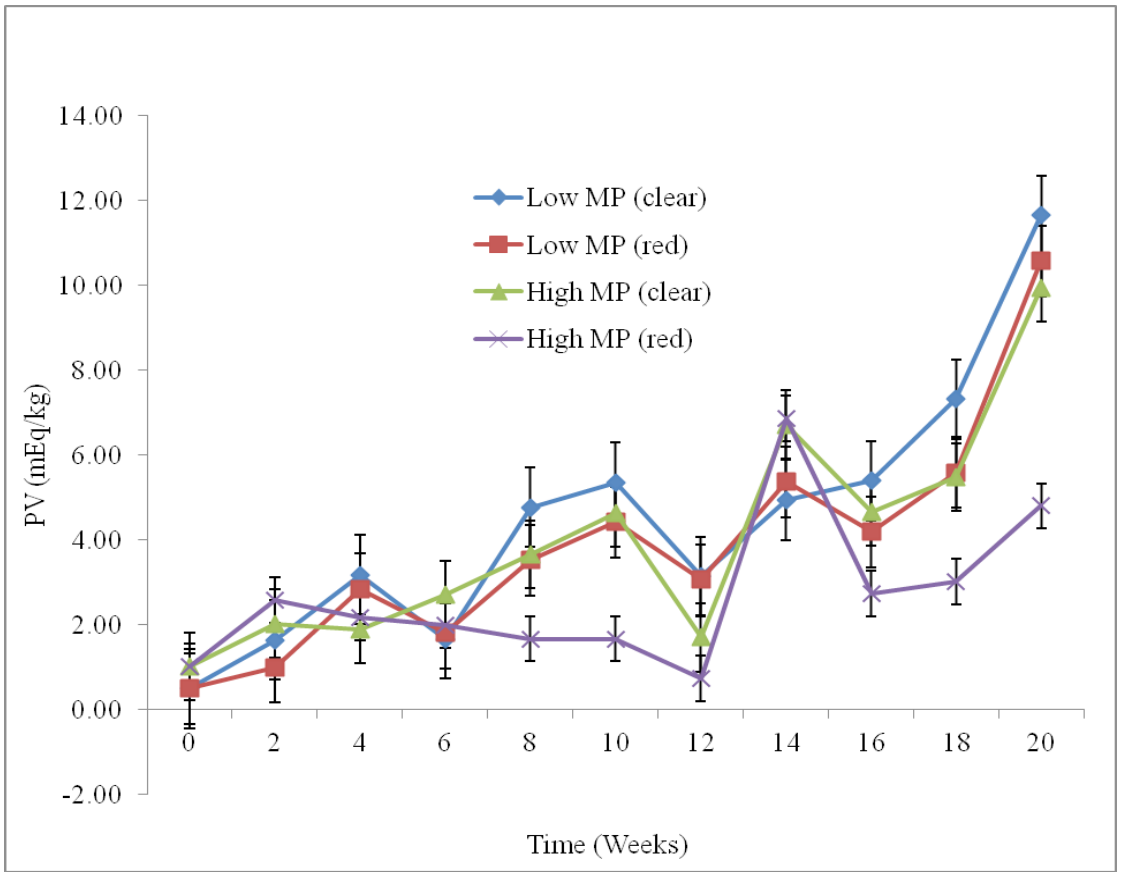

Fig. 2: Changes in peroxide value during storage of refined Nile perch viscera oil in different packages

or terminate the onset of oxidative deterioration processes because of their free radical quenching ability[27]. There is, however, possibility of improving the oil stability by means of oxygen scavengers and antioxidants as has been used on rainbow trout fillets[28].

CONCLUSION

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

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