Nile tilapia (Oreochromis niloticus) fried in recycled palm oil: implications for nutrition and health

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ABSTRACT
Fish constitutes a occasional food for the Sidama people of Hawassa, the capital city of Ethiopia’s Sidama Region and the site of a large endorheic lake. Freshly-caught fish, especially Nile tilapia or koroso in Local name, are typically fried prior to consumption. Despite the sensory qualities, fried foods are not always advisable due to the impact of frying on the nutritional quality of food. This study was designed to assess the nutritional quality of Nile tilapia that had been fried in the same palm oil over six consecutive frying cycles. The raw fish were purchased from the Lake Hawassa fish market and fried at Hawassa University by simulating local preparation methods. A gas chromatography-mass spectrophotometer (GCMS) was used for the fatty acid profile analysis and a total of 22 fatty acids were elucidated. The nutritional quality indices of fatty acids was determined by calculating the recommended formula and JMP pro 13 was used for statistical analysis. The study results revealed that the tilapia fried in the oldest, most used oil, cycles 3–6, contained high amounts of saturated and trans fatty acids, as well as high atherogenic and thrombogenic indices; however, it was also lower in essential and cis fatty acids, the hypocholesterolemic/hypercholesterolemic ratio, the per-oxidizability index, and the nutritive value index. Conversely, fish prepared earlier (cycles 1–3) with fresher oil were higher in essential fatty acids and cis fatty acids, while the hypocholesterolemic/hypercholesterolemic ratio, the per-oxidizability index, and the nutritive value index were also high. Fish fried in an earlier cycle were also low in saturated and trans-fatty acids, with a lower atherogenic index, and thrombogenic index. Therefore, it can be concluded that repeatedly using the same frying oil to prepare Nile tilapia contributed to the loss of nutritional value. Results suggest that palm oil should be limited to no more than three frying cycles to maximize nutritional intake in of fish consumption.

Introduction
Fish is a highly nutritious food source and vital for human health. [1] Fish consumption is popular worldwide; it is recommended by nutritionists and health care workers as a contributing factor in the prevention of cardiovascular diseases. [2] A variety of processing methods are used, due to both taste preferences and safety concerns. However, fish is a perishable product and food handlers need to pay careful attention to safety and quality factors during preparation and consumption. At Hawassa Lake in Ethiopia, three common fish preparations are available; sliced raw fish, fried fish, and fish soup. However, fried fish consumption is the most common preparation style of all at the lakeside.

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Using the same oil to fry fish over multiple cycles can degrade and diminish the nutritional quality, thereby decreasing the benefits of fish consumption to human health.\textsuperscript{[3]} Similarly, deep-frying causes oxidation and generates free radical compounds, is positively correlated with cardiovascular diseases, and considered a risk factor for chronic diseases and a more limited life span of consumers.\textsuperscript{[4,5]} The higher consumption of energy-dense food, particularly foods high in saturated trans-fat (STF), can cause weight gain and increased risk of cardiovascular disease. There is a correlation between a balanced diet and the prevention of chronic diseases, and a balanced diet also plays a critical role in maintaining health throughout the life span.\textsuperscript{[6–9]}

In the last two decades, the WHO Dietary Guidelines (1998) were shifted to emphasize the type of fat to be consumed and promote the ideal fatty acid profile.\textsuperscript{[5]} There is no clear cutoff point for the effect of fried food consumption on human health; however, consumers should consider the risk factors related to the heavy consumption of deep-fried foods. In the meantime, risk assessments and control systems for reducing trans fat should continue. To that end, this study was designed to (1) determine the fatty acid profile of fried Nile tilapia that has been fried in the same oil of different ages, from fresh to six frying cycles old, (2) calculate the nutritional quality index for the consumption of fried tilapia and the impact on human health, and (3) recommend a management system for the use of oil and food preparation.

**Methods and materials**

**Description of the sample collection area**

Hawassa is the capital city of the Sidama regional state of Ethiopia and home to the Sidama people. The city of Hawassa is 275 km from Addis Ababa, Ethiopia’s capital, and is known for Lake Hawassa, a lake which is approximately 27 m deep, 95sq km wide, and 16 km in length, from northeast to southwest, and 8 km from east to west.\textsuperscript{[10]} Geographically, Hawassa Lake is situated at 6 033’–7 033’ N and 38 022’–38 029’ E. The lake is an endorheic one, however, there is inflow from the factory via the Tikur wuha river.\textsuperscript{[11]} In Amharic, the lake is named Yefiker Hayek or lovely lake, as it is economically beneficial for the community. There are more than three fish species, and Nile tilapia (Oreochromis niloticus) or is the dominant taxon.\textsuperscript{[12]} Three types of fish products are made at the lake shoreline; raw sliced fish, fried fish, and fish soup. Currently, 18 unions actively participate in the fish market, and the most commonly adopted business is providing fried fish for the consumer. The fish that are most likely to be fried, and the oil most used for frying, are Nile tilapia and palm oil, respectively.\textsuperscript{[12]}

