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Original Article

Effect of different cooking processes on the fatty acid profile of grass carp (*Ctenopharyngodon idella*) fillets during chill storage

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Abstract: The effects of different cooking methods (deep fat frying, boiling and steaming) on lipid content and fatty acid composition of grass carp (*Ctenopharyngodon idella*) fillets during chill storage were investigated. Fillet samples were cooked and then stored at + 4°C for 4 days. The control and the cooked fillet samples were analyzed for their chemical characteristics. Twelve fatty acids were identified with 39.11, 15.37 and 45.52 g/ 100 g of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), respectively. The n-3/n-6 ratios of raw, deep fried, boiled and steamed samples were 1.12, 0.10, 1.45 and 0.72, respectively. The pattern of changes in fatty acid groups was different in fried, boiled and steamed samples after 1 and 4 days of chill storage. The EPA+DHA/C:16 ratio was higher in boiled and boiled-chill stored samples than steamed and fried samples. EPA+DHA/C:16 ratio for cooked, stored for 1 day and then 4 days were as 0.051, 0.003 and 0.017 for fried, 0.492, 0.583 and 0.489 for boiled and 0.247, 0.037 and 0.149 for steamed samples, respectively. These results showed that the boiled process is better than other cooking processes on the FA pattern of grass cap.

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Introduction

Different aspects of beneficial effects of fish lipid on human health have been widely established (Arts et al., 2001; Connor, 2000) because of its long chain polyunsaturated fatty acids of n-3. Various environmental and biological factors and different processing method can effect on the content of lipid and composition of fatty acids of fish species (Sigurgisladóttir and Pálmadóttir, 1993; Love, 1997). It is well-known that changes in lipid content and fatty acid composition in frying process are higher than other cooking processes (Gladyshev et al., 2006; Gladyshev et al., 2007; Bakar et al., 2008; Larsen et al., 2010). Therefore, the pattern changes of long chain polyunsaturated omega-3 fatty acids are different based on fish species and cooking procedure (García-Arias et al., 2003; Gladyshev et al., 2007; Bakar et al., 2008; Larsen et al., 2010; Nikoo et al., 2010a).

The cooking process, chill storage and reheating is a common practice in large catering operations, restaurants, and homes (Bakar et al., 2008). The changes in lipid content and fatty acid composition have studied in cook-chill-reheat or cook-freeze-reheat by García-Arias et al. (2003), Bakar et al. (2008) and Nikoo et al. (2010a, b). Although there are many references regarding the effects of cooking on fish fatty acid composition, data related to the effect of cooking and chill storage on fatty acid composition and lipid content of grass carp (*Ctenopharyngodon idella*) is limited. Therefore, this research was aimed to study the effects of different cooking processes on lipid content and fatty acid profile of grass carp fillets during chill storage.

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Table 1. Lipid content of raw and cooked of grass carp fillets (% basis on wet weight)

samples	Raw	Cooked		
		Deep fried	Boiled	Steamed
Lipid content (%)	1.44	26.7	1.94	1.94

Materials and methods

Sample preparation: Twenty fresh grass carp with weight of about 1.5 kg were purchased from a local market (Zabol, Iran). They were transported in isothermal iceboxes to the laboratory 5 hrs after catching. Then, they were cleaned and filleted and same weight of fillets (about 100 g) were used for cooking process (deep fat frying, boiling and steaming). The cooked samples were placed in moisture-impermeable plastic bags, stored in chill room (+4°C) and analyzed on 0, 1 and 4 days. The experiences were performed with three replicates.

Cooking process: Fish fillets were fried in frying oil (Sunflower oil, Bahar, Iran) using a deep fryer (Aaura, Tefal, Iran) for 6-7 min which was preheated to 180ºC. The fillets were boiled in a small water under 85-90ºC for 10-15 min (Gladyshev et al., 2006). For steaming, samples were placed in a steamer (Panasonic, Japan) and steamed for 5–6 min. After cooking all samples were drained gently on a stainless steel grills and air cooled.

Chemical analysis: Soxhlet apparatus with diethyl ether as solvent was used to measuring the total lipid content (AOAC, 2000) and lipid extraction was performed based on Bakar et al. (2008). Lipid samples were converted to their constituent fatty acid methyl esters according to Metcalf et al. (1966). Analysis of fatty acid methyl esters was performed by a Unicam 4600 with a bpx 70 capillary column (30.0 m X 0.25 mm i.d.) and quantified by FID detector. The split ratio was 10:1. The GC condition was as follows: injection port temperature was 300ºC and FID temperature was 350ºC. Oven temperature program was set at an initial temperature of 160ºC for 6 min, then raised to 180ºC at 20ºC min^{-1} and held for 9 min and again was raised to 190ºC at 20ºC min-1 and held for 14 min (Metcalf et al., 1966). The carrier gas was helium. The column flow rate was 1.9 mL min^{-1}. In the detector, helium gas flow rate was 30 mL min^{-1}. The sample size was 1 μL.

