Effects of frying by different frying oils on fatty acid profile of silver carp (*Hypophthalmichthys molitrix*)

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Abstract
The study aims to determine the influence of frying (shallow and deep) with olive, canola and sunflower oil on fatty acid composition of silver carp. Frying by olive oil and canola oil increased the monounsaturated fatty acids (MUFA) significantly (p<0.05) that consequently decreased saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) and ω-3 fatty acids. Frying by sunflower oil increased PUFA significantly (p<0.05), which caused to decrease in SFA and MUFA. The ω-6/ω-3 ratio of control samples (0.224) after shallow fat frying and deep frying, increased to 1.287 and 0.615; to 2.290 and 1.538; and to 9.381 and 5.950 by olive oil, canola oil and sunflower oil, respectively. Results suggest that frying oil can change the fatty acid composition of fish. The changes are depending to the kind of frying oil and method of frying which used.

Keywords: Frying, Fatty acid composition, ω-6/ω-3 ratio, Silver carp
Introduction
The presence of some particular fatty acids such as omega-3 fatty acids (EPA and DHA) in fish is the reason of beneficial effect of fish consumption. The consumption of fish is inversely associated with ischemic heart disease, arrhythmic death, other heart diseases, kidney disorders, arthritis, diabetes, cancer, cholesterol level and hypertension (Pepping, 1999; Von Schacky et al., 1999; Horrocks, 1999; Mozaffarian et al., 2003). However, lipid content and fatty acid composition in fish vary from species to species, age, sex and diet (Sigurgisladottir and Palmadottir, 1993). In addition to these biological factors, processing also affects fish lipid content and composition (Hoffman et al., 1994; Aubourg, 1999). Many comparative studies confirmed that cooking procedures could influence fat variations. Furthermore, the fat content of raw fish can influence fat exchanges and interactions between the culinary fat and fish lipid when frying (Gall et al., 1983; García-Arias et al., 2003; Al-Saghir et al., 2004; Bakar et al., 2008; Larsen et al., 2010). After partially water evaporation in frying process, penetration of culinary oil cause to change in lipid content and composition of fish. García-Arias et al. (2003) had reported that shallow fat frying significantly affected the fatty acid composition of sardine (S. pilchardus), which increasing oleic and linoleic acids and decreased eicosapentaenoic and docosahexaenoic acids. Changes in the fatty acid composition of S. guttatus were found in fried samples. The content of C16:0, C18:1 n-9 c and C18:2 n-6 c increased significantly after frying, while the content of other fatty acids especially C15:0, C18:3 n-3, C20:3 n-6 and C20:5 n-3 decreased (Bakar et al., 2008). It also reported that deep frying showed a significant increase in omega-6 fatty acids of King Salmon fillets due to uptake of linoleic acid from frying oil (Larsen et al., 2010). Candela et al. (1997) had reported an increase in ratio of ω-6/ω-3 fatty acids between 21.75 and 26.85 times after frying which giving rise to a negative effect on the benefits related to intake of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA). It also reported that consumption of fried fish did not reduce the risk of fatal ischemic heart disease (IHD) due to changes in fatty acid composition and n-6/n-3 ratio by frying oil (Mozaffarian et al., 2003). The people primary hypothesis was that consumption of fish meals would be beneficial for their health. However, preparation method and frying oil may greatly affect fish potential benefits. So this work aimed to study how shallow and deep-fat frying in olive, canola and sunflower oil can affect fatty acid composition of silver carp which is a predominant species in carp poly-culture.

Materials and methods
Sample preparation
Fresh samples of silver carp (Hypophthalmichthys molitrix) were obtained from a local fish market during autumn 2009. Fish were transported to the fisheries laboratory (Zabol University) in ice containing boxes. Upon arrival to laboratory, the fish were washed under running tap water, headed, gutted, cleaned and rewashed. Then they were cut to slices
with 2.0 cm thickness. Slices (with mean weight of 65.00 g) of 10 fish were randomly divided in 7 homogenous groups. One group of fish slices was analyzed immediately and was used as the control (fresh-raw) samples. The other groups were fried by the methods of shallow fat frying and deep fat frying with different oils. After frying, the fish slices were drained gently on stainless steel grills and allowed to be air cooled. The bones and skins of fish were removed. All samples in each group were homogenised using a kitchen blender. All assays were conducted on triplicate samples of the homogenates.

Frying oils
Olive oil (Extra virgin olive oil, Plaza De Espana, Spain), canola oil (Mahidasht Kermanshah, Iran) and sunflower oil (Nina, Iran) used for shallow and deep fat frying with major fatty acid content as mentioned in Table 1.