**Sample collection and preparation**

Sampling was carried out during the dry season. Nile Tilapia fish \((n = 24)\), weighing 150 g on average, were collected from Lake Hawassa’s Gudumale Park where the fish is purchased and distributed immediately after capture. The fish were dissected at the lakeside and cleaned, descaled, and eviscerated manually using a sterile knife.\textsuperscript{[13]} Fish samples of 110 g were placed into plastic bags, kept in an icebox at a temperature of 4°C with a fish/ice ratio of 1:2, and transported to the Hawassa University Food Science laboratory.

Palm oil, from the palm Elaeis guineensis, was used to fry each sample, following the same process as found at Hawassa Lake. Palm oil is the most common oil import for Ethiopia and is always available in Hawassa city markets at low cost; 50 birr (1.1 USD) for two liters of palm oil versus 200 birr (4.8 USD) for two liters of sunflower oil. Palm oil is made up of approximately 48% saturated fatty acids (SFA) and 52% unsaturated fatty acids (UFA). The most abundant SFA in palm oil is palmitic acid at 85%, whereas oleic acid is the largest UFA component at 88%.\textsuperscript{[14]}
**Fish frying method**

The fish samples weighing approximately 110 g, and about 20 cm in length, 5 cm in width, and 20 mm in thickness, with a temperature of 25°C, were deep-fried. The frying temperature was set and maintained at 170°C for the entire frying cycle. Each cycle was conducted for 10 minutes and the period between each frying cycle was 30 seconds. The first cycle with fresh oil was labeled C1, while the last or 6th cycle, completed with the same palm oil, was labeled C6. Cycles 2–6 were labeled in sequential order from C2 through C6. The palm oil-fish ratio was two litters of palm oil for frying 24 fish, and four tilapia samples were fried within each cycle. All fried fish were prepared in the same way as they are in Hawassa's fish market, i.e., once fried, the fish were suspended for 15 minutes to drain excess oil. The fish were then prepared for analysis based on standard recommendations.[15] After removing the central vertebra backbone, the fried fish were oven-dried at 60°C for 72 hr, transferred into desiccators, and cooled for 30 minutes. Fried fish were ground to 0.3 mm and the powder was stored in desiccators for analysis. Samples were homogenized by passing them twice through a mincer with 4 mm holes and the resulting material was mixed thoroughly. Uniformity of the homogenate was ensured by further mixing. The resultant homogenate was packed into several small sterile containers and stored at 0°C until analysis was performed. All experimental procedures were conducted in duplicate.

**Fatty acid profiles**

The fatty acid profile was determined using a gas chromatography-mass spectrophotometer (GCMS) and the lipid was extracted following standard procedures.[16] The extraction process was conducted using the following steps; (1) extraction flasks were cleaned and dried in a drying oven (Memmert) at 105°C for an hour, (2) flasks were cooled in a desiccator containing granular silica gel for thirty minutes and then weighed, (3) the deep-fried samples were homogenized and weighed in a conical flask, and kept dry for an hour at 105°C, (4) the flask was cooled to room temperature, 50 ml of 4 M hydrochloric acid was added, and the flask was boiled for an hour, (5) water (150 ml) was added, and the solution was filtered through fluted filter paper and washed until a neutral reaction on litmus paper was achieved, (6) filter paper was dried with its content for 1 hr at 105°C and inserted into the extraction thimble of the Soxhlet apparatus, (7) fat extraction was continued with hexane using soxhlet for 6 hr, and (8) hexane was evaporated using a rotary evaporator after extraction and was kept for derivatization.

Exactly 200 mg of oil was weighed into the bottom of the screw-capped tube that is Teflon-lined. Then 2 ml of 2 N KOH was added in methanol. The tube was closed tightly and heated in a boiling water bath at 80°C for 1 hr and cooled. Then 5 ml of 5% HCl was added in methanol. The tube was tightly closed and heated in boiling water at 80°C for 1 hr. After cooling, 5 ml of hexane and 5 ml of water were added into the tube and mixed. Next hexane was collected from the top layer after 30 seconds in a centrifuge. The solvent layer was washed by diluting potassium bicarbonate to remove excess acid and dried with anhydrous sodium or magnesium sulfate. Potassium bicarbonate was recovered after removal of the solvent by evaporation under reduced pressure on a rotary film evaporator. Then the extract was transferred into a GC vial through injection. All of the analyses were carried out in triplicate. Finally, the fatty acid methyl esters (FAME) of the fish fats were then analyzed by capillary gas chromatography. Helium was used as the carrier gas at 14 psi. Relative quantities were expressed as the weight percent of total fatty acids identified via comparison of retention times to known FAME standards.

