Effect of frying oil stability over repeated reuse cycles on the quality and safety of deep-fried Nile tilapia fish (Oreochromis niloticus): a response surface modeling approach

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ABSTRACT

Fresh water fish is considered a source of good quality proteins and essential fats. Frying is among widely used fish preparation techniques globally. Deep-frying oil quality with repeated uses, has been a concern. This research investigated the influence of frying oil stability (expressed as levels of peroxide values (0.2, 1.2 meq/kg) and free fatty acids (0.05, 0.13% palmitic acid)) over repeated uses (1,6 cycles) on the oxidative stability and essential nutrients of fried fish using a response surface approach, with the objectives of generating information relevant for the improvement of community health outcomes, with a special focus of this particular research was on trends, but not optimization. The frying oil and fried fish quality were adequately explained by response surface model and supported by principal component analysis. The result showed that the oxidative stability of both the frying oil and fried fish were deteriorating over the reuse cycles of the frying oil (with increasing trends of unhealthy fats, saturated and trans) corresponding to decreasing trends in the healthy fat components and vitamin A). It was also indicated that the essential fats (omega 3, omega 6, cis, vitamin A), were decreased while the risky fats (saturated, trans) were increased along with the frying cycles. The result revealed the urgent need for regulating frying oil and fried food qualities, particularly in developing countries.

Introduction

Deep-fat frying, food completely immersed in hot oil, is one of the oldest and popular food preparation techniques, with the fried food quality heavily depending on the food being fried, frying oil and the number of times it is used.\[1\] Vegetable oils are widely used in food frying and they can be deteriorated by repeated heating that leads to lipid oxidation, which is a common practice in many food cultures as it reduces the cost of food preparation. Thermal oxidation results in new compounds which are potentially hazardous to consumers.\[2\] Peroxide values and free fatty acids are commonly used indicators of oil degradation by oxidation.\[3,4\] High peroxide values (PV > 10 meq/kg) and free fatty acid levels (FFA > 1%) were reported in actively used and discarded frying oils and these levels were not acceptable as per common global standards.\[5,6\] Prolonged consumption of the repeatedly heated oil has been shown to increase blood pressure and total cholesterol, causing vascular inflammation as well as vascular changes which predispose to atherosclerosis in consumers.\[2\]

A recent study in animal model confirmed that free fatty acids can be a risk factor for the formation of atherosclerotic plaque in coronary arteries of rats after being exposed to free fatty acids for 150 days.\[7\] The oxidative stability of the frying oils in repeated cycles of uses were observed to decrease as indicated by the increases in peroxide values, and levels of trans, saturated, and free fatty
acids. Other factors that play key roles in the deterioration of frying oils include the nature of the food matrix, fatty acid profiles of the frying oils and the number of reuse cycles of the oil in frying. The levels of peroxide values in fried fish in increased reuse cycles was reported to be abnormally high regardless of adding antioxidant agents such as vitamin E.

Fish is an important protein source in the urban and semi-urban settings in Ethiopia, where diverse species are commonly produced in freshwater bodies (rivers, lakes, and ponds), with the largest number of endemic species reported to be from the Blue Nile. Ethiopia has the potential of 52000 tons of fish production per annum. Nile tilapia (Oreochromis niloticus) is the most popular fish of freshwater catches (60%). Fish frying in Hawassa and many other places in Ethiopia, is usually done in a traditional way, under hardly controlled safety requirements with repeated use of the frying oil. The current study investigated the oxidative stability and nutritional composition of deep-fried Nile tilapia fish (O. niloticus) catches from Hawassa Lake (Ethiopia) as influenced by the number of reuse cycles of refined and bleached palm oil and the load of peroxide values and free fatty acids that accumulated over repeated uses ranging from 1 to 6 times. Response surface methodology (RSM) was used to characterize the oxidative stability and important nutritional composition of deep-fried Nile tilapia fish. The response surface effect of an augmented design with 12 runs including duplicates for estimating the errors, was used. To the author's best knowledge, there is no report so far with the use of advanced designs (response surface methodology, in explaining the influences of oxidative status of the frying oils over frying cycles on the nutritional composition and stability of fried fish. The objective of this study was to generate data on the relations of the frying oil stability and fried food quality over repeated use cycles of frying oils.

