The Effects of Deep-Frying, Refrigerated Storage and Reheating on the Fat Content, Oxidation and Fatty Acid Composition of the Fish Rutilus frisii kutum

Mehdi Nikoo1, Mohamad Reza Ghomi1, Eshagh Zakipour Rahimabadi1, Sooottwat Benjakul4 and Behzad Javadian1

1Food Laboratory, Mazandaran University of Medical Sciences, Sari, Iran
2Department of Fisheries, Islamic Azad University- Tonekabon Branch, Tonekabon, 46817, Iran
3Department of Fisheries, University of Zabol, Zabol, Iran
4Department of Food Technology, Faculty of Agro-industry, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Abstract

Fatty acid distribution in the different parts of kutum, Rutilus frisii kutum and the effect of frying and refrigerated storage followed by reheating on lipid oxidation and fatty acid composition in different parts of body flesh were evaluated. Lipid content increased after frying and reheating. In general, saturated fatty acids were higher in anterior parts, while the contents of polyunsaturated fatty acids increased from the anterior to the posterior parts. Frying decreased the contents of unsaturated fatty acids but increased the contents of saturated fatty acids. After refrigerated storage, followed by microwave reheating, the content of polyunsaturated fatty acids decreased, whereas the content of saturated fatty acids increased. The peroxo value increased after frying, compared with that of raw sample. However, the lower PV was obtained in fried-chilled-reheated samples. Frying increased the TBA values and reheating enhanced this increment. Therefore, the frying as well as the refrigerated storage and reheating had the impact on fatty acid composition as well as oxidative stability of kutum fillets.

Keywords: Lipid oxidation; Fatty acids; Frying; Reheating; Rutilus frisii kutum

Introduction

The potential health benefits of n-3 polyunsaturated fatty acids especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids have been demonstrated [1,2]. N-3 fatty acids cannot be synthesized in human body and must be supplied through diet [3]. It is well known that aquatic ecosystems are the main source of polyunsaturated fatty acids and human obtain principal parts of EPA and DHA through consuming fish and aquatic products [1]. Nutritional values of fish regarding their fatty acid profiles have been studied in many commercially important freshwater or marine bony fish species [4,5,6]. Nevertheless, fish are usually eaten as cooked and fatty acid composition of raw fish may not reflect the nutritive value of cooked counterpart [7]. Recently, the improvement of palatability and less preparation time of foods have drastically increased the consumption of fried foods associated with the increased dietary fat intake [8]. However, frying produce some modifications in foods such as changes in the fatty acid composition and lipid oxidation [9]. Although the effects of frying on fatty acid composition of fish have been studied, the information regarding the impact of common practice starting from frying, chilled storage, followed by reheating in the large catering operations, restaurants and houses on lipid oxidation and fatty acid composition is limited [10]. Different parts of fish may have different beneficial health effects as determined by varying contents of n-3 fatty acids [11]. Therefore, the aims of the present study was to assess: 1) the effect of frying, chilled storage followed by microwave reheating on the lipid content, fatty acid composition and lipid oxidation of kutum fillets, and 2) to determine the fatty acid composition in different parts of fried and reheated fillets.

Materials and Methods

Sample preparation

Samples of kutum, Cyprinidae (n=10, weight: 1000-1200 g) were purchased from a local market (Ghaemshahr, Iran) and transported to the laboratory in ice containing boxes with fish to ice ratio of 1:2 (w/w). Upon arrival, the fish were first beheaded, eviscerated and washed with tap water to remove adhering blood and slime. They were then cut into slices of 1 cm thickness. Slices of three different parts of body (head, middle and tail parts) were divided into three homogenous groups (group 1, 2 and 3). Group 1 was used as the control (fresh-raw samples). The other 2 groups were subjected to frying. One group was analyzed immediately after frying and the other group was stored at 4°C for two days [10] and then reheated by microwave oven before analysis.

