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# Effects of different cooking methods on minerals, vitamins and nutritional quality indices of grass carp (*Ctenopharyngodon idella*)

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**Abstract**

This study aimed to evaluate the nutritional value (proximate composition, fatty acid profiles, vitamins and minerals) contents and also nutritional quality indices (NQI)) of grass carp (*Ctenopharyngodon idella*) prepared according to common consumer techniques: raw, poached, steamed, microwaved, pan-fried and deep-fried (in olive oil). In comparison to raw fish fillets, when grass carp was cooked there was an increase in protein, lipid and ash contents. Cooking methods had no significant effect on total n-3 fatty acids except for frying fillets. Lowest and highest content of n-3 was shown in deep-fried and pan-fried samples, respectively. Total n-6 fatty acid of cooked samples increased in comparison to raw samples. Na, K, Mg, P and Zn contents of boiled fish fillets significantly decreased. None of cooking methods had a significant effect a vitamin D. However, vitamin A, $B_1$ and $B_3$ contents of cooked fish significantly decreased.

**Keywords**: Cooking method, Grass carp, Fatty acids, Vitamins, Minerals, Nutritional quality indices.

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## Introduction

Grass carp (*Ctenopharyngodon idella*, family Cyprinidae) is one of the main fresh water fish species and highly demanded aquaculture species in Iran. Among the cultivated fishes, grass carp, also called farmed white fish, has received great attention because of its similarity to Caspian white fish in Iran. The muscle of fish contains important amounts of macronutrient and micronutrient which are beneficial for human health. Cooking methods can lead to a loss of the nutritional value of foods. The proper cooking methods are important for preserving maximum nutritional value such as proximate composition, vitamins, minerals and the fatty acids composition. Fatty acids are one of the most important healthy aspects of fish consumption (Uran and Gokoglu, 2014). The fatty acids in fish based on the number of double bonds are saturated fatty acids (SFA), mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) (Larsen *et al*., 2010; Moradi *et al*., 2011), but it is clear that individual fatty acids within these groups have distinct biological properties and health effects (Marimuthu *et al*., 2012). Seafoods are the only excellent source of the long chain polyunsaturated fatty acids (LC-PUFAs) such as arachidonic acid (ARA), eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (Lund, 2013) which have markedly different biological functions and physiological properties compared to the shorter chain PUFAs such as α- linolenic acid and linoleic acid (Larsen *et al*., 2010). The polyunsaturated fatty acids are considered to be susceptible to oxidation during heating compared with saturated fatty acids (Weber *et al*., 2008; Hosseini *et al*., 2014). However, in some studies, the EPA and DHA contents remained stable in different species of fish during different cooking methods (Al-Saghir *et al*., 2004; Gladyshev *et al*., 2006).

The nutritional quality index (NQI) of fatty acids profile and their biological functions have necessary health effects of fatty acids of fish (Hosseini *et al*., 2014). NQI is calculated by several indices of fatty acids composition including indices of atherogenicity (IA) and the thrombogenicity (IT) (Turan *et al*., 2007; hypocholesterolemic/ hypercholesterolemic fatty acid ratio (HH) (Testi *et al*., 2006); EPA+ DHA, PUFA/ SFA- stearic acid (Unsan, 2007); PUFA/ SFA ratio (Kalogeropoulos *et al*., 2004; Marques *et al*., 2010); n-3/n-6 PUFA ratios (Marques *et al*., 2010) and ARA/ EPA and UFA/ SFA ratios (Larsen *et al*., 2010).

Fish is a perfect source of vitamins and minerals which can be affected by different cooking methods (Ersoy and Özeren, 2009). There has been continuous research on the retention of vitamins and minerals by different cooking methods (Gokoglu *et al*., 2004; Ersoy and Özeren, 2009; Marimuthu *et al*., 2012; Hosseini *et al*., 2014). Therefore, it is important to determine nutritional value of fish cooked in one way or another namely steaming, poaching, pan-frying, deep-frying and

microwave. The aim of this study was to determine the proximate composition, vitamins and mineral contents and nutritional quality indices in the raw and cooked grass carp (*C. idella*).

## Materials and methods

*Sample preparation and cooking*

The fish, *Ctenopharyngodon idella*, was alive purchased from a farm in Khorramshahr City, Khozestan Province, Iran. Fish were slaughtered and transported to the laboratory of Khorramshahr University of Marine Science and Technology within 2-3 hrs. after being caught. The mean weight and length of fish were 1400±0.25 g and 47.33±0.05 cm, respectively. At the time of arrival at the laboratory, fresh fish were washed with cold water and filleted and cut into slices with a thickness of 1 cm by hand, and then the fish samples were cooked using AOAC 976.16 procedure (Larsen *et al*., 2010). Different cooking procedures were selected as common processing methods used by consumers. These were poaching, steaming, microwaving, pan-frying and deep-frying.