**Indexes of lipid quality**

Using the fatty-acid composition data, the atherogenicity and thrombogenicity indices were calculated as follows:
Atherogenicity index (AI)
This is defined as the ratio of the sum of the main SFAs and that of the main classes of UFA.\textsuperscript{[17]} The following equation was applied:

$$IA = \frac{[(12 : 0 + 4 \times C14 : 0) + C16 : 0]}{(\sum^M UFAs + \sum^P UFAn6 + PUFA n3)}$$  \hspace{1cm} (1)

Thrombogenicity index (TI): This is the relationship between pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (MUFAs, PUFAs – n6, and PUFAs – n3).\textsuperscript{[17]}

$$IT = \frac{C14 : 0 + C16 : 0 + C18 : 0}{(0.5 \sum^M UFAs + 0.5 \times PUFA n6 + 3 \times PUFA n3 + (PUFA n3/PUFA n6)}$$  \hspace{1cm} (2)

Hypocholesterolaemic index (hHI)
This can be defined as the cholesterol/saturated fat (CSI) index. Low CSI indicates low saturated fat and cholesterol, and low atherogenicity.\textsuperscript{[17]}

$$hHI = \frac{(C18:1n - 9 + C20:1(n - 9) + 22:5(n - 3) + 22:6(n - 3) + C18:3n - 6 + 20:4(n - 6) + 18:2(9, 12) + 20:5(n - 3))}{((C14:0 + C16:0))}$$  \hspace{1cm} (3)

Nutritive Value Index\textsuperscript{[17]}

$$NVI = \frac{C18 : 0 + C18 : 1}{C16 : 0}$$  \hspace{1cm} (4)

Peroxidizability Index\textsuperscript{[18]}

$$PI = \text{mononoicacid} \times 0.025 + \text{dienoicacid} \times 1 + \text{trienoicacid} \times 2 + \text{tetraconoicacid} \times 4 + \text{pentoicacid} \times 6 + \text{hexanoicacid} \times 8$$  \hspace{1cm} (5)

Data analyses
Analyses were performed twice for all samples and closely agreeing replicates were obtained. The data were analyzed using JMP pro 13 with one-way ANOVA. The means were separated using Tukey’s HSD test at $p < .05$. Principal component analysis (PCA) was used for identifying the main fatty acids and nutritive quality index concerning the frying cycle, and R software was used for correlation analysis.

Results and discussion

Identified fatty acids in fish fried with different cycle
A total of 22 fatty acids were identified in the fried Nile tilapia fish (Table 1). The identified saturated fatty acids are lauric acid (12:0), myristic acid (14:0), palmitoleic acid (16:0), stearic Acid (18:0), methyl stearate (19:0), arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0). Palmitoleic acid (16:1 n-7), cis-10-heptadecenoic acid (17:1 n-10), oleic acid (18:1 n-9), gondoic acid (20:1 n-9) and linoleic acid (18:2 n-6) are identified cis fatty acids. Docosapentanoic acid (22:5 n-3), docosahexaenoic acid (22:6 n-3), and eicosapentaenoic acid (20:5 n-3) are identified omega-3 fatty acids. Linoleic acid (18:2 n-6), gamma-linolenic acid (18:3 n-6), and arachidonic acid (20:4 n-6) are identified omega-6 fatty acids. Finally, Trans-13-octadecenoic acid (18:1 n-13) and trans-10-heptadecenoic acid 17:2(8,10) were identified as trans fatty acids in raw Nile tilapia (koroso in Sidamo) and in samples that had been fried in the market.\textsuperscript{[19]}
Table 1. Fatty acids in Nile tilapia (Oreochromis niloticus) fried with re-used palm oil.

| Fatty acid Formula | Level of saturation | Frying cycle using same palm oil |
|--------------------|---------------------|----------------------------------|
|                    |                     | C1 | C2 | C3 | C4 | C5 | C6 |
| 12:0               | Saturated           | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 14:0               |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 15:0               |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 16:0               |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 17:0               |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 18:0               |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 19:0               |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 20:0               |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 22:0               |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 24:0               |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 16:1 n-7           | cis monounsaturated | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 17:1 n-10          |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 18:1 n-9           |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 20:1 n-9           |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| √18:2 n-6          | √cis Polysaturated  | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 18:1 n-13          | trans monounsaturated | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| √17:2(8,10)        | √trans Polysaturated | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 22:5 n-3           | Omega-3             | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 22:6 n-3           |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 20:5 n-3           |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 18:3 n-6           | Omega-6             | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |
| 20:4 n-6           |                     | ✓  | ✓  | ✓  | ✓  | ✓  | ✓  |