The output of this research will potentially be used to improve the quality and safety of deep-fried fish and other foods, which might include setting regulations, planning, and implementing public awareness programs on good frying practices. Information in this manuscript may help in shifting in the frying oil reuse cycles or even fish cooking methods and positively contributes to general public health and sustainable and healthy cooking and eating patterns in terms of food safety. The findings presented in this article will also give highlights on the gaps in food preparation and consumption patterns in different cultures and associated risks to the health of the consumers.

Materials and methods

Materials

Tilapia fish species (n = 24) averagely weighing about 150 g were collected from Lake Hawassa around Gudumale Park, Ethiopia during the dry season. The fish catches were immediately slaughtered following acceptable practices as per the Ethiopian laws and recommendations for animals used as food (Section 10.2 of the Ethiopian National Research Ethics Review Guidelines). A percussive mechanical stunning method, reported to be a humane slaughtering technique (Lines & Spence, 2014) was used. Fish were immediately cut and left to bleed (killed) before it got back to stability. The slaughtered fish were dissected, cleaned, descaled, and eviscerated manually using sterile plastic knives. The edible part (flesh and skin) was transferred into plastic bags and kept in an icebox at a temperature of 4°C with a fish/ice ratio of 1:2 and then transported to the laboratory.

Frying oil

Refined palm oil was purchased from the local market. The frying methods and conditions used by the local restaurants and street food vendors around Lake Hawassa were simulated in the laboratory with similar cooking oil, frying temperature, frying time and frying utensils, but with the frying cycle limited to 6 times. Samples of the frying oil after each cycle of frying were taken and analyzed for peroxide values and free fatty acid levels.
**Frying method**

The eviscerated fish samples (weighing 110 g on average) were deep-fried in palm oil for 10 min for each frying cycle (frying cycle 1–6). All fried fish were drained for 15 seconds to remove excess oil.

**Oxidative stability of fried fish**

Free fatty acid: Free fatty acid (FFA) content indicates the acid value in oily foods. FFA indicates the hydrolysis of triglycerides. FFA was determined by titrimetric method according to one described by.\(^4\) FFA level was calculated as the weight of potassium hydroxide (mg) needed to neutralize the organic acids present in 1 g of oil using phenolphthalein indicator. The titration was performed using an automated titration apparatus (AD-2 Automatic titrator, Shanghai, China).

Peroxide values: Peroxide value (PV) was determined by the titration procedure. Homogenized fried fish samples (5 g) were weighed in duplicates, into 250 mL glass-stoppered Erlenmeyer flasks. Acetic acid chloroform solution (CH\(_2\)COOH–CHCl\(_3\)) of 30 mL was added and swirled to dissolve it. Saturated KI solution of 0.5 mL was added from Mohr pipe and was left standing with occasional shaking for 1 min. Water of 30 mL was added and the resultant solution was titrated slowly with 0.1 M Na\(_2\)S\(_2\)O\(_3\) with vigorous shaking until the yellow color was almost gone. Starch solution (1\%) of 0.5 mL was added and titration was continued with shaking vigorously to release all I\(_2\) from the CHCl\(_3\) layer until blue color disappeared. The determination was repeated with 0.01 M Na\(_2\)S\(_2\)O\(_3\). Determination of PV in blanks were done and results were subtracted from the values for experimental samples. Peroxide value (mill equivalent peroxide/kg oil) was given by:

\[
PV\left(\frac{\text{meq}}{\text{kg}}\right) = \frac{S \times M \times 1000}{m_s}
\]

where S = volume (mL) of Na\(_2\)S\(_2\)O\(_3\) (blank corrected) and M = molarity of Na\(_2\)S\(_2\)O\(_3\) solution and m\(_s\) = mass of fish samples.