Steaming and poaching: the fish fillets were placed in a stainless steel steamer above a stainless steel pot of boiling water (500 mL) and cooked with the lid on for 5 min and 30 s, and 3 min and 30s, respectively. After cooking, the fillets were placed on absorbent paper towels.

Pan-frying: the fish fillets were added to a frying pan (180 ºC) bone side down for 3 min, then skin side down for 3 min (no oil added). For the fillet samples, each short side was cooked for a further minute. Total cooking time was 10 min per sample.

Deep-frying: the fish fillet samples were placed in a wire mesh basket and immersed in olive oil in a deep fryer for 5 min at 180 ºC. After frying, the basket was shaken and the samples placed on absorbent paper towels.

Microwaving: the fish fillet samples were individually placed on a ceramic plate and cooked on 100% power (high) for 40 s (CE3260E Model, SAMSUNG). After cooking, the samples were placed on absorbent paper towels.

After the cooking process, the samples were cooled to room temperature and the skin and backbones of the samples were removed. All fish in each lot group were homogenized using a kitchen blender and analyzed to determine proximate composition, fatty acids composition, minerals and vitamins contents. All assays were conducted on triplicate samples of the homogenates.

*Proximate composition*

Proximate composition of cooked and uncooked fish was measured in triplicate for moisture, proteins, lipids and ash contents. The moisture content of fish was determined by drying the meat in an oven at 105 ºC until a constant weight was obtained (AOAC, 2002). The crude protein was measured by converting the nitrogen content determined by Kjeldahl's method (6.25 × N) (AOAC, 2002). The lipid content was extracted by the AOAC (2002) method using the soxhlet system. Ash

content was determined gravimetrically in a muffle furnace by heating at 525 ºC for 24 h (AOAC, 2002).

*Fatty acid profile*

Fatty acids were extracted from fish samples according to the method demonstrated by Folch *et al*., (1957) with modification. Fatty acids of the extracts were then converted to fatty acid methyl esters (FAMEs). FAMEs were analyzed using a Phillips GC-PU4400 (Phillips Scientific, Cambridge, UK) equipped with a fused silica capillary polar column (BPX70, 60 m×0.32 mm ID, 0.25-µm film thickness, SGM, Victoria, Australia) and a flame ionization detector (FID). The temperature of the injector and FID were 240 and 280 ºC, respectively.

*Analysis of vitamins*

*B1 and B3 vitamins*

Water soluble vitamins (vitamins $B_1$ and $B_3$) were measured according to Ersoy and Özeren (2009) by High-Performance Liquid Chromatography (HPLC) (KNUAER, Germany) methods. The HPLC condition is expressed as: wavelength: 245 nm, flowing rate: 1.0 mL $min^{-1}$, injection volume: 20 L, mobile phase: 1000 mL phosphate solvent, 360 mL methanol, pressure: 150-160 bar, running time: 22min.

*Vitamins A and D*

Lipid soluble vitamins (A and D) were determined by HPLC (Thermo Scientific Specta SYSTEM, Thermo Fisher Scientific Inc., Waltham MA, USA) equipped with RP analytical column ODS2 Hypersil ™ 250×4.6 mm, 5 µm (Thermo fisher Scientific Inc., Waltham, MA, USA) based on studies conducted by Stancheva and Dobreva (2013). Vitamins A and D were monitored by UV detection at $\lambda_{max}$=325 nm and $\lambda_{max}$=265 nm, respectively.

*Analysis of minerals*

To determine the minerals content (Na, K, Ca, Mg, Fe, Mn, Cu and Zn), the samples were digested in $HNO_3$ and were measured by atomic absorption spectrophotometry (GBC Savant A, Australia) (AOAC, 2002). The P content was measured by Spectrophotometer after color change in Barton solution (Uran and Gokoglu, 2014). The results were expressed as absorbance at 430 nm. Standard curves were used for the determination of the elements in question.

*Statistical analyses*

Significant differences between means were determined by one- way analysis of variance (ANOVA) using SPSS16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Duncan's test was used to compare the means. A significance level of $p<0.05$ was used.

**Results**

The proximate composition of raw grass carp fillets after different cooking processes is shown in Table 1. The moisture, protein and fat contents of grass carp fillets were significantly affected by all the cooking methods. Microwave (69.66%) and deep-fried (59.33%) cooked samples had the

highest and lowest moisture content. The protein contents of grass carp fillets were significantly increased in all the cooking methods. Microwave fillets had the highest protein contents (24.84%). None of cooking methods had been a significant effect on fat contents except for deep-frying fillets. There was no significant difference in the ash content of fillets between raw and cooked samples.