Notes:
1. Cycle 1 or C1 is fresh palm oil,
2. C2 = second use,
3. C3 = third use,
4. C4 = fourth use,
5. C5 = fifth use,
6. C6 = sixth or final use of palm oil

Fatty acid composition of fish fried at different cycles

In this study, 10 SFAs were identified, and palmitic acid was found in the highest percentage of these (Table 2). Further, palmitic acid, methyl stearate, and myristic acid were found in the highest amount (percentage) as compared to the rest of the fatty acids. The mean percentage for palmitic acid, methyl stearate, and myristic acid ranged from 31.02–36.87%, 4.67–4.90%, and 1.78%–2.30%, respectively (Table 2). The study also identified five cis fatty acids. Oleic acid (37.28–34.2%), linoleic acid (11.55–11.01%), and palmitoleic acid (4.91–2.11%) shared the highest percentage of the cis fatty acid profile in the fried fish (Table 2). Trans-13-octadecenoic acid and trans-10-heptadecenoic acid were identified, and the trans-13-octadecenoic acid was found in the highest percentage, ranging from 0.80–1.16% (Table 2).

Our study also revealed the existence of omega-3 and omega-6 fatty acids in the fried Nile tilapia samples (Tables 1 and 2). Omega-3 fatty acids, docosapentaenoic acid, docosahexaenoic acid, and eicosapentaenoic acid, were found in the fried tilapia, whereas omega-6 fatty acids, including linoleic acid, gamma-linolenic acid, and arachidonic acid were also present (Tables 1 and 2). A comparable amount of all omega-3 fatty acids was found, although the amount decreased from C1 to C6. Concerning omega-6 fatty acids, linoleic acid ranged from 11.55–11.01%, but was also the highest such acid as compared to gamma-linolenic acid, and arachidonic acid (Table 2). The study results also demonstrated that fish fried in the higher frying cycle, where oil had been used repeatedly, contained higher saturated and trans fatty acid, but lower cis and essential fatty acids.

The higher SFAs, and the lower essential fatty acids, in the subsequently higher frying cycle could be due to the fact that the fish was fried with palm oil, as palm oil is known for its higher SFAs. Regarding cis and trans fatty acids, as the oil is used repeatedly for frying purposes, the probability of oil becoming saturated is high, and the increasing temperature accelerates cis-trans conversion or
Table 2. Comparison of fatty acid composition across the frying cycles.