**Sample preparation for chemical analysis**

The fried fish samples were prepared according to the method stated by Weber et al. (2008)\(^18\). After removing the central backbone, the fried fish samples were oven-dried at a temperature of 60°C for 72 h, then transferred into desiccators and cooled for 30 min. The fried and dried fish samples were then milled to a 0.3 mm size. The dried samples were homogenized by mixing them thoroughly. The resultant homogenate samples were packaged into several small, polyethylene bags and stored at 0°C until required for further analysis.

**Nutritional quality of fried fish**

Vitamin A content: Vitamin A was determined using a high-performance liquid chromatography (HPLC) using a method described by Gesto et al. (2012)\(^19\) with minor modifications. Hydroquinone (100 g), 95% ethanol and 25 mL KOH solution were added into a 250 mL flask containing 0.1 mg of homogenized fish samples. The flask was placed in a boiling water bath set at a temperature of 53 ± 2°C with regular shaking. The flask was removed from the water bath and cooled to room temperature. Meanwhile, 50 mL of hexane was added and contents was shaken. Similarly, the second extraction was conducted. The content of total vitamin A in the sample being analyzed was calculated from the peak height (or areas) of retinol in the injected volume (20 μL) of the sample solution against the standard.

\[
\text{Retinol} \left(\frac{\text{mg}}{\text{kg}}\right) = \frac{\text{Instrument reading} \times \text{extract volume} \times \text{dilution factor}}{\text{Weight of sample(Kg)}}
\]
Fatty acid profiles: Fatty acid profile was determined by gas chromatography-mass spectrophotometer (GC-MS). Lipid was extracted following the standard procedure developed by Christiansen [20] and used recently by Petrović.[21] A homogenized fried fish sample of 5 g was weighed into a conical flask and dried for 1 h at 105°C. The flask was cooled to room temperature and 50 mL of 4 M hydrochloric acid was added to it. The solution was boiled for 1 hr. Water (150 mL) was added, and then the solution was filtered by fluted filter paper and washed until neutral reaction on litmus paper was observed. The filter paper was dried for 1 hr at 105°C and inserted into the extraction thimble of the Soxhlet apparatus. Fat extraction was continued with hexane using Soxhlet for 6 h. Then, hexane was removed using a rotary evaporator after extraction and it was kept for derivatization. Oil samples of 200 mg was weighed into the bottom of a screw-capped Teflon-lined tube and then 2 mL of 2 N KOH (in methanol) was added. The tube was closed tightly and heated on shaking water bath at 80°C for 1 hr. The tube was then cooled and 5 mL of 5% HCl was added in methanol. The tube was closed tightly again and heated on shaking water bath at 80°C for 1 hr. After cooling, 5 mL of hexane and 5 mL of water were added to the tube and mixed, and then hexane was collected from the layer after short centrifugation. The solvent layer was washed by dilute potassium bicarbonate to remove excess acid and dried over anhydrous sodium sulfate. Potassium bicarbonate was recovered after removal of the solvent by evaporation under reduced pressure on a rotary film evaporator. The extract was then injected into a GC vial, where the fatty acid methyl esters (FAME) of the fish fats were analyzed by capillary gas chromatography (7890B GC). Helium was used as the carrier gas at a pressure of 14 psi and at column flow of 1 mL/min. The GC conditions during analysis included intel temperature of 250°C, injection volume of 1 μL, split ratio of 35, with a detector of 5977B MS and a column specification of DB-5 MS, 30 m × 0.25 mm × 0.25 μm. Relative quantities were expressed as a weight percent of total fatty acids identified via comparison of retention times to known FAME standards.[21]

Data analyses

Duplicate data of oxidative stability, fatty acid profiles and vitamin A contents of fish samples as influenced by frying oil samples of different reuse cycles were analyzed using JMP pro13 (SAS product) The response surface effects of the frying oil oxidative stability on the oxidative stability and nutritional quality of the fried fish muscles, was looked into. Response surface profilers were reported and a pairwise correlation analysis among parameters of relevant associations were used in augmenting the discussion.