Statistical analysis: The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. The significance of results was at 5%. All data are expressed as mean ± S.D.

Results

The total lipid content of raw grass carp fillet was 1.44% (based on wet weight). Deep frying significantly (P<0.05) increased the fillet lipid content to 26.7%. There was slight increase in lipid content of boiled and steamed samples (Table 1).

The fatty acid composition of raw samples is shown in Table 2. Twelve fatty acids were identified with 39.11, 15.37 and 45.52 g/100 g of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), respectively. In raw samples, the abundance of fatty acids (in decreasing order) were palmitic acid (C16:0, with 29.27 %), oleic acid (C18:1 n-9, with 14.65 %), linoleic acid (C18:2 n-6, with 12.44 %), docosahexaenoic acid (C22:6 n-3, with 9.20 %) and Arachidonic acid (C20:4 n-6, with 9.02 %). N-3 and n-6 fatty acids constitute about 52.06 and 47.14 % of PUFAs, respectively, exhibiting an n-3/ n-6 ratio of 1.12.

The abundance of fatty acid groups (in decreasing order) in fried, boiled and steamed samples were MUFAs > PUFAs > SFAs, PUFAs > SFAs > MUFAs similar to raw samples and MUFAs > SFAs > PUFAs, respectively (Table 3). In boiled samples, content of higher polyunsaturated fatty acids was decreased, while C18:3 (n-3) significantly increased (P<0.05). The patterns of changes in fatty acid groups was different in fried, boiled and steamed samples after 1 and 4 days of chill storage (Table 3). After 1-day storage of fried samples, the SFAs levels were increased significantly (P<0.05),

Table 2. Fatty acid composition of grass carp fillets changes after cooking and chill storage.

Fatty acid	(g/100 g fatty acids)									
	Raw fillet	Fried			Boiled			Steamed		
		Day 0	Day 1	Day 4	Day 0	Day 1	Day 4	Day 0	Day 1	Day 4
C14:0	2.920 ± 0.040 ^{a}	0.663 ± 0.035 ^{dA}	0.350 ± 0.005 ^{C}	0.539 ± 0.020 ^{B}	1.441 ± 0.009 ^{bC}	2.534 ± 0.050 ^{A}	2.219 ± 0.045 ^{B}	1.351 ± 0.020 ^{cB}	0.618 ± 0.015 ^{C}	1.746 ± 0.025 ^{A}
C16:0	29.276 ± 0.097 ^{a}	25.777 ± 0.085 ^{bC}	31.189 ± 0.100 ^{A}	27.743 ± 0.093 ^{B}	25.578 ± 0.050 ^{cC}	25.884 ± 0.080 ^{B}	29.156 ± 0.105 ^{A}	25.296 ± 0.035 ^{dC}	28.878 ± 0.073 ^{A}	27.277 ± 0.070 ^{B}
C16:1	0.000 ± 0.000 ^{d}	1.406 ± 0.003 ^{cA}	0.000 ± 0.000 ^{B}	0.000 ± 0.000 ^{B}	1.893 ± 0.005 ^{bC}	15.512 ± 0.052 ^{B}	16.443 ± 0.090 ^{A}	6.017 ± 0.035 ^{aA}	0.000 ± 0.000 ^{C}	3.717 ± 0.025 ^{B}
C18:0	6.916 ± 0.092 a	3.860 ± 0.065 ^{cA}	2.235 ± 0.030 ^{C}	2.951 ± 0.050 ^{B}	5.424 ± 0.041 ^{bA}	4.522 ± 0.022 ^{B}	3.512 ± 0.005 ^{C}	7.015 ± 0.065 ^{aA}	3.443 ± 0.020 ^{C}	4.969 ± 0.015 ^{B}
C18:1 (n-9)	14.657 ± 0.098 ^{c}	33.723 ± 0.105 ^{aA}	33.267 ± 0.150 ^{B}	32.760 ± 0.087 ^{C}	14.844 ± 0.125 ^{cC}	23.149 ± 0.085 ^{A}	21.607 ± 0.105 ^{B}	31.974 ± 0.220 ^{bB}	32.373 ± 0.070 ^{A}	31.724 ± 0.055 ^{B}
C18:2 (n-6)	12.438 ± 0.050 ^{c}	30.754 ± 0.520 ^{aB}	31.052 ± 0.061 ^{B}	32.579 ± 0.055 ^{A}	13.647 ± 0.045 ^{bA}	5.178 ± 0.090 ^{B}	4.403 ± 0.100 ^{C}	11.457 ± 0.083 ^{dC}	30.174 ± 0.155 ^{A}	16.998 ± 0.105 ^{B}
C18:3 (n-3)	5.820 ± 0.034 ^{b}	1.896 ± 0.092 ^{dB}	1.764 ± 0.065 ^{B}	2.690 ± 0.030 ^{A}	15.772 ± 0.090 ^{aA}	2.931 ± 0.011 ^{C}	3.783 ± 0.035 ^{B}	4.032 ± 0.022 ^{cB}	3.441 ± 0.045 ^{C}	6.918 ± 0.096 ^{A}
C20:4 (n-6)	9.018 ± 0.301 ^{a}	0.160 ± 0.035 ^{dA}	0.052 ± 0.010 ^{B}	0.118 ± 0.026 ^{AB}	6.693 ± 0.051 ^{bA}	2.934 ± 0.012 ^{B}	1.949 ± 0.105 ^{C}	2.865 ± 0.030 ^{cA}	0.000 ± 0.000 ^{C}	1.820 ± 0.028 ^{B}
C22:1 (n-9)	0.713 ± 0.003 ^{c}	0.447 ± 0.010 ^{dA}	0.000 ± 0.000 ^{C}	0.159 ± 0.025 ^{B}	1.030 ± 0.015 ^{bB}	0.952 ± 0.016 ^{C}	1.213 ± 0.020 ^{A}	3.738 ± 0.050 ^{aA}	0.000 ± 0.000 ^{B}	0.000 ± 0.000 ^{B}
C20:5 (n-3)	6.208 ± 0.016 ^{a}	0.800 ± 0.005 ^{dA}	0.091 ± 0.010 ^{C}	0.151 ± 0.035 ^{B}	3.953 ± 0.055 ^{bC}	4.927 ± 0.021 ^{A}	4.762 ± 0.009 ^{B}	1.387 ± 0.012 ^{cB}	0.511± 0.005 ^{C}	3.525 ± 0.035 ^{A}
C22:5 (n-3)	2.836 ± 0.032 ^{a}	0.000 ± 0.000 ^{cA}	0.000 ± 0.000 ^{A}	0.000 ± 0.000 ^{A}	1.097 ± 0.017 ^{bC}	1.312 ± 0.030 ^{B}	1.444 ± 0.040 ^{A}	0.000 ± 0.000 ^{cB}	0.000 ± 0.000 ^{B}	0.777 ± 0.010 ^{A}
C22:6 (n-3)	9.197 ± 0.029 ^{a}	0.515 ± 0.015 ^{dA}	0.000 ± 0.000 ^{C}	0.310 ± 0.005 ^{B}	8.628 ± 0.060 ^{bC}	10.165 ± 0.095 ^{A}	9.509 ± 0.026 ^{B}	4.869 ± 0.060 ^{cA}	0.563 ± 0.035 ^{B}	0.529 ± 0.019 ^{B}