<table>
<thead>
<tr>
<th>Frying oils</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1</th>
<th>C18:2</th>
<th>C18:3</th>
<th>C20:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil</td>
<td>10.64</td>
<td>3.59</td>
<td>76.61</td>
<td>1.72</td>
<td>0.62</td>
<td>-</td>
</tr>
<tr>
<td>Canola oil</td>
<td>5.21</td>
<td>2.60</td>
<td>58.31</td>
<td>22.03</td>
<td>5.65</td>
<td>1.25</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>7.45</td>
<td>3.49</td>
<td>33.48</td>
<td>52.46</td>
<td>1.30</td>
<td>-</td>
</tr>
</tbody>
</table>

Frying procedure
The procedure for shallow fat frying was based on Bakar et al. (2008) method. Deep-fat frying was carried out in a 3 L capacity deep-fryer (Tefal, Iran). The temperature of the frying oil was set at 180 ± 2°C which was monitored with a metal thermometer. The fillets were fried until the core temperature was about 65-70°C.

Lipid extraction
The procedure used for the lipid extraction was based on Kinsella et al. (1977). About 50 g of fish muscle were homogenized in a warring blender for 2 min with a mixture of 50 ml chloroform and 100 ml methanol. One volume of chloroform (50 ml) and distilled water (50 ml) were added to the mixture and blended for 30 sec, respectively. The homogenate was then filtered, and the filtrate collected, and transferred to a reparatory funnel to allow for phase separation. The lower fraction was collected and filtered. It was then transferred to a rotary evaporator for evaporation. The sample was then collected for the fatty acid analysis.

Fatty acid analysis
Lipid samples were converted to their constituent fatty acid methyl esters by the method of Timms (1978). Lipid sample (0.2 g) in triplicates were weighed and diluted in 4 ml hexane followed by the addition of 0.2 ml sodium methoxide in a sealed tube. The mixture was then shaken using a vortex for 10 s and left for about 30 min until it separated into two phases. The top layer, FAME was then taken for analysis. Analysis of fatty acid methyl esters was performed on a Shimadzu GC, 17A with a J & W scientific DB high polar capillary column (60.0 m x 0.322 mm i.d) and quantified by FID detector. The GC conditions were as

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follows: injection port temperature was 250ºC; flame ionization detector temperature was 260ºC. Oven temperature was isotherm of 195 ºC for 45 min. The carrier gas was helium. The column flow rate was 0.9 ml/min. The sample size injected for each analysis was 1 ml. Samples were manually injected into the GC port. Compounds were identified by comparison with the retention times of known standards (Fluka 12 component FAME mix and also two pure FAMEs).

Statistical analysis
The data were analyzed using the one way analysis of variance test (ANOVA). The Tukey’s test was used for mean comparison when a significant variation was found by the ANOVA test. The significance of results was at 5%. The software used was Minitab, release 13.

Results
The fatty acids profiles of raw and fried samples of silver carp are shown in Table 2. Twenty fatty acids were detected in raw and fried samples. Raw silver carp showed considerable amounts of palmitic, stearic, palmitolic, oleic, linoleic, linolenic, eicosapentaenoic and docosahexaenoic acid (Table 2). Fatty acids composition responds differently to frying oils. Higher content of oleic acid was found in fried samples by olive oil (3.328 and 3.086 times higher in shallow and deep fat frying, respectively) in comparison with raw samples, which caused to decrease in the content of other fatty acids with exception of linoleic and stearic acids content (Table 2). In fried samples by canola oil, the increase was happened in the content of oleic acid (C18:1) and linoleic acid (C18:2). Changes in fried samples by sunflower oil were different due to higher increment in the content of linoleic acid (7.876 times higher in both methods of frying) and oleic acid in comparison with raw samples. In present study, moisture content reduced from 75.65 % to 64.85, 68.21 and 66.52 % after shallow fat frying with olive, canola and sunflower oils and to 43.50, 56.26 and 51.78 % after deep fat frying, respectively. Instead total lipid content increased from 2.63 % to 3.11, 3.46 and 3.40 % after shallow fat frying with olive, canola and sunflower oils and to 4.23, 4.15 and 4.18 after deep fat frying, respectively.