| Fatty acid formula | C1 | C2 | C3 | C4 | C5 | C6 |
|-------------------|----|----|----|----|----|----|
| C12:0              | 0.27 ± 0.028 | 0.32 ± 0.02 | 0.32 ± 0.01 | 0.35 ± 0.04 | 0.43 ± 0.021 | 0.43 ± 0.035 |
| C14:0              | 1.78 ± 0.14 | 1.87 ± 0.71 | 1.81 ± 0.24 | 2.30 ± 0.76 | 2.30 ± 0.25 | 2.30 ± 0.29 |
| C15:0              | 0.26 ± 0.07 | 0.28 ± 0.04 | 0.31 ± 0.01 | 0.36 ± 0.014 | 0.36 ± 0.026 | 0.37 ± 0.04 |
| C16:0              | 31.02 ± 1.42 | 32.56 ± 1.23 | 34.95 ± 0.9 | 35.89 ± 1.32 | 36.99 ± 0.96 | 36.87 ± 0.12 |
| C17:0              | 0.51 ± 0.028 | 0.50 ± 0.02 | 0.55 ± 0.02 | 0.67 ± 0.028 | 0.65 ± 0.01 | 0.71 ± 0.07 |
| C18:0              | 0.15 ± 0.05 | 0.15 ± 0.06 | 0.19 ± 0.02 | 0.21 ± 0.021 | 0.24 ± 0.020 | 0.28 ± 0.012 |
| C19:0              | 4.67 ± 0.71 | 5.68 ± 0.82 | 6.55 ± 1.12 | 6.72 ± 0.99 | 6.73 ± 0.74 | 6.90 ± 0.86 |
| C20:0              | 0.67 ± 0.04 | 0.88 ± 0.09 | 0.54 ± 0.02 | 0.48 ± 0.041 | 0.48 ± 0.042 | 0.56 ± 0.02 |
| C22:0              | 0.13 ± 0.014 | 0.18 ± 0.01 | 0.26 ± 0.02 | 0.38 ± 0.01 | 0.36 ± 0.026 | 0.39 ± 0.01 |
| C24:0              | 0.20 ± 0.09 | 0.37 ± 0.02 | 0.26 ± 0.01 | 0.26 ± 0.012 | 0.28 ± 0.042 | 0.30 ± 0.03 |
| 16:1 n-7           | 4.91 ± 0.69 | 3.54 ± 0.45 | 2.54 ± 0.28 | 2.58 ± 0.49 | 2.31 ± 0.71 | 2.11 ± 0.38 |
| 17:1 n-10          | 0.63 ± 0.07 | 0.32 ± 0.014 | 0.25 ± 0.03 | 0.14 ± 0.021 | 0.18 ± 0.014 | 0.106 ± 0.02 |
| 18:1 n-9           | 37.28 ± 0.68 | 36.96 ± 0.96 | 36.02 ± 0.8 | 35.47 ± 0.86 | 34.16 ± 0.97 | 34.20 ± 0.75 |
| 20:1 n-9           | 0.52 ± 0.14 | 0.41 ± 0.035 | 0.32 ± 0.02 | 0.36 ± 0.06 | 0.28 ± 0.07 | 0.22 ± 0.02 |
| 18:2 n-6           | 11.55 ± 1.16 | 11.30 ± 0.9 | 11.02 ± 1.0 | 10.04 ± 0.82 | 10.93 ± 0.96 | 11.01 ± 0.90 |
| 18:1 n-13          | 0.80 ± 0.14 | 1.10 ± 0.28 | 1.09 ± 0.21 | 1.13 ± 0.34 | 1.12 ± 0.50 | 1.16 ± 0.38 |
| 17:2(8,10)         | 0.02 ± 0.002 | 0.09 ± 0.004 | 0.12 ± 0.01 | 0.11 ± 0.003 | 0.14 ± 0.07 | 0.17 ± 0.06 |
| 22:5 n-3           | 0.97 ± 0.16 | 0.76 ± 0.06 | 0.97 ± 0.03 | 0.60 ± 0.04 | 0.40 ± 0.025 | 0.5 ± 0.042 |
| 22:6 n-3           | 0.92 ± 0.09 | 0.68 ± 0.05 | 0.65 ± 0.02 | 0.63 ± 0.08 | 0.44 ± 0.028 | 0.51 ± 0.028 |
| 20:5 n-3           | 0.78 ± 0.07 | 0.64 ± 0.014 | 0.45 ± 0.06 | 0.34 ± 0.03 | 0.41 ± 0.017 | 0.25 ± 0.07 |
| 18:3 n-6           | 0.76 ± 0.06 | 0.36 ± 0.03 | 0.28 ± 0.01 | 0.22 ± 0.002 | 0.16 ± 0.014 | 0.14 ± 0.002 |
| 20:4 n-6           | 0.85 ± 0.024 | 0.72 ± 0.056 | 0.65 ± 0.06 | 0.40 ± 0.014 | 0.34 ± 0.003 | 0.27 ± 0.012 |

Notes:
1. Each cycle represents four fish fried in the same oil, and there are six separate cycles using the same oil (C1-C6).
2. All values are an average of means ± standard deviations for the 4 fish fried in each cycle from 1–6.
3. There are no significant differences of means in comparisons of C1-C3, and C4-C6.
4. However, all comparisons of values in cycles 1-3, compared to values in C4-C6, are significantly different at the .05 level.

isomerization.\textsuperscript{[20–22]} Therefore, more \textit{trans}-fatty acids could be generated, but at the same time, both saturated and \textit{trans} fatty acids were strongly negatively correlated with essential and \textit{cis} fatty acids at the 0.05 and the 0.01 level, with \textit{r} \textit{calc} = −0.999 (Figure 2).

**Nutritional quality index (NQI) of Nile tilapia fried in different cycles**

Including saturated, unsaturated, \textit{cis}, and \textit{trans}-fatty acids, the nutritional quality index of fish fried with the same palm oil over six frying cycles is presented in Table 3. The total SFAs, \textit{cis} fatty acids, \textit{trans}-fatty acids, omega-3 fatty acids, and omega-6 fatty acids of fish fried with recycled palm oil (C1-C6) ranged from 39.87–49.48%, 54.89–47.65%, 0.83–1.35%, 2.65–1.26%, and 13.16–11.42%, respectively. Nutritional value and healthiness of fat for human consumption are judged by $\Sigma$PUFA/SFA and $\Sigma$ω-6/ω-3.\textsuperscript{[23]} Similarly, a balanced intake of dietary $\Sigma$UFA/SFA and $\Sigma$PUFA/SFA is very important in regulating serum cholesterol.\textsuperscript{[24]} It has also been recommended that a ratio of $\Sigma$PUFA/SFA greater than 0.45 should be considered in human diets for prevention of cardiovascular (CVD) and chronic diseases such as cancer; however, a ratio of and $\Sigma$PUFA/SFA below 0.45 is considered undesirable for consumption due to its potential to induce an increase of cholesterol in the blood.\textsuperscript{[25]} The study revealed that a $\Sigma$PUFA/SFA ratio for the fish fried with recycled palm oil used six times is varied from 0.39–0.26%. Thus, the repeated use of the frying oil decreases the ratio of $\Sigma$PUFA/$\Sigma$SFA, such that the consumption of fish fried with recycled oil could have a negative impact on human health.