Values are mean ± standard deviation of two determinations.
Capital letters (A, B, C) in the same line indicate significant differences (P<0.05) of storage.
Small letters (a, b, c, d, e) in the same line indicate significant differences (P<0.05) of treatment.

Table 3. Changes in fatty acid groups of grass carp fillets after cooking and chill storage.

Fatty acid	(g/100 g fatty acids)									
	Raw fillet	Fried samples			Boiled samples			Steamed samples		
		Day 0	Day 1	Day 4	Day 0	Day 1	Day 4	Day 0	Day 1	Day 4
∑ SFA	39.112	30.300	33.774	31.233	32.443	32.940	34.887	33.662	32.939	33.992
∑ MUFA	15.370	35.576	33.267	32.919	17.767	39.613	39.263	41.729	32.373	35.441
∑ PUFA	45.517	34.125	32.959	35.848	49.790	27.447	25.850	24.610	34.689	30.567
∑ n-3	24.061	3.211	1.855	3.151	29.450	19.335	19.498	10.288	4.515	11.749
∑ n-6	21.456	30.914	31.104	32.697	20.340	8.112	6.352	14.322	30.174	18.818
n-3/ n-6	1.121	0.104	0.060	0.096	1.448	2.380	3.069	0.718	0.150	0.624

whereas slight decrease were observed in MUFAs and PUFAs. A significant increase were found in MUFAs levels of boiled samples after 1 days of storage (P<0.05), while the content of PUFAs were significantly decreased (P<0.05) (Table 3). Longer storage of steamed samples in chill room was led to little increase in SFAs, significant increase in MUFAs and also significant decrease in PUFAs, respectively (P<0.05) (Table 3).

Discussion

The average lipid content of raw samples in this study was slightly less than the values reported by Wu and Mao (2008) and Ojagh et al. (2009) for grass carp. Among the biological and environmental factors, diet has a great effect on the lipid content of fish flesh (Sigurgisladóttir and Pálmadóttir, 1993). Based on the classification by Suriah et al. (1995), grass carp may be classified as lean fish with lipid content below 5%.