**Table 1: Proximate composition of raw and cooked grass carp.**

| Cooking method | Moisture (%) | Protein (%) | Fat (%) | Ash (%) |
|---|---|---|---|---|
| Raw | 74.33±1.85$^{a}$ | 17.19±0.79$^{c}$ | 1.78±0.17$^{b}$ | 1.45±0.05$^{a}$ |
| Poached | 65.33±1.85$^{bc}$ | 19.81±1.44$^{bc}$ | 1.59±0.28$^{b}$ | 1.14±0.15$^{a}$ |
| Steamed | 68.00±1.52$^{ab}$ | 18.95±0.71$^{bc}$ | 1.86±0.20$^{b}$ | 1.27±0.06$^{a}$ |
| Microwaved | 69.66±1.45$^{ab}$ | 24.84±0.59$^{a}$ | 1.96±0.41$^{b}$ | 1.78±0.33$^{a}$ |
| Pan- fried | 67.33±0.88$^{ab}$ | 21.74±0.89$^{ab}$ | 1.57±0.39$^{b}$ | 1.54±0.06$^{a}$ |
| Deep- fried | 59.33±4.05$^{c}$ | 21.69±1.36$^{ab}$ | 7.88±1.13$^{a}$ | 1.76±0.48$^{a}$ |

Results are mean± standard error of triplicates.
Means within the same column having different superscripts are significantly different ($p$<0.05).

The most important fatty acids composition in grass carp fillets is shown in Table 2. Oleic acid (C 18:1) was the largest proportions of fatty acids presented in the raw samples, followed by palmitic acid (C 16:0) and palmitoleic acid (16:1). The most important saturated fatty acids were myristic acid (C 14:0), palmitic acid (C 16:0), stearic acid (C 18:0), and lignoceric acid (C 24:0). Heat treatments effect differently on fatty acids profiles. The SFA was significantly different between cooking methods. Palmitic acid (C 16:0) was the main fatty acids among the SFA. MUFA, with the largest amount of total fatty acids, was dominated by Oleic acid (C 18:1) in raw fish. Poaching grass carp fillets caused a significant decrease ($p$<0.05) of MUFA contents. Pan-frying significantly increased the total PUFA contents. The omega-3 fatty acid contents of grass carp is shown in Table 2. Results showed that the omega-3 fatty acid contents (as % of total fatty acids) in raw fish was 2.95%. The most important omega- 3 fatty acids analyzed in grass carp were α-linolenic acid (C 18:3 n-3, ALA), eicosapentaenoic acid (C 20:5 n-3, EPA) and docosahexanoicacid (C 22:6 n-3, DHA). Total n-3 content was significantly increased in the pan-fried compared to other cooking methods. Table 2 shows the omega-6 fatty acids content of grass carp. Linoleic acid (C 18:2 n-6) is the most abundant omega-6 fatty acid in grass carp fillet. None of cooking methods had a significant effect on n-6 content.

**Table 2: Fatty acid composition of raw and cooked grass carp fillets.**

| Fatty acid | Raw | Poached | Steamed | Microwaved | Pan- fried | Deep- fried |
|---|---|---|---|---|---|---|
| C14 | 2.06±0.04$^{bc}$ | 2.18±0.03$^{b}$ | 1.95±0.08$^{c}$ | 2.28±0.08$^{b}$ | 2.59±0.03$^{a}$ | 1.92±0.10$^{c}$ |
| C16 | 22.65±0.19$^{cd}$ | 23.05±0.04$^{bc}$ | 22.27±0.29$^{d}$ | 23.28±0.08$^{ab}$ | 23.73±0.20$^{a}$ | 21.19±0.03$^{e}$ |
| C18 | 3.28±0.03$^{c}$ | 3.73±0.05$^{b}$ | 3.45±0.04$^{c}$ | 3.36±0.13$^{c}$ | 4.24±0.04$^{a}$ | 3.40±0.03$^{c}$ |
| C24 | 0.07±0.01$^{b}$ | 0.06±0.00$^{b}$ | 0.05±0.01$^{b}$ | 0.15±0.01$^{a}$ | 0.06±0.01$^{b}$ | 0.08±0.01$^{b}$ |
| ∑ SFA | 28.03±0.21$^{c}$ | 29.01±0.09$^{b}$ | 27.72±0.32$^{c}$ | 29.02±0.29$^{b}$ | 30.71±0.19$^{a}$ | 26.58±0.11$^{d}$ |
| C 16:1 | 12.77±0.16$^{a}$ | 11.61±0.29$^{bc}$ | 12.22±0.06$^{ab}$ | 11.95±0.16$^{bc}$ | 12.10±0.11$^{abc}$ | 11.40±0.37$^{c}$ |