A recent study of omega-3 and omega-6 fatty acids indicated that the highest proportion of PUFA is not necessarily healthy, unless there is a balance in the ratio of ω-3/ω-6.\textsuperscript{[23]} Essential fatty acids (Omega-3, omega-6) and the ratio of ω-6/ω-3 are critical in controlling the hypocholesterolemic index and regulating the thrombogenic and atherogenic indices, respectively. Red meat, e.g., beef, and meat-derived products are characterized by a low atherogenic index and high h/H index.\textsuperscript{[25]} Meat products with low thrombogenicity decrease the threat of atrial fibrillation.\textsuperscript{[25]}

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Table 3. Comparison of nutritional quality indices of lipids in fish fried with repeated cycle.

| Fatty acid Indices | C1       | C2       | C3       | C4       | C5       | C6       |
|-------------------|----------|----------|----------|----------|----------|----------|
| ∑SFA              | 39.87 ± 0.063 | 43.02 ± 0.02 | 46.03 ± 0.02 | 47.96 ± 0.33 | 49.22 ± 0.056 | 49.48 ± 0.12 |
| ∑MUFA             | 44.14 ± 0.03  | 42.33 ± 0.10 | 40.22 ± 0.01 | 39.68 ± 0.35 | 38.05 ± 0.05  | 37.79 ± 0.02  |
| ∑PUFA             | 15.85 ± 0.06  | 14.55 ± 0.02 | 14.14 ± 0.05 | 12.34 ± 0.07 | 12.82 ± 0.04e | 12.86 ± 0.03  |
| ∑MUFA/SFA         | 1.10 ± 0.06  | 0.98 ± 0.04a | 0.87 ± 0.031a | 0.82 ± 0.03a | 0.77 ± 0.031a | 0.76 ± 0.01a  |
| ∑PUFA/SFA         | 0.39 ± 0.04  | 0.34 ± 0.03  | 0.30 ± 0.02  | 0.26 ± 0.01  | 0.26 ± 0.02  | 0.26 ± 0.02  |
| ω-3               | 2.65 ± 0.01  | 2.08 ± 0.07  | 1.65 ± 0.07  | 1.56 ± 0.021 | 1.24 ± 0.021 | 1.26 ± 0.07  |
| ω-6               | 13.16 ± 0.0001 | 12.38 ± 0.05 | 11.95 ± 0.001 | 10.66 ± 0.01 | 11.43 ± 0.014 | 11.42 ± 0.001 |
| ω-3/ω-6           | 0.20 ± 0.02  | 0.16 ± 0.01  | 0.13 ± 0.001 | 0.14 ± 0.01  | 0.10 ± 0.002 | 0.11 ± 0.001  |
| ω-6/ω-3           | 4.96 ± 0.0003 | 5.95 ± 0.009 | 7.24 ± 0.002 | 6.83 ± 0.005 | 9.27 ± 0.01  | 9.06 ± 0.01   |
| Σcis              | 54.89 ± 0.08 | 52.53 ± 0.09 | 50.15 ± 0.07 | 48.59 ± 0.07 | 47.86 ± 0.06 | 47.65 ± 0.06  |
| Σtrans            | 0.83 ± 0.15  | 1.19 ± 0.02  | 1.21 ± 0.014 | 1.24 ± 0.001 | 1.27 ± 0.001 | 1.35 ± 0.02   |
| IA                | 0.64 ± 0.001 | 0.71 ± 0.003 | 0.78 ± 0.001 | 0.87 ± 0.03  | 0.91 ± 0.01  | 0.92 ± 0.002  |
| IT                | 0.89 ± 0.001 | 1.02 ± 0.001 | 1.18 ± 0.001 | 1.28 ± 0.006 | 1.38 ± 0.001 | 1.38 ± 0.002  |
| h                 | 53.63 ± 0.02 | 51.83 ± 0.02 | 50.36 ± 0.01 | 48.06 ± 0.36 | 47.12 ± 0.05 | 47.10 ± 0.014 |
| H                 | 32.8 ± 0.014 | 34.43 ± 0.07 | 36.76 ± 0.07 | 38.19 ± 0.42 | 39.29 ± 0.001 | 39.17 ± 0.12  |
| h/H               | 1.63 ± 0.001 | 1.59 ± 0.003 | 1.36 ± 0.002 | 1.25 ± 0.02  | 1.19 ± 0.001 | 1.20 ± 0.004  |
| NVI               | 1.20 ± 0.001 | 1.13 ± 0.001 | 1.03 ± 0.002 | 0.99 ± 0.009 | 0.92 ± 0.001 | 0.93 ± 0.003  |
| PI (%)            | 35.45 ± 0.05 | 29.88 ± 0.67 | 29.02 ± 0.02 | 23.86 ± 0.15 | 21.40 ± 0.14 | 22.03 ± 0.44  |