In raw silver carp, SFA was the most abundant fatty acid (36.810 % of total fatty acids) followed by PUFA (32.745 % of total fatty acids) and MUFA (30.745 % of total fatty acids) (Table 3). This arrangement was changed by frying which was dependent on frying oil and frying method. In frying with olive oil, MUFA fatty acids increased significantly and became most abundant fatty acids (70.234 and 63.871 % of total fatty acids in shallow and deep fat frying, respectively) followed by SFA fatty acids (19.518 and 22.518 %, respectively). The increase in MUFA fatty acids after frying by canola oil also happened but the changes in PUFA fatty acids content were not significant. In Samples fried by canola oil, MUFA fatty acid was the most abundant (56.656 and 55. 240 % of total fatty acids in shallow and deep fat frying, respectively) which followed by PUFA fatty acids. The trends of changes were different in fried samples with sunflower oil. In sunflower oil, PUFA fatty acids increased drastically. In these samples, PUFA fatty acids was the most abundant
fatty acids (51.511 and 49.531 % of total fatty acids in shallow and deep fat frying, respectively) which followed by MUFA fatty acids (Table 3). The PUFA composition and ω-6/ω-3 fatty acids ratio of silver carp was of particular interest in this study due to their importance in human health. Results showed a higher ω-3 fatty acid content in raw fish. The most abundant fatty acids in PUFA were DHA (10.332 % of total fatty acids) and EPA (8.935% of total fatty acids) followed by C18:3 and C18:2. The ω-6/ω-3 ratio in raw fish was around 0.224. Fried samples in olive oil showed a significant (p<0.05) decrease in ω-3 fatty acids content which caused to increase in ω-6/ω-3 fatty acids ratio to 1.287 and 0.615 in shallow and deep fat frying, respectively. In fried samples in canola oil, not only the content of ω-3 fatty acids decreased, but also the content of ω-6 due to the effect of frying oil increased and caused to higher increase in ω-6/ω-3 fatty acids ratio in compare to olive oil (2.290 and 1.538, in shallow and deep fat frying, respectively). Using sunflower oil for frying caused to great increase in the content of ω-6 fatty acids and increased the ratio of ω-6/ω-3 fatty acids to 9.381 and 7.127 in shallow and deep fat frying, respectively. Using sunflower oil for frying caused to great increase in the content of ω-6 fatty acids and increased the ratio of ω-6/ω-3 fatty acids to 9.381 and 7.127 in shallow and deep fat frying, respectively. Similar results have been reported by Candela et al. (1998) with elevated ω-6/ω-3 ratio in using of sunflower oil to frying of sardines, mackerel and salmon. However, there are some reports with very smaller changes in this ratio when using sunflower oil (Gladyshev et al., 2006; Gladyshev et al., 2007; Larsen et al., 2010). The changes in shallow fat frying were higher than deep fat frying in all samples.

**Discussion**
The fatty acid profiles of products during frying processes became similar to those of the culinary fat used. Similar results were found in frying of fish by olive oil (García-Arias et al., 2003), sunflower oil (Candela et al., 1998; Larsen et al., 2010), palm oil (Bakar et al., 2008), soybean, canola oil (Weber et al., 2008) and also frying the different breaded foods (Miranda et al., 2010), due to oil absorption in frying process that in turn dilutes the concentration of other fatty acids. Present results showed that the major fatty acids of frying oil were observed by fish slices which caused to heterogeneous changes in the content of other fatty acids. As the initial lipid content of fish (Ågren and Hänninen, 1993) and method of frying (Larsen et al., 2010) are determinable in fatty acids composition changes, special care must be done in selecting the frying oil with regard to health benefits of ω-6/ω-3 ratio. It has been reported that vegetable oils rich in ω-6 PUFAs should be avoided in pan and deep-fat frying (Ågren and Hänninen, 1993). Current study showed different effects of olive, canola and sunflower oils (with different profile) on fatty acid composition of silver carp. While olive oil and canola oil with higher content of C18:1, increased the content of this fatty acid in fish slices, sunflower oil increased the content of C18:2 fatty acid and subsequently the content of ω-6 PUFAs. Great increase in content of ω-6 PUFAs during frying by sunflower oil had negative effect on ω-6/ω-3 ratio. Larsen et al. (2010) have reported a lower influence of pan frying in fatty acid composition than deep fat frying in king salmon.
Table 2: Effect of frying by different methods and different oils on fatty acid composition (g/100 g of total fatty acids) of *H. molitrix*