| **Table 2 continued:** | | | | | | |
|---|---|---|---|---|---|---|
| C 18:1 | 34.29±4.12$^{b}$ | 34.88±4.08$^{b}$ | 35.90±3.63$^{b}$ | 36.99±2.50$^{ab}$ | 42.53±5.45$^{ab}$ | 51.04±6.48$^{a}$ |
| ∑ MUFA | 47.16±4.18$^{ab}$ | 46.50±3.79$^{b}$ | 48.12±3.62$^{ab}$ | 49.00±2.46$^{ab}$ | 54.63±5.47$^{ab}$ | 62.44±6.78$^{a}$ |
| C 18 2w6 | 8.10±0.59$^{a}$ | 9.39±0.55$^{a}$ | 9.50±1.90$^{a}$ | 10.27±1.14$^{a}$ | 9.54±0.50$^{a}$ | 9.70±0.89$^{a}$ |
| C 20 4w6 | 0.75±0.04$^{b}$ | 0.54±0.27$^{b}$ | 0.73±0.05$^{b}$ | 0.70±0.06$^{b}$ | 1.20±0.11$^{a}$ | 0.73±0.04$^{b}$ |
| ∑ n6 | 8.85±0.60$^{a}$ | 9.93±0.31$^{a}$ | 10.25±1.14$^{a}$ | 10.97±1.16$^{a}$ | 10.75±0.42$^{a}$ | 10.43±0.93$^{a}$ |
| C 18:3 n3 | 2.28±0.09$^{a}$ | 2.18±0.14$^{a}$ | 2.21±0.11$^{a}$ | 2.32±0.10$^{a}$ | 2.19±0.12$^{a}$ | 2.55±0.16$^{a}$ |
| C 20: 5 n3 | 0.21±0.02$^{bc}$ | 0.15±0.03$^{bc}$ | 0.13±0.01$^{c}$ | 0.24±0.03$^{b}$ | 0.82±0.03$^{a}$ | 0.17±0.01$^{bc}$ |
| C 22:6 n3 | 0.46±0.04$^{b}$ | 0.44±0.08$^{b}$ | 0.37±0.02$^{b}$ | 0.42±0.02$^{b}$ | 0.73±0.06$^{a}$ | 0.43±0.06$^{b}$ |
| ∑ n3 | 2.95±0.13$^{b}$ | 2.83±0.25$^{b}$ | 2.72±0.14$^{b}$ | 3.22±0.21$^{b}$ | 3.88±0.14$^{a}$ | 2.80±0.19$^{b}$ |
| ∑ PUFA | 11.81±0.48$^{b}$ | 12.77±0.10$^{ab}$ | 12.98±1.00$^{ab}$ | 14.20±0.95$^{ab}$ | 14.63±0.43$^{a}$ | 13.23±1.13$^{ab}$ |

Results are mean± standard error of triplicates.
Means within the same row having different superscripts are significantly different ($p$<0.05).

**Table 3: Nutritional quality indices (NQI) of raw and cooked grass carp fish.**

| Nutritional index | Raw | Poached | Steamed | microwaved | Pan-fried | Deep-fried |
|---|---|---|---|---|---|---|
| PUFA/SFA | 0.41±0.01$^{a}$ | 0.43±0.00$^{a}$ | 0.46±0.03$^{a}$ | 0.49±0.03$^{a}$ | 0.48±0.03$^{a}$ | 0.47±0.01$^{a}$ |
| UFA/SFA | 2.09±0.16$^{b}$ | 2.03±0.12$^{b}$ | 2.20±0.12$^{b}$ | 2.84±0.26$^{a}$ | 2.17±0.04$^{b}$ | 2.25±0.20$^{b}$ |
| n-3/n-6 | 0.33±0.03$^{a}$ | 0.28±0.03$^{a}$ | 0.27±0.04$^{a}$ | 0.26±0.00$^{a}$ | 0.30±0.04$^{a}$ | 0.35±0.02$^{a}$ |
| EPA+DHA | 0.59±0.09$^{b}$ | 0.59±0.12$^{b}$ | 0.50±0.03$^{b}$ | 0.61±0.07$^{b}$ | 0.66±0.05$^{b}$ | 1.56±0.09$^{a}$ |
| HH$^{*}$ | 1.86±0.18$^{b}$ | 1.88±0.16$^{b}$ | 2.01±0.13$^{b}$ | 2.70±0.26$^{a}$ | 2.00±0.06$^{b}$ | 2.17±0.24$^{ab}$ |
| AI$^{**}$ | 0.52±0.03$^{a}$ | 0.53±0.03$^{a}$ | 0.48±0.01$^{a}$ | 0.38±0.03$^{a}$ | 0.79±0.29$^{a}$ | 0.49±0.04$^{a}$ |
| TI$^{***}$ | 0.75±0.04$^{a}$ | 0.78±0.04$^{a}$ | 0.73±0.02$^{a}$ | 0.59±0.05$^{b}$ | 0.72±0.03$^{ab}$ | 0.68±0.04$^{ab}$ |
| ARA/EPA | 3.64±0.58$^{ab}$ | 3.66±1.98$^{ab}$ | 6.08±1.25$^{a}$ | 4.17±0.31$^{ab}$ | 3.04±0.55$^{ab}$ | 1.45±0.11$^{b}$ |
| DHA/EPA | 2.15±0.11$^{bc}$ | 2.95±0.02$^{a}$ | 2.95±0.27$^{a}$ | 2.46±0.34$^{ab}$ | 1.81±0.15$^{c}$ | 0.89±0.05$^{d}$ |