RESULTS

Principal components analysis

A principal component analysis (PCA) of the frying oil quality parameters (PV and FFA) along the repeated use of the oil was analyzed and the analysis output is presented in Figure 1. The PCA analysis was based on correlation (Figure 1 (A)) and covariance (Figure 1 (B)) associations. The result shows that 93.98% of the overall variation in the fried fish quality parameters were explained by the two components on correlation basis, where component 1 alone contributed 89.3% (Figure 1(A)). It was also revealed that 98.23% of variations in the fish quality parameters were explained by the two components on the covariance basis, where component 1 alone contributed 97.8% of the association. PV, FFA and fatty acid groups knew to be associated with increased risks of coronary heart disease,[22] saturated and trans, were aligned with the oxidative stability indicators of frying oil with both the correlation and covariance PCA. The health-promoting essential fatty acids[23]; cis, omega-3 and 6 fatty acids as well vitamin A were seen to be influenced less by the frying oil quality in both PCA types (Figure 1).
A separate pairwise Pearson’s correlation analysis also corresponded to the PCA results in the present study (data not shown) along the increasing oil reuse cycles. A significant (p < .05), strong and negative correlations were reported between the saturated fats and essential nutrients (r = −0.978, −0.879 and −0.859 for omega 3, omega 6, cis fats and vit. A, respectively). Similarly, a Pearson’s correlation coefficients (r) of −0.888, −0.709, −0.894, and −0.699 were found between the trans fats against omega 3, omega 6, cis fats and vit. A, respectively. There were also significant negative correlations between the indicators of oxidative stability indicators of both the frying oil and fried fish samples and the essential nutrients of the fish. Significant positive correlations were reported between possible pairs of the individual essential nutrients (omega and cis fats as well as vit. A). Correspondingly, significant, strong and positive correlation coefficients were obtained for the possible pairs of the FFA and PV of both the frying oil and fried fish muscles, as well as the saturated and trans fats.

**Characteristics of the response surface model**

The response surface effect of the oxidative quality of frying oil (peroxide values, PV and free fatty acids, FFA) along the increasing number of reuse cycles, on the oxidative stability, fatty acid profile and vitamin A contents of fried Nile tilapia fish was significant (p < .05, Table 1). The augmented response surface model was also shown to be adequate in explaining the dependence of oxidative stability and fatty acid profiles of fried Nile tilapia fish, which was evidenced by the values of coefficient of determination (R² and R²(adj.)) ranging from 0.789 to 0.999 (Table 1).

| Responses        | Model significance (p-value) | Model adequacy |
|------------------|------------------------------|----------------|
| Fish PV          | <0.0001                      | 0.992          | 0.982 |
| Fish FFA         | <0.0001                      | 0.999          | 0.999 |
| Saturated fats   | <0.0001                      | 0.999          | 0.999 |
| Trans fats       | <0.0002                      | 0.990          | 0.967 |
| Cis fats         | <0.0001                      | 0.992          | 0.981 |
| Omega 3 fats     | <0.0003                      | 0.983          | 0.963 |
| Omega 6 fats     | 0.0117                       | 0.923          | 0.830 |
| Vitamin A        | 0.0195                       | 0.904          | 0.789 |

Figure 1. Principal components on correlation [a] and covariance [b] basis; FA = fatty acids, FFA = free fatty acids, PV = peroxide values.
**Response surface analysis**