Notes:
- Each cycle represents four fish fried in the same oil, and there are six separate cycles using the same oil (C1-C6).
- All values are an average of means ± standard deviations for the 4 fish fried in each cycle from 1–6.
- There are no significant differences of means in comparisons of C1-C3, and C4-C6.
- However, all comparisons of values in Cycles 1–3, compared to values in C4-C6, are significantly different at the .05 level.
- Abbreviations: ∑SFA, saturated fatty acid; ∑MUFA’s, monounsaturated fatty acid; ∑PUFA, polyunsaturated fatty acid; DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; IA, atherogenic index; IT, thrombogenic index; h/H, hypocholesterolemic/Hypercholesterolemic index; NVI, nutritive value index; PI, Peroxidizability index.

A ratio of ω-6/ω-3 that is less than 4 indicates that a diet has a desirable quantity of omega-3 and omega-6 fatty acids for reduction of cardiovascular diseases.[23] This study revealed that the ratio of omega-6 and omega-3 fatty acids in fish fried with the same palm oil, used for six frying cycles, ranged from 4.96–9.06%. Thus, the ω-6/ω-3 ratio for fish fried in recycled palm oil was higher than the standard. Therefore, fish fried in palm oil that has been used repeatedly does not contain the optimal quantity of omega-3 and omega-6 fatty acids; consequently, it can be considered unhealthy.

The ∑MUFA/SFA, ∑PUFA/SFA, and ∑ω-6/ω-3 are not the only indicators concerning healthy amounts of fatty acids in food. It is also important to evaluate omega-3 fatty acids, and the indices of atherogenicity, thrombogenicity, nutritive value, as well as the hypocholesterolemic/hypercholesterolemic ratio. Omega-3 fatty acids decrease the concentration of low-density lipoprotein cholesterol in plasma and this is recognized as the most important essential fatty acid in the human diet as it is the precursor to the formation of eicosanoids.[26–28] In this study, the amount of omega-3 fatty acids of fish fried with recycled palm oil, from C1-C6, ranged from 2.65–1.26%. The highest amount of omega-3 fatty acids was observed in cycles 1–3, while the lower omega-3 fatty acids were measured in cycles 4–6. The latter process may occur because omega-3 fatty acids are likely to oxidize when exposed to high-temperatures, due to the presence of a (pi) bond.[28]

**Nutritional quality index (NQI) of Nile tilapia fish fried with different cycles**

The atherogenic, thrombogenic, hypocholesterolemic/hepercholesterolemic, nutritive value, and the peroxidizability indices (PI) of fish, fried with recycled palm oil across six cycles, are shown in Figure 1. A high atherogenicity index indicates that there is adhesion of lipids to cells of the immunological and circulatory system; thus, there is an aggregation of plaque. Conversely, a low atherogenicity index means that coronary diseases are less likely to occur. The SFAs, myristic and palmitic acid, are among the most atherogenic agents.[29]
The index of thrombogenicity indicates the tendency to form clots in the blood vessels.\textsuperscript{2} Stearic acid is thought to be neutral concerning atherogenicity, but is instead considered to be thrombogenic.\textsuperscript{30} Higher indices for atherogenicity and thrombogenicity are responsible for the formation of atheroma and stimulate the aggregation of platelets in the cardiovascular system.\textsuperscript{31–33} Hence, lower stearic acid values are desirable in the prevention of cardiovascular disorders.
Results of the current study revealed that the atherogenicity and thrombogenicity values for Nile tilapia, fried with recycled palm oil, ranged from 0.64–0.92% and 0.89–1.38%, respectively. The repeated use of the same palm oil for frying fish increases the amount of atherogenicity and thrombogenicity; consequently, the consumption of foods prepared with palm oil used in multiple frying cycles can be considered unhealthy.

The ratio of hypocholesterolemic and Hypercholesterolemic indices, and the nutritive value index, are additional indicators for the effect of specific fatty acids on cholesterol metabolism. A higher value for each of these indices is considered desirable; in other words, nutritionally higher h/H and NVI values are more beneficial for human health. The h/H ratio and NVI values were significantly affected by the frying cycles, with a range of 1.63–1.20% and 1.20–0.93% across C1-C6, respectively. Recycling palm oil for fish frying decreases the value of the h/H ratio and NVI. Thus, frying cycles C1-C3 are healthier than cycles C4-C6; the h/H ratio and NVI were high in the lower frying cycle, a result which is considered healthy. The highest NVI value in C1-C3 is due to the fact that fish fried in fresher palm oil had the highest proportion of stearic acid (C 18:0) to oleic acid (18:1) and the lowest percentage of palmitic acid (C 16:0).