Results are mean± standard error of triplicates.
Means within the same row having different superscripts are significantly different ($p$<0.05).
$^{*}$ HH= (C18:1n9+ C18:2n6+ C20:4n6+ C18:3n3+ C20:5n3+ C22:5n3+ C22:6n3)/ (C14:0+ C16:0)
$^{**}$ AI= [C12:0+ 4(C14:0)+ C16:0]/ [MUFA+ n-3 PUFA+ n-6 PUFA]
$^{***}$ TI= [C 14:0+ C16:0+ C18:0]/ [0.5 MUFA+ 0.5 (n-6 PUFA)+ 3 (n-3 PUFA+ n-3 PUFA/ n-6 PUFA]

Table 4 shows vitamins A (retinol), D (Calciferol), $B_1$ (thiamin) and $B_3$ (niacin) contents of the samples. Vitamin A content of raw fish was found to be 0.55 mg $100^{-1}$ g. There was no significant difference in vitamin A contents of different cooked fish except for frying fillets ($p$< 0.05). Vitamin D content of raw fish was found to be 0.04 mg $100^{-1}$ g. None of cooking methods had a significant effect the content of vitamin D. Vitamin $B_1$ content of raw fish was found to be 0.08 mg $100^{-1}$ g. Vitamin $B_1$ content was significantly reduced in all cooking methods. Vitamin $B_3$ content of raw fish was found to be 2.08 mg $100^{-1}$ g. Pan-frying and microwave cooking had no significant effect on vitamin $B_3$ contents, but poaching, steaming and deep-frying had significant effect.

**Table 4: Vitamins content of raw and cooked grass carp fillets.**

| Cooking method | A | D | B1 | B3 |
|---|---|---|---|---|
| Raw | 0.55±0.03$^{c}$ | 0.04±0.01$^{a}$ | 0.08±0.01$^{a}$ | 2.08±0.08$^{a}$ |
| Poached | 0.45±0.05$^{c}$ | 0.07±0.00$^{a}$ | 0.02±0.01$^{c}$ | 1.01±0.02$^{b}$ |
| Steamed | 0.43±0.03$^{c}$ | 0.09±0.01$^{a}$ | 0.05±0.03$^{b}$ | 1.00±0.05$^{b}$ |
| Microwaved | 0.57±0.12$^{bc}$ | 0.04±0.00$^{a}$ | 0.05±0.02$^{b}$ | 2.11±0.03$^{a}$ |
| Pan-fried | 1.50±0.02$^{a}$ | 0.04±0.01$^{a}$ | 0.04±0.06$^{b}$ | 1.99±0.16$^{a}$ |
| Deep-fried | 0.80±0.12$^{b}$ | 0.05±0.04$^{a}$ | 0.05±0.02$^{b}$ | 0.63±0.08$^{c}$ |

Results are mean± standard error of triplicates.
Means within the same column having different superscripts are significantly different ($p$<0.05).

Mineral contents of raw and cooked grass carp fillets are shown in Table 5. The Na content of raw sample was 558.00 mg $kg^{-1}$. There was no significant difference in the Na content of raw, poached, steamed and pan-fried fillets. The K content of raw fish was 1169.33 mg $kg^{-1}$. The K content decreased significantly after all cooking processes except for microwaved cooking method. The Mg, Cu, Ca and Fe contents of raw grass carp was found to be 144.33, 0.10, 224.66 and 13.75 mg $kg^{-1}$, respectively. None of cooking methods had a significant effect on Mg, Cu and Ca contents. The P content of grass carp fillet was 2523.00 mg $kg^{-1}$. The Mn content of raw fish was 0.66 mg $kg^{-1}$. None of cooking methods had a significant effect on Mn content except for microwave cooking method. The Zn content of raw fish was 16.40 mg $kg^{-1}$. The Zn content of fillets was significantly decreased after cooking.