Frying oil reuse cycle and accumulating PV versus quality of fried fish: The number of reuse cycles (1,6) of frying oil with the increasing accumulation of PV (0.2, 1.2) resulted in steadily decreasing levels of cis- (Figure 2 [A]), omega 3 [B], omega 6 fats [C], and vitamin A levels [D]. The observed decrease in the essential nutrients of fried fish with increasing oil reuse cycles, is caused by thermal destruction of mono and polyunsaturated fatty acids due to the presence of double bonds in their structure. During frying of foods, the frying oil undergoes three deleterious reactions: hydrolysis caused by water, oxidation, and thermal alteration caused by oxygen and heat.[24] The deterioration of the frying oil along the higher reuse cycles were also evidenced with increasing levels of the PV, FFA and risky fat components (saturated and trans) in the fried fish muscles (Figure 3 [A-D]). The PV and FFA levels were seen increasing (0 to 3.94 meq/kg and 0.595 to 4.88% of total fat, respectively, Figure 3 [A & B]) with the repeated use of the frying oils (1,6). Similarly, the saturated and trans-fats (Figure 3 [C&D]), were increasing steadily with the frying oil reuse cycles and the accumulating PV, which also exhibited high association in the PCA (Figure 1). The current work, therefore, explains for the first time, the relationships between the frying oil use cycle and its oxidation status with the food being fried in it (Nile Tilapia), using a response surface model of fairly good adequacy.

It is also interesting to note that the essential nutrients had dependances on the frying use cycles and PV of the frying oil that was explained by a curvature indicated by the contours (Figure 2). The indicators of fish oxidative stability (FFA, PV) and the risky fats (saturated and trans) were also observed depending on the oil quality with some high-order model indicated by the curving contour (Figure 3). This is likely due to the synergistic effects of the deteriorations in the frying oil quality on the fish nutritional quality and safety.

![Figure 2](image-url) - Influences of frying oil reuse cycle and accumulating peroxide values on essential nutrients of fried fish; FA = fatty acids; PV = peroxide values.
Fried fish quality as influenced by frying oil reuse cycles and accumulating FFA: The nutritional quality of fried fish was influenced by the number of reuse cycles (0,6) of the frying oil and the resulting accumulation of FFA (0.048, 0.126) (Figure 4). Essential nutrients: cis, omega 3 and 6, as well as vitamin A were decreasing with increasing frying oil use cycles and FFA levels (Figure 4 [A-D]). The decrease in the essential nutrients (cis and omega fats as well as vit. A), paralleled the deteriorating status of the oxidative stability of the fish and increasing levels of its risky fats (saturated and trans-fats) as detailed in Figure 5. This was also indicated by the PCA results (Figure 1), with the FFA, PV and the risky fats (saturated and trans) aligned with the frying oil reuse cycles and resulting FFA and PV in the oil.

A more pronounced curvature in the response surface plots of the essential fats and vitamin A levels as influenced by the accumulating levels of FFA over the increased reuse of the frying oils (Figure 4) are shown, indicating greater influence of the FFA on the nutrients. The observed trends and associations between FFA and essential nutrients might be due to the reactive natures of the former causing chemical changes and damages in the later as reported by. Similar trends of curving were observed in the prediction profiler plots (data not shown) many of these parameters.

Discussion

Deep fat frying is a widely used method of preparing ready to eat foods with likeable aroma and texture both at traditional and industrial levels. Deep frying of foods like fish is known to be a risk factor for increased obesity, coronary heart disease (CHD) and many forms of cancer due to excess caloric intake from the fat and also due to increasing levels of risky fat components (FFA, PV, saturated and trans fats) in the expense of polyunsaturated and cis essential fats as indicated in the present research. The risk factors from deep-fried fish and other foods are exacerbated by the practice of reusing the frying oil, which keeps on generating more of the FFA, PV, and saturated as well as trans fatty acids. It is therefore, a good idea to discourage deep fat frying and shift to other healthier food preparation
Figure 4. Fried fish safety and quality as influenced by frying oil reuse cycles and accumulating FFA; FA = fatty acids; FFA = free fatty acids.