<table>
<thead>
<tr>
<th>Fatty acid composition</th>
<th>Silver carp raw</th>
<th>Shallow fat frying</th>
<th>Deep frying</th>
<th>Shallow fat frying</th>
<th>Deep frying</th>
<th>Shallow fat frying</th>
<th>Deep frying</th>
<th>Shallow fat frying</th>
<th>Deep frying</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Olive oil</td>
<td>Canola oil</td>
<td>Sunflower oil</td>
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<tr>
<td></td>
<td></td>
<td>Shallow fat frying</td>
<td>Deep frying</td>
<td>Shallow fat frying</td>
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<td>Shallow fat frying</td>
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<td>Shallow fat frying</td>
<td>Deep frying</td>
</tr>
<tr>
<td>C14:0</td>
<td>2.400 ± 0.040 a</td>
<td>0.738 ± 0.005 c</td>
<td>0.909 ± 0.060 b</td>
<td>0.524 ± 0.045 cd</td>
<td>0.817 ± 0.072 bc</td>
<td>0.447 ± 0.009 d</td>
<td>0.631 ± 0.065 c</td>
<td></td>
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</tr>
<tr>
<td>C14:1</td>
<td>-</td>
<td>0.215 ± 0.000 a</td>
<td>-</td>
<td>0.146 ± 0.000 b</td>
<td>-</td>
<td>0.107 ± 0.002 c</td>
<td>-</td>
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<tr>
<td>C15:0</td>
<td>0.752 ± 0.010 a</td>
<td>0.224 ± 0.000 b</td>
<td>-</td>
<td>0.194 ± 0.010 c</td>
<td>-</td>
<td>0.145 ± 0.000 d</td>
<td>-</td>
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</tr>
<tr>
<td>C16:0</td>
<td>21.395 ± 0.735 a</td>
<td>13.359 ± 0.395 c</td>
<td>15.037 ± 0.605 b</td>
<td>8.335 ± 0.500 e</td>
<td>10.382 ± 0.060 d</td>
<td>8.816 ± 0.092 e</td>
<td>10.654 ± 0.590 d</td>
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<tr>
<td>C16:1</td>
<td>6.478 ± 0.087 a</td>
<td>2.535 ± 0.154 c</td>
<td>3.459 ± 0.083 b</td>
<td>1.534 ± 0.025 e</td>
<td>2.593 ± 0.095 c</td>
<td>1.269 ± 0.010 e</td>
<td>1.887 ± 0.156 d</td>
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</tr>
<tr>
<td>C17:0</td>
<td>1.545 ± 0.009 a</td>
<td>0.314 ± 0.003 c</td>
<td>0.409 ± 0.001 b</td>
<td>0.333 ± 0.006 c</td>
<td>0.317 ± 0.002 c</td>
<td>0.198 ± 0.003 d</td>
<td>0.306 ± 0.000 c</td>
<td></td>
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</tr>
<tr>
<td>C17:1</td>
<td>0.943 ± 0.008 a</td>
<td>0.284 ± 0.003 d</td>
<td>0.391 ± 0.000 b</td>
<td>0.216 ± 0.005 f</td>
<td>0.324 ± 0.001 c</td>
<td>0.165 ± 0.004 g</td>
<td>0.261 ± 0.000 e</td>
<td></td>
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</tr>
<tr>
<td>C18:0</td>
<td>4.214 ± 0.032 a</td>
<td>3.826 ± 0.062 ab</td>
<td>3.938 ± 0.045 a</td>
<td>3.059 ± 0.021 b</td>
<td>3.158 ± 0.023 ab</td>
<td>3.624 ± 0.028 ab</td>
<td>3.833 ± 0.015 ab</td>
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<tr>
<td>C18:1</td>
<td>19.774 ± 0.150 e</td>
<td>65.812 ± 2.005 a</td>
<td>59.559 ± 1.030 b</td>
<td>54.178 ± 0.980 c</td>
<td>51.464 ± 1.020 c</td>
<td>32.480 ± 2.005 d</td>
<td>31.275 ± 0.525 d</td>
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</tr>
<tr>
<td>C18:2</td>
<td>5.299 ± 0.088 e</td>
<td>5.012 ± 0.039 e</td>
<td>4.498 ± 0.047 e</td>
<td>19.492 ± 0.198 c</td>
<td>16.103 ± 0.750 d</td>
<td>46.018 ± 1.090 a</td>
<td>41.738 ± 1.766 b</td>
<td></td>
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</tr>
<tr>
<td>C18:3</td>
<td>7.476 ± 0.012 a</td>
<td>2.724 ± 0.015 e</td>
<td>3.938 ± 0.040 c</td>
<td>6.441 ± 0.164 b</td>
<td>7.191 ± 0.220 a</td>
<td>2.527 ± 0.080 e</td>
<td>3.312 ± 0.311 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C20:0</td>
<td>1.789 ± 0.085 a</td>
<td>0.529 ± 0.004 