**Table 5: Minerals content of raw and cooked grass carp fillets.**

| Mineral (mg $kg^{-1}$) | Raw | Poached | Steamed | Microwaved | Pan- fried | Deep- fried |
|---|---|---|---|---|---|---|
| Na | 558.00±12.70$^{cd}$ | 575.33±38.68$^{cd}$ | 497.33±69.86$^{d}$ | 824.00±60.04$^{a}$ | 648.00±17.32$^{bc}$ | 724.66±6.35$^{ab}$ |
| K | 1169.33±39.83$^{a}$ | 830.66±23.67$^{c}$ | 718.66±0.66$^{d}$ | 1133.33±19.05$^{a}$ | 694.00±23.09$^{d}$ | 954.00±47.34$^{b}$ |
| Mg | 144.33±5.48$^{ab}$ | 138.66±1.76$^{ab}$ | 131.33±14.43$^{b}$ | 160.00±11.54$^{a}$ | 122.00±2.30$^{b}$ | 132.00±1.15$^{b}$ |
| P | 2523.00±62.61$^{a}$ | 2451.19±149.19$^{a}$ | 1706.75±146.89$^{b}$ | 2418.81±93.18$^{a}$ | 2737.95±55.18$^{a}$ | 1496.73±34.26$^{b}$ |
| Mn | 0.66±0.09$^{b}$ | 0.47±0.00$^{b}$ | 0.62±0.11$^{b}$ | 0.98±0.08$^{a}$ | 0.66±0.12$^{b}$ | 0.48±0.08$^{b}$ |
| Cu | 0.10±0.01$^{ab}$ | 0.05±0.01$^{b}$ | 0.10±0.01$^{ab}$ | 0.13±0.02$^{a}$ | 0.10±0.01$^{ab}$ | 0.06±0.01$^{b}$ |
| Zn | 16.40±0.05$^{a}$ | 12.84±0.39$^{ab}$ | 13.71±0.22$^{b}$ | 14.46±0.37$^{b}$ | 14.49±1.21$^{b}$ | 11.36±0.12$^{b}$ |
| Ca | 224.66±82.56$^{ab}$ | 280.66±14.43$^{a}$ | 180.00±42.72$^{ab}$ | 268.00±2.30$^{a}$ | 188.66±38.68$^{ab}$ | 120.66±3.75$^{b}$ |
| Fe | 13.75±1.44$^{a}$ | 15.50±0.57$^{a}$ | 18.25±0.14$^{a}$ | 17.58±2.16$^{a}$ | 17.75±1.73$^{a}$ | 14.00±1.15$^{a}$ |

Results are mean± standard error of triplicates.
Means within the same row having different superscripts are significantly different ($p$<0.05).

## Discussion

The moisture content of raw fillets was 74.33%, showing a decrease after cooking because of the denaturation of protein structure and evaporation of water during cooking (Delfieh *et al*., 2013). The fat content significantly increased in deep-frying cooking method compared to other cooking methods; due to the oil penetration after evaporating water during frying (Rosa *et al*., 2007; Ersoy and Özeren, 2009; Koubaa *et al*., 2012). The total lipid content of the fish samples was inversely related to the moisture content (Larsen *et al*., 2010; Hosseini *et al*., 2014). The increase of proteins, fats and ash content was found to reduce moisture content (Ersoy and Özeren, 2009).

A relative pattern of fatty acids content presented in raw fillet was MUFA>SFA>PUFA. These findings are in agreement with those reported by Neff *et al*. (2014) for freshwater common carp and Weber *et al*. (2008) for silver catfish. However, it was found that polyunsaturated fatty acids constitute the highest amount of total fatty acids in rainbow trout (Testi *et al*., 2006). The lowest SFA content was observed in deep-fried fillets. Weber *et al*. (2008), Larsen *et al*. (2010) and Uran and Gokoglu (2014) showed that deep-fried fillets in vegetable oil had the lowest SFA content. The results of