Figure 5. Fried fish safety and quality as influenced by frying oil reuse cycles and accumulating FFA; FA = fatty acids; FFA = free fatty acids; PV = peroxide values.
techniques, such as air frying, steaming, or microwaving, which have been reported to be safer.\textsuperscript{[26–28]}

Fish cooking methods other than the deep fat frying are safer as they are not associated with reuse of frying oils.

The absence of clear standards and regulations on specific deep-fried foods, such as fish at global, regional, and national levels, presents momentous challenge to the health of the general public. The FFA and PV levels reported in the present research for fried fish are within the acceptable ranges of the Codex standards for named vegetable oils.\textsuperscript{[29]} However, this does not guarantee safety when the practices of the oil reuse in certain cultures is almost indefinite number of times by adding fresh oil to the darkened and viscous residues. This was the observation of the authors in the study area, Hawassa lake and of course many other traditional fishery spots in Ethiopia. This might also be a common practice in several food cultures in Africa, Asia, and other developing world, where fish consumption is important. The risk factor associated with fried fish is concerning for the fact that aquaculture and fish consumption is being promoted to large proportions of the populations in developing countries as a sustainable and cost-effective dietary intervention against the widely prevalent protein-energy-malnutrition and food insecurity.\textsuperscript{[30,31,32]} The result of the present study and the trending practices of expanding aquaculture as a source of good quality animal proteins, implies the necessity of global regulatory measures on the deep fat frying technologies and fried food qualities. The current study shows that the lower the frying reuse cycle, the better the food quality and safety can be and the residual oil after frying might be used as a cooking oil for other foods rather than discarding which would otherwise bring an environmental pollution concern.

Other cooking methods than deep fat frying were reported to result in foods of better oxidative stability and lipid loads,\textsuperscript{[27–29,33–35]} Non-frying cooking methods are healthier for consumers and need to be promoted. Regulatory frameworks should be worked out for deep fat fried foods, particularly those commercially served as street foods in developing countries.

Researches in recent years have also reported the potentials of spices and herbs in different forms in improving frying oil qualities due to their rich natural antioxidant contents. Şahin et al. (2017)\textsuperscript{[36]} and Redondo-Cuevas et al. (2017) reported that the use of various spices increased the oxidative stability of different vegetable oils by 18 to 128.91%, which implies that the use of spices together with selection of appropriate types of oils may help in stabilizing the fried food quality. The application of different spice in the culinary cultures of developing world might fit into this strategy and help in reducing the risk factors associated with fried foods like fish. A need for paradigm shift in regulating frying oil quality and increasing its stability, through the use of antioxidants and developing technologies that creates partial vacuum during frying to avoid oxidation reactions to the fats was suggested by.\textsuperscript{[28]} It was also suggested that researches looking into genetic development of vegetables with more stable oils are developed and used in the integrated approach to controlling frying oil quality and safeguarding the consumers.

Other advanced solutions may be explored from the emerging encapsulation technologies. For instance, the use of rosemary extracts microencapsulated with fish oil was studied by Yeşilsu and Özyurt (2019)\textsuperscript{[37]} where the microencapsulation of the natural antioxidant from the herb improved the stability of fish oil. It is important to investigate the microencapsulation techniques for frying oils as well. Oxidative stability of electro sprayed glucose syrup capsules were also reported to increase the stability of oils,\textsuperscript{[38]} which could also be explored for frying oils in the future, at least as part of the integrated approaches to be devised in improving oxidative stability of frying oils.

**Conclusion**

The response surface effect of the frying oil quality on the oxidative stability and essential nutrients of fried fish was statistically significant, with fairly good model fitness adequacy. The model indicated the deteriorating quality of fish (oxidative status and nutritive relevance), which were also supported by the principal component analysis. Saturated and trans fats of the fried fish muscles were steadily increasing in the expense of the essential nutrients (omega and cis fats, as well as vit. A). The unsaturated fats are degrading and converting to saturated and trans fatty acids by the high and
repeated application of frying heat. The lipids have also been further degrading into FFA and PV both in the frying oil and the fried fish, especially at the higher cycles of frying oil uses.

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