Principal component analysis (PCA)

Principal Component Analysis (PCA) showed that the fried fish from C4, C5, and C6 were aligned together with ΣSFAs, Σtrans fatty acids, IA, and IT (Figure 1). Similarly, fried fish from C1, C2, and C3 were in the same alignment with Σω-6/ω-3, Σomega-3, Σomega-6, ΣPUFA/SFA, h/H, NVI, and PI (%). These results suggest that recycling palm oil while frying food items such as fish could lead to loss of the nutritive quality, and therefore impact consumer health. Conversely, fried fish from the first through third frying cycles, C1-C3, are likely to have minimal influence on the nutritional quality of the fish, and therefore, limited influence on consumer health. Again, fried fish from C1-C3 were high in essential fatty acids, and low in the atherogenic and thrombogenic index, as compared to fried fish in the higher frying cycles. Similar results have been obtained for Caspian white fish or Caspian roach, *Caspian kutum*, based on using the same oil to prepare multiple batches for consumption.14,35

The peroxidizability index of fish fried with recycled palm oil is presented in Table 3. This index (PI) indicates the relationship between the fatty acid composition of a tissue and its susceptibility to oxidation. The PI index assesses the stability of PUFA included in food products and protects from possible oxidation. A higher PI value is equivalent to a greater protective potential for coronary artery disease. An excessive intake of PUFA leads to oxidative stress, whereby the lipid is susceptible to lipid peroxidation.24–26 Oxidative stress is associated with the formation of lipid peroxide and has been suggested to contribute to pathological processes in aging and diseases such as atherosclerosis. The peroxidizability index of fish fried with recycled palm oil, across C1-C6, is significantly affected, such that the value ranged from 35.45–22.08%. The continued use of the same palm oil for frying fish decreases the peroxidizability index; a lower index was obtained for fish fried in C4-C6, while a higher index was found in C1-C3. The higher peroxidizability index for fish fried during C1-C3 indicates the presence of PUFA, and PUFA are susceptible to autoxidation. The higher tendency for lipid oxidation was found in other kinds of marine fish also fried in the same oil.15

Correlation analysis of the nutritional quality index of fried fish

The nutritional qualities of fried fish, prepared using the same oil in different cycles, are shown in Figure 2. Results suggest that SFAs, the atherogenic index, the thrombogenic index, and trans fatty acids are all positively correlated, (r = 0.9 and 0.99). Similarly, monounsaturated fatty acids, essential fatty acids (e.g., omega-3 and omega-6 fatty acids), cis fatty acid, the hypocholesterolemic index, the nutritive value, and the per-oxidizability index were positively correlated with each other, but were negatively correlated with SFAs, the atherogenic index, the thrombogenic index, and trans fatty acids.
Limitations of the current study

In the current study, fat content was extracted by using the Soxhlet method, a standard process for such an analysis. The study compared the fatty acid-related nutritional quality indices of fried samples implying that a comparison had been made across the frying cycles with raw fish as well. However, the fatty acid profile for the raw sample was not included within this study as it was previously published. Also, the palm oil that is used for frying is not ingested by the consumer and in this analysis, the fatty acid profile of palm oil was not assessed. Also, we did not assess oils other than palm oil, nor did we assess other fish species from Lake Hawassa prepared in an identical way. Nevertheless, because our results are robust and similar outcomes have been obtained in other studies with other fish species, we are confident that the specific use of the same palm oil to fry different batches of tilapia can override or mask the health benefits that might accrue by using fresh oil.

Conclusions and recommendations

Nile tilapia, fried in palm oil, had a high amount of SFAs, including palmitic acid, methyl stearate, and myristic acid. By contrast, the UFA linoleic acid is a common component of tilapia as compared to the other cis and omega-6 fatty acids. There are also trans-13-octadecenoic acid and trans-10-heptadecenoic acid present. Omega-3 fatty acids are also found in Nile tilapia; however, the amount present was highly influenced by the frying cycle. Saturated and trans fatty acids were correlated positively. Similarly, unsaturated and cis fatty acids were found to be positively correlated. Regarding the nutritional quality index, fish prepared in fresher oil, during cycles 1–3, had a minimal influence on consumer health as compared to fish fried in older oil, from cycles 4–6. Thus, we recommend limiting the number of frying cycles that use the same oil. Further analyses about the impact on the health and nutrition on consumers, using a variety of fish species prepared in different types of oil over multiple cooking cycles, should be conducted. In a country where food insecurity rates are high, consumers need to feel confident about the food they consume; they should be able to maximize nutritional intake, while at the same time maintaining rather than damaging their health status.

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