this study are in agreement with the values presented by Turan *et al*. (2007), Delfieh *et al*. (2013) and Hosseini *et al*. (2014), for different aquatic species. Fatty acids composition of olive oil has changed MUFA content of deep-fried fillets compared to raw fillets. Similar results were found in previous studies in deep-fried fillets by Gall *et al*. (1983); Weber *et al*. (2008) and Ansorena *et al*. (2010). However it was found that MUFA content of some fish species have not been change in fried fillets compared with raw fillets (Hosseini *et al*., 2014). Olive oil is one of the popular frying oil in Iran and it is a good source of oleic acid (C 18:1) (Sioen *et al*., 2006; Jalarma Reddy *et al*., 2015; Portarena *et al*., 2015). The total fatty acids content of virgin olive oil used in this research was an SFA (14.81%), MUFA (74.07%) and PUFA (11.11%) where the percentage of PUFA was smaller than other fatty acids. Deep-fried fillets absorbed olive oil during cooking which resulted in increased levels of the major oleic acid in the deep-fried fillet. Similar observations were found by Ansorena *et al*. (2010). In deep-fried fillets, PUFA was in the highest amount (Table 1). The increase in the amount of PUFA must be explained by losing moisture during deep-frying (Sioen *et al*., 2006) and antioxidant properties of olive oil (Jalarma Reddy *et al*., 2015). In this study, raw and cooked grass carp fillets had significantly less n-3 PUFA than n-6 PUFA (Table 2). These results are in agreement with those of Vlieg and Body (1988) and Moradi *et al*. (2011), who reported that the levels of n-6 PUFA of fresh water fish generally contained higher proportions than marine water fish. EPA and DHA fatty acids are found in fish lipids, but not in vegetable oil (Türkkan *et al*., 2008). The DHA/ EPA ratio in raw fish was 2.15%. EPA and DHA content did not significantly differ between cooked and uncooked fillets, except pan-fried fillet. Linolenic acid (C 18:3 n-3), abundant in vegetable sources, is the most abundant omega-3 fatty acids of grass carp fillet because they are herbivorous fish (Larsen *et al*., 2010). Deep-fried of grass carp fillets caused a significant decrease of n-3 content due to the oil absorption during frying. Similar results were found in previous studies in fried kutum (Hosseini *et al*., 2014), silver cat fish (Weber *et al*., 2008), seabass (Türkkan *et al*., 2008) and red mullet (Koubaa *et al*., 2012).

The peroxidisability index (PI) was calculated by Testi *et al*. (2006) to determine the relationship between fatty acids composition and its susceptibility to oxidation as follows: PI= (0.025×momoenes)+ (1×dienes) + (2×trienes) + (4×tetraenes) + (6×pentaenes) + (8×hexaenes). In this study, PI index was 25.83. Hosseini *et al*. (2014) and Testi *et al*. (2006) reported that a high value of PI index indicates a higher sensitivity of fatty acids to oxidation. This study showed that grass carp fillet was less sensitive to oxidation during cooking. Unusan (2007) and Weber *et al*. (2008) reported that the lower PI index of the samples may be related to the low total n-3 content of grass carp flesh.

The PUFA/SFA and UFA/SFA ratios are the major parameters currently used to assess the nutritional quality of seafood (Larsen *et al*., 2010). WHO recommended a >0.4 PUFA/SFA ratio for a healthy diet (WHO, 2003). A diet containing high PUFA/SFA and UFA/SFA ratios reduces atherogenicity and thrombogenicity (Fehily *et al*., 1994). Deep-fried samples were statistically higher in UFA/SFA ratio compared to other cooking methods, due to absorption of oil when the fish fillets are fried (Table 3). There have been several reports on the effects of various PUFA/SFA and UFA/SFA ratios of fatty acids of fish fillets on lipid metabolism (Kalogeropoulos *et al*., 2004; Marques *et al*., 2010; Hosseini *et al*., 2014). The SFA and MUFA caused an increase in low-density lipoprotein cholesterol (LDLC) and high-density lipoprotein cholesterol (HDLC) of serum, respectively (Fehily *et al*., 1994; Kalogeropoulos *et al*., 2004; Hosseini *et al*., 2014). The shorter chain-length fatty acids (C6:0, C8:0 and C10:0) have little or no effects on serum LDLC compared with chain length (C12:0, C14:0 and C16:0) (Woollett and Dietschy, 1994).

The n-3/n-6 ratio in raw grass carp fish was 0.33 (Table 3). Hosseini *et al*. (2014) reported that n-3/n-6 ratio ranged from 0.24 to 4.1 in different fish species. The n-3/n-6 ratio in all fish samples was in the range recommended by WHO (Gladyshev *et al*., 2006). Simopoulos (2002) and Osman *et al*. (2001) reported that the n-3/n-6 ratio of 1:1 or 1:1.5 is considered to be optimal for nutritional index. ARA/EPA ratio is a better nutritional quality index compared to the n-3/n-6 ratio (Hosseini *et al*., 2014). Larsen *et al*. (2010) reported that the increase of ARA/EPA ratio decreases the nutritional quality of fish oil. Deep-fried cooking decreased the ARA/EPA ratio significantly ($p$<0.05) and the other cooking methods had no significant effects on this index.
The long chain n-3 fatty acids are obviously precursors of hormones known as eicosanoids which have important biological functions (Hosseini *et al*., 2014). The American Heart Association generally recommended a daily intake of EPA+DHA about 500- 1000 mg to reduce the risk of death from coronary heart disease, which may be reached by consuming at least two servings of fatty fish per week (Larsen *et al*., 2010; Hosseini *et al*., 2014; Neff *et al*., 2014). The results of this study indicated that deep-frying cooking method had the highest EPA+DHA index compared to other methods.