After frying, fat content of fillet samples significantly (P<0.05) increased. This result is in agreement with those of Garcia-Arias et al. (2003), Gokoglu et al. (2004), Weber et al. (2008) and Hakimeh et al. (2010), although the value obtained in this study was higher. The increase of total lipid

content was not significant in boiled and steamed samples. Similar findings have reported by Weber et al. (2008) and Gokoglu et al. (2004) for boiling and by Hakimeh et al. (2010) for steaming. Cooking induces water loss in the food, which in turn increases its lipid content (Hoffman et al., 1994; García-Arias et al., 2003). Fat increase in fried sample could be due to oil penetration into the food after water is partially lost by evaporation. Ågren and Hänninen (1993) have concluded that additional oil in frying, mainly determines the small and lean fish lipid content.

The fatty acid profile in this study was similar to that found by Ojagh et al. (2009) and Wu and Mao (2008) for grass carp, although in present study, the content of PUFAs and MUFAs were higher and lower, respectively.

Based on the results, the content of C18:1 n-9c and C18:2 n-6c increased significantly after frying, while the content of other fatty acids decreased, especially docosahexaenoic acid and Arachidonic acid. García-Arias et al. (2003) and Bakar et al. (2008) reported similar observations for shallow fat frying where the cooking process had significantly affected the fatty acid composition of fish, increasing oleic and linoleic acids and decreasing eicosapentaenoic and docosahexaenoic acids. It has reported that fatty acid profiles of fish in frying processes became similar to those of the culinary fat used (Arias et al., 2003; Bakar et al., 2008).

The saturated fatty acid content decreased significantly in boiled samples. This could be explained by the fact that SFAs are largely represented in neutral lipids and are more prone to migration (Enser et al., 1996; Badiani et al., 2002).

In steamed samples, higher polyunsaturated fatty acid content decreased significantly, whereas the content of C18:1 (n-9) was increased. This finding is not in agreement with finding of Bakar et al. (2008) and Larsen et al. (2010) who reported no differences between steamed and raw samples. The n-3/n-6 ratio of deep fried, boiled and steamed samples were 0.10, 1.45 and 0.72, respectively.

The longer storage of fried samples in chill room was caused a slight decrease in SFAs and MUFAs and slight increase in PUFAs. The changes in fatty acid profile during storage in chill room could be due to changes in SFA and MUFA content which are neutral lipids and more prone to migration (Enser et al., 1996; Badiani et al., 2002) and also oxidation progress during storage (Bakar et al., 2008; Nikoo et al., 2010a).

The SFAs levels increased after 4 days of storage of boiled samples, while the levels of PUFAs decreased. Nikoo et al. (2010b) observed that the content of polyunsaturated fatty acids of *Rutilus frisii kutum* decrease, whereas the content of saturated fatty acids increased after 2 days of refrigerated storage. A significant decrease and increase of MUFAs and PUFAs were observed for the steamed King Mackerel after 1-day of storage, respectively, while the decrease of SFAs was not significant (Bakar et al., 2008).

Nutritional value of lipids is determined not only by the composition of fatty acids, but also by their ratio (Simopoulos, 1999; Schmitz and Ecker, 2008). Imbalance in the ratio of n-6 and n-3 acids (which should be about 3-5:1) can contribute to the development of cancer and the various kinds of inflammation (El-Badry et al., 2007). This research demonstrates that, the n-3/n-6 ratio decreased to 0.060 and then increased to 0.096 after 1 and 4 days of storage in fried samples. This ratio increased to 2.380 and 3.069 after 1 and 4 days of storage in boiled samples. In steamed samples, n-3/n-6 ratio decreased to 0.150 and then increased to 0.624, respectively. These results indicate that the fatty acid composition of fried sample was affected by the frying oil agreeing with previous studies (Amira et al., 2010; Kitson et al., 2009).

The EPA+DHA/C:16 ratio was higher in boiled and boiled- chill stored samples than steamed and fried samples. EPA+DHA/C:16 ratio of cooked, stored for 1 day and then 4 days were 0.051, 0.003 and 0.017 for fried samples, 0.492, 0.583 and 0.489 for boiled samples and 0.247, 0.037 and 0.149 for steamed

sample, respectively.

Some researchers reported that there was a significant effect of frying on the EPA and DHA levels in fried fish (Özogul et al., 2009; Gladyshev et al., 2006). The decrease in the EPA and DHA levels after frying may have been resulted from susceptibility of highly unsaturated fatty acids (HUFA) to oxidation during heating. Also, this reduction may have been caused by the absorption of oil during frying.

The results show that cooking and chill storage affect the total lipid and FA profile of samples. This study concluded that boiling method retain the fatty acid composition such as n-3 better than other cooking methods. Therefore it is recommended for cooking of grass carp.

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