Frying and reheating

The fish fillets were deep-fried for 5 min at 160°C in soybean oil containing 120 ppm TBHQ (Kesh V Sanat Shomal Co., Iran) in a deep fryer (HDF-510, Hamilton, Iran). Fatty acid compositions (g/100g of total fatty acids) of the oil used were: C16:0 (22.7), C16:1 (15.7), C17:0 (1.88), C17:1 (1.27), C18:0 (20.38), C18:1 (4.58), C18:2 (6.03), C18:3 (2.72), C18:4 (3.24), C20:0 (0.97), C20:1 (1.10), C20:2 (2.21), C20:3 (5.10), C22:0 (0.74), C22:1 (2.18), C20:5 (5.15), C22:6 (4.05). Fillet to oil ratio was approximately 250 g/L (w/v). The internal pan of the fryer was washed, cleaned and dried after each batch of frying. After frying, the fillets were drained for about 2 minutes at room temperature.

Reheating of fried samples stored at 4°C for 2 days was performed using a microwave oven (MCO 3515, Major, Iran) set at a frequency

© 2010 Nikoo M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
of 2450 MHZ for 4 min. The internal temperature of all samples was between 75 and 80°C.

Lipid extraction and fatty acid analysis

Lipid was extracted according to Kinsella et al. [12] Fifty g of fillets were homogenized in a waring blender (32BL79, New Hartford, Connecticut, USA) for 2 min with a mixture of 50 ml chloroform and 100 ml methanol. Then 50 ml of chloroform were added and further homogenized. Finally 50 ml of distilled water were added to the mixture and blended for 30 sec. The homogenate was filtered through a whatman No. 4 filter paper into a decanter. The lower fraction was then collected and filtered. It was then transferred to a rotary evaporator (Rotavapor R-114, BÜCHI, Switzerland) for solvent evaporation. Lipid content was expressed as gram per 100 g wet muscle.

Fatty acid methyl ester (FAME) was prepared following the method of [13]. Lipid samples (0.2 g) were weighed and diluted with 4 ml of hexane followed by the addition of 0.2 ml of sodium methoxide in a sealed tube. The mixture was then shaken using a vortex for 1 s and left for about 30 min until it separated into two phases. The top layer, FAME, was then taken for analysis by using Trace GC (Thermo Finnigan, Italy). The GC conditions were as follow: capillary column (Bpx-70 60 m x 0.25 mm i.d.x0.25μm), the split ratio was 80:1. Injection port temperature was 250°C; flame ionization detector temperature was 270°C. Oven temperature was set at 194°C for 90 minutes. Flow rate of carrier gas (helium) was 1 ml min-1 and the makeup gas was N2 (30 ml/min). The sample size injected for each analysis was 1 μL.

Determination of peroxide and thiobarbituric acid values

Peroxide value (PV) was measured according to PORM (1995). About 0.3 g of fat was put into a 250 ml flask with stopper. Sample was dissolved in 10 ml chloroform-acetic acid mixture by shaking. Then 1ml of saturated KI solution was added and immediately stoppered and stand in the dark for 5 min. After that, 20 ml distilled water was added and shook. Sample was titrated with 0.01 N, Na2S2O3 solution until yellow color almost disappears. Thereafter, 1ml of 1.5% starch solution was added and titration continued until dark blue color was disappeared. A blank test was carried out, without oil. Peroxide value (meq per 1000g) determined by the formula: 1000 (V1-V2) / W; Where V1 is the volume (in ml) of the sodium thiosulphate solution of normality N used for determination, V2 is the volume of the sodium thiosulphate solution used for the blank test, W is the weight (in g) of the test portion, N is the normality of the sodium thiosulphate solution.

TBA value was determined according to the method of Kim [14]. Lipid samples (0.2-0.4 g) were placed in a test tube with stopper. Three drops of antioxidant, butylated hydroxyl toluene solution was then added to the sample. This was followed by the addition of 1 ml of TBA solution and 17 ml of TCA solution. Nitrogen gas was then flushed into the test tube and immediately stoppered. Samples were heated at 100°C in a boiling water-bath for 30 min. Thereafter, samples were cooled in tap water to room temperature. Then, 5 ml of chloroform was added to sample and mixed for a few seconds with a vortex mixer. Fifteen ml of the color solution was transferred to a centrifuge tube and centrifuged for 10 min at 3000 rpm (BH-1200, Behdad, Tehran, Iran). The absorbance of supernatant was read at 532 nm. A blank was also carried out in the same manner except the lipid was omitted.

Statistical analysis

The experiment was run in triplicate with the different lots of sample. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan multiple range test. All data are expressed as mean ± S.D. The significance of results was at 5%. The software used was Minitab, release 14 (Unscrambler, Version 6.11, CAMO A/S, Norway). Principle components analysis (PCA) was used to visualize and reduce the data.

Results and Discussion

The lipid content in different parts of body is shown in Table 1. Result showed no difference in the lipid content in different parts of body. The average lipid content was higher than the value (1.52%) reported for kutum caught from a dam lake in Turkey [6]. This higher content of lipid might be due to the presence of skin on fillets in this study [11]. Moreover some biological and environmental factors such as age, sex, maturity, seasons, location, temperature and especially diet can affect the lipid content of fish flesh [15]. Based on the classification by Suriah et al. [16], kutum may be classified as lean fish with lipid content below 5%. After frying, fat content of fillet samples significantly increased. This result is in agreement with those of Garcia-Arias et al. [17] and Weber et al. [18] who reported that sardine fillet and silver catfish fillet had the increase in lipid content after frying. Fat increase can be due to oil penetration into the food after water is partially lost by evaporation [19].

Table 1: Changes in lipid (%), peroxide (PV, Meq O2 / kg oil) and thiobarbituric acid (TBA) value (mg malonaldehyde/kg) of raw, fried and fried-refrigerated stored-reheated fillets of kutum. Data are expressed as means±S.D. Rows that have no common letters are significantly different (P<0.05).

| Table 2: Distribution of fatty acids (g/100 g of total fatty acids) of raw, fried and fried-refrigerated stored-reheated fillets of kutum. | Data are expressed as means±S.D. Rows that have no common letters are significantly different (P<0.05). |
The initial peroxide (PV) value of the tail, middle and head parts of body was 2.56, 2.39 and 2.83 Meq O₂ / kg oil, respectively (Table 1). PV in all parts tested increased after frying (P<0.05). PV of chilled-reheated middle and tail parts increased to some extent, in comparison with those of fried samples (P<0.05). On the other hand, the increase in PV was found in the head portion after chilled storage with the subsequent reheating (P<0.05). For TBA value, chilled-reheated samples had the higher TBA values, compared with fried sample and raw samples, respectively. The increase in PV and TBA value suggested that progressive lipid oxidation in the fried sample. Those changes were more pronounced in the chilled-reheated sample. The prooxidants released during frying might accelerate the oxidation during 2 days of storage. The reheating also resulted in the enhancement of oxidation in the fish fillet. It has reported that unstable primary oxidation products, hydroperoxides, are decomposed rapidly into secondary oxidation products such as aldehydes and ketones [20]. Increase in PV and TBA values of fried and chill-reheated samples also reported by [10] which indicated that lipid oxidation took place during frying and reheating process. As reported by Al-Saghir et al. [21], in addition of heat treatment, the kind of cooking oil also can alter the peroxide value. Frying and chilled storage followed by reheating also increased the TBA value, indicating that the secondary products of oxidation increased during the procedure. Similar results were found by Baker et al. [10]. Although the lipid oxidation occurred during frying or subsequent storage and reheating, PV and TBA value were lower than acceptability level for human consumption reported by Huss [22]. The level of 8 meq O₂/kg of oil is the limit of acceptability of PV and TBA. Also, the C22:6/C16:0 ratio decreased in all fried and reheated samples of kutum, suggesting that thermal oxidation was induced during frying, chilling and reheating procedure.

Fatty acid profiles of raw, fried and fried-chilled-reheated sample are shown in Tables 2 and 3. In raw samples, the abundant fatty acids in the descending order were C16:0>C18:0>C16:1>C20:3>C18:1. Saturated fatty acids represented the most dominant class of fatty acids in the descending order were C16:0>C18:0>C16:1>C20:3>C18:1. The n-6/n-3 ratio in raw fish was 0.24, 0.27 and 0.27 in head, middle and tail respectively. Similar result was reported by Özogul et al. [6] and Pirestani et al. [26] for kutum. Among the freshwater fish studied by Özogul et al. [6], kutum showed the lowest n-6/n-3 ratio (0.21). The content of n-6 and n-3 fatty acids also decreased. In general, the changes in PV and TBA value were higher in anterior compared to posterior samples. The increase in PV and TBA value suggested that progressive lipid oxidation in the fried sample. Those changes were more pronounced in the chilled-reheated sample. The prooxidants released during frying might accelerate the oxidation during 2 days of storage. The reheating also resulted in the enhancement of oxidation in the fish fillet. It has reported that unstable primary oxidation products, hydroperoxides, are decomposed rapidly into secondary oxidation products such as aldehydes and ketones [20]. Increase in PV and TBA values of fried and chill-reheated samples also reported by [10] which indicated that lipid oxidation took place during frying and reheating process. As reported by Al-Saghir et al. [21], in addition of heat treatment, the kind of cooking oil also can alter the peroxide value. Frying and chilled storage followed by reheating also increased the TBA value, indicating that the secondary products of oxidation increased during the procedure. Similar results were found by Baker et al. [10]. Although the lipid oxidation occurred during frying or subsequent storage and reheating, PV and TBA value were lower than acceptability level for human consumption reported by Huss [22]. The level of 8 meq O₂/kg of oil is the limit of acceptability of PV and TBA. Also, the C22:6/C16:0 ratio decreased in all fried and reheated samples of kutum, suggesting that thermal oxidation was induced during frying, chilling and reheating procedure.

Fatty acid profiles of raw, fried and fried-chilled-reheated sample are shown in Tables 2 and 3. In raw samples, the abundant fatty acids in the descending order were C16:0>C18:0>C16:1>C20:3>C18:1. Saturated fatty acids represented the most dominant class of fatty acids followed by monounsaturated and polyunsaturated fatty acids and polyunsaturated fatty acids increased from anterior to the posterior samples of body (Table 2). It has reported that fatty acid profiles of fish in frying processes became similar to those of the culinary fat used [24,25] have reported that oil in frying media mainly determines the fatty acid composition of small and lean fish. In this study, polyunsaturated fatty acid content of kutum was not affected by soybean oil used for deep-fat frying. The changes in long chain fatty acids can also be related to thermal oxidation [17]. Saturated fatty acids (C17:0, C20:0 and C22:0) increased during chilled storage or reheating process, while there were no changes in C16:0 and C18:0 (Table 3). Moreover their contents increased from anterior to posterior body parts. Monounsaturated fatty acids of fried sample were not changed after chilling and reheating and no differences were found among three parts. Polyunsaturated fatty acids decreased in the middle and tail parts in chilled and reheated samples. Being similar to fried slices, their contents were higher in anterior compared to posterior body parts.

The n-6/n-3 ratio in raw fish was 0.24, 0.27 and 0.27 in head, middle and tail respectively. Similar result was reported by Özogul et al. [6] and Pirestani et al. [26] for kutum. Among the freshwater fish studied by Özogul et al. [6], kutum showed the lowest n-6/n-3 ratio (0.21). The content of n-6 and n-3 fatty acids also decreased. In general, the changes in PV and TBA value were higher in anterior compared to posterior samples.

### Table 3: Fatty acid composition (g/100 g of total fatty acids) of raw, fried and fried-refrigerated stored-reheated fillets of kutum.

|       | head | middle | tail  |
|-------|------|--------|------|
| C16:0 | 3.14±0.03a | 3.31±0.02b | 3.91±0.02b |
| C18:0 | 3.18±0.02b | 3.36±0.02b | 3.83±0.02b |
| C16:1 | 2.93±0.02b | 2.96±0.02b | 3.31±0.02b |
| C18:1 | 3.47±0.02b | 3.50±0.02b | 4.03±0.02b |
| C20:1 | 3.56±0.02b | 3.60±0.02b | 4.14±0.02b |
| C20:2 | 3.66±0.02b | 3.70±0.02b | 4.24±0.02b |
| C20:3 | 3.76±0.02b | 3.80±0.02b | 4.32±0.02b |
| C22:1 | 3.86±0.02b | 3.90±0.02b | 4.42±0.02b |
| C22:6 | 3.96±0.02b | 4.00±0.02b | 4.52±0.02b |

Values are means and S.D. of triplicate. Means with the same small letter within a row were not significantly different at P<0.05 level for head, middle and tail in different treatments.
were slightly higher in n-3 fatty acids. The n-6/n-3 ratios of fried head, middle and tail samples were 1.04, 0.96 and 1.13-fold higher than those of raw samples, respectively. Similar results were reported by Gladyshev et al. [27] in frying of trout and by Larson et al. [28] and Weber et al. [18] in frying of salmon and silver catfish respectively.

Principle component analysis (PCA) was also carried out for all treatments (Figure 1). PC1 (90.1%) and PC1 (6.9%) together explained 97.0% for the effect of different parts of fish body in raw, fried and chill-reheated treatments. As shown in Figure 1, the first component separated all fried-chilled-reheated samples from all raw samples. The fried-chill-reheated samples were in positive side of PC1. Fried-chill-reheated samples were in positive side of PC1. Fried-chill-reheated samples were separated all fried-chilled-reheated samples from all raw samples. The fried-chill-reheated samples were in positive side of PC1. Fried-chill-reheated samples were separated all fried-chilled-reheated samples from all raw samples. The fried-chill-reheated samples were in positive side of PC1. Fried-chill-reheated samples were characterized by the higher contents of C18:0, C16:0, C17:0 and C20:0. The higher contents of C17:0 and C20:0 were found in fried-fried-chill-reheated samples were in positive side of PC1. Fried-chill-reheated samples were separated all fried-chilled-reheated samples from all raw samples. The fried-chill-reheated samples were in positive side of PC1. Fried-chill-reheated samples were characterized by the higher contents of C18:0, C16:0, C17:0 and C20:0. The higher contents of C17:0 and C20:0 were found in fried-chill-reheated samples than from fried samples (Table 3).

In conclusion raw, fried and fried-refrigerated stored-refrigerated slices fillets of kutum.

References

1. Arts MT, Ackman RG, Holub BJ (2001) Essential fatty acids in aquatic ecosystem: a crucial link between diet and human health and evolution. Canadian Journal of Fisheries and Aquatic Science 58: 122-137.

2. Conner WE (1997) The beneficial effects of omega-3 fatty acids: Cardiovascular disease and neurodevelopment. Curr Opin Lipidol 8: 1-3.

3. Bang HO, Dyerberg J, Hirjone N (1976) The composition of food consumed by Greenland Eskimos. Acta Medica Scandinavica 200: 69-73.

4. Ho BT, Paul DR (2009) Fatty acid profile of Tra catfish (Pangasius hypophthalmus) compared to Atlantic salmon (Salmo salar) and Asian seabass (Lates calcarifer). International Food Research Journal 16: 501-506.

5. Huyh MD, Kitts DD (2009) Evaluating nutritional quality of pacific fish species from fatty acid signatures. Food Chemistry 114: 912-918.

6. Özogul Y, Özogul F, Alagonz S (2007) Fatty acid profiles and fat contents of commercially important seawater and freshwater fish species of Turkey: A comparative study. Food Chemistry 103: 217-223.

7. Candela, M, Astiasaran I, Bello J (1996) Deep fat frying modifies high-fat fish lipid fraction. Agricultural and Food Chemistry 46: 2793-2796.

8. Saguy IS, Pinthus EJ (1995) Oil uptake during deep-fat frying: Factors and mechanism. Food Technology 49: 142-145.

9. Sánchez-Muniz FJ, Viejo JM, Medina R (1992) Deep frying of sardines in different culinary fats. Changes in the fatty acid composition of sardines and frying fats. Journal of Agricultural and Food Chemistry 40: 2252-2256.

10. Bakar J, Zakipour Rahimabadi E, Cheman YB (2008) Lipid characteristics in cooked-chill-reheated fillets of Indo-Pacific King Mackerel (Scomberomorus guatatus). Food Science and Technology 41: 2144-2150.

11. Katlikou P, Hughes SI, Robb DHF (2001) Lipid distribution within Atlantic salmon (Salmo salar) fillets. Aquaculture 202: 89-99.

12. Kinsele JA, Shimp JL, Mai J, Weihrach J (1977) Fatty acid content and composition of freshwater fish. J Am Oil Chem Soc 54: 424-429.

13. Timms RE (1978) Artefact peaks in the preparation and gas liquid chromatographic determination of methyl esters. Australian Journal of Dairy Technology 33: 4-6.

14. Kim LL (1992) Extraction of lipids. C-2 In Laboratory manual of analytical methods and procedures for fish and fish products, (K. Miwa and L. Suji, eds.), Singapore: Marine Fisheries research Department, Southeast Asian Fisheries Development Center.

15. Cellick M, Diler A, Kieukkulinez A (2005) A comparison of the proximate composition and fatty acid profiles of zander (Sander lucioperca) from two different regions and climatic conditions. Food Chemistry 92: 637-641.

16. Suriah AB, Huah TS, Hassan Q, Duad NM (1995) Fatty acid composition of some Malaysian freshwater fish. Food Chemistry 54: 45-49.

17. García-Arias MT, Pontes EN, Garcia-Linares MC, Garcia-Fernandez MC, Sanchez-Muniz FJ (2003) Cooking-frying-reheating (CFR) of Sardine (Sardinia pilchardus) fillets. Effects of different cooking and reheating procedures on the proximate and fatty acid composition. Food Chemistry 83: 349-356.

18. Weber J, Bochi VC, Ribeiro CP, Victorio AM, Emanuellie T (2008) Effects of different cooking methods on the oxidation, proximate and fatty acid composition of Silver catfish (Rhamdia quelen) fillets. Food Chemistry 106: 140-146.

19. Saguy IS, Dana D (2003) Integrated approach to deep fat frying: engineering, nutrition, health and consumer aspects. Journal of Food Engineering 56: 143-152.

20. Cho E, Min DB (2007) Chemistry of deep-fat frying oils. Journal of Food Science 72: R77-R86.

21. Al-Saghir S, Thurner K, Wagner KH, Frisch G, Luf W, et al. (2004) Effects of different cooking procedures on lipid quality and cholesterol oxidation of farmed salmon fish (Salmo salar). J Agric Food Chem 52: 5290-5296.

22. Huss HH (1988) Fresh fish quality and quality changes. FAO, Rome, Italy.

23. Gruger EH Jr (1967) Fatty acid composition. In Fish oils, their chemistry, Technology, Stability, Nutritional properties and uses, (M.E. Stansby, ed.) pp. 3-31. AVI Publishing Co., Westport CT.

24. Miranda JM, Martinez B, Pérez B, Antón Y, Vázquez BI, et al. (2010) The effects of industrial pre-frying and domestic cooking methods on the nutritional compositions and fatty acid profiles of two different fried breaded foods. Food Science and Technology 43: 1271-1276.

25. Ågren JJ, Hänninen O (1993) Effects of cooking on the fatty acids of three freshwater fish species. Food Chemistry 46: 377-382.

26. Pirestani S, Sahari MA, Barzegar M (2010) Fatty acids changes during frozen storage in several fish species from south Caspian Sea. J Agr Sci Tech 12: 321-329.

27. Gladyshev ML, Sushchic NN, Gubanenko GA, Demiriecheva SM, Kalachova GS (1993) Effects of cooking on the fatty acids of three fish species. Canadian Journal of Fisheries and Aquatic Science 50: 122-137.

28. Huss HH (1988) Fresh fish quality and quality changes. FAO, Rome, Italy.

29. POIRM (1995) Determination of Peroxide Value. Methods of test for palm oil and palm oil products. Palm Oil Research Institute of Malaysia, Selangor, Malaysia.