The effect of specific fatty acids on cholesterol metabolism was shown by hypocholesterolamic/ hypercholesterolamic fatty acids ratio (HH) (Santos- Silva *et al*., 2002). In this study, HH value was 1.86 in raw grass carp. Testi *et al*. (2006), Filho *et al*. (2010), and Hosseini *et al*. (2014) observed that the ratio of HH ranged from 0.25 to 4.83 in fish and fish products. There were no significant differences in the HH ratio between cooked and raw fillets except for microwave cooking method.

Atherogenicity index (AI) and Thrombogenic index (TI) are two

indices proposed by Ulbricht and Southgate (1991). The AI and TI ranged 0.33-2.37 and 0.01- 1.18 in different seafood products, respectively (Kalogeropoulos *et al*., 2004; Rosa *et al*., 2007; Turan *et al*., 2007; Filho *et al*., 2010, Delfieh *et al*., 2013; Hosseini *et al*., 2014). Hosseini *et al*. (2014) reported that the higher content of AI and TI indices indicate the lower lipid quality of sea food. It was found that diet with low AI and TI indices could have lower risk of coronary heart disease (Rosa *et al*., 2007; Hosseini *et al*., 2014). In this study, AI and TI of raw fillet were 0.52 and 0.75, respectively. Cooking treatment had no significant effect on AI value. Cooked and uncooked fillets had no significantly different TI index, except for microwave method.

Higher content of vitamin A was observed in grass carp fillets cooked by frying methods. A study by Ersoy and Özeren (2009) showed that fried cooked samples had higher retention of vitamin A. Cooking methods had no significant effect on vitamin D. Vitamin $B_1$ content of all cooking methods significantly decreased. This result was similar to the $B_1$ content of kutum roach described by Hosseini *et al*. (2014). Among cooked samples, poached samples showed the highest loss of vitamin $B_1$, due to leaching of thiamin into water. Lang (1970) reported that niacin is a stable vitamin and resistant to the heat and oxidation. Microwaved and pan-fried methods did not significantly affect the vitamin $B_3$ content. Maximum moisture content in the fillets is more concentrated in water-soluble vitamins (Ersoy and Özeren, 2009).

The highest Na content was observed in microwaved fillet (824.00 mg $kg^{-1}$) due to the high loss of water occurring during microwave and frying cooking (Ersoy and Özeren, 2009; Koubaa *et al*., 2012). The lowest and the highest Na content was observed in microwave and poached fillets by Gokoglu *et al*. (2004), respectively. The highest K content was observed in microwave cooking method. Hosseini *et al*. (2014) observed that K content of the grass carp fillet cooked with poaching method significantly decreased, while other cooking methods had no significant effect on K content. Conversely, Ersoy and Özeren (2009) and Rosa *et al*. (2007) showed that K content was significantly increased in all cooking methods. There was no significant difference in the Mg content of fillet between raw and cooked samples. According to Gokoglu *et al*. (2004), the Mg content of cooked rainbow trout had no significant effect on microwave cooked samples. After poaching and deep-frying, P content of grass carp was significantly decreased, which was similar to the results found by Gokoglu *et al*. (2004). None of the cooking methods had significant effects on the Cu content except for poaching method. Similarly, Ersoy and Özeren (2009) reported that the Cu content of cooked African catfish was found to be insignificant. Zn content of cooked fish significantly decreased. This result is in accordance with the reports of Gokoglu *et al*. (2004) for all cooking methods. Hosseini *et al*. (2014) showed that none

of the cooking methods had a significant effect on the Zn content of kutum roach. There was no significant effect in Ca content of fillet between raw and cooked samples. This result is similar to that of Badiani *et al*. (2013). None of the cooking methods had a significant effect on the Fe content. The similar result has been reported by Gokoglu *et al*. (2004).

The nutritional quality indices showed that steaming cooking methods were good preservation of fatty acids. Despite changes in PUFA/ SFA, UFA/ SFA, n-3/n-6, EPA+DHA ratios during different cooking methods, all fish samples fell within the recommended range. Among all cooking methods, the microwave and poaching methods are the suitable cooking methods with less vitamins and minerals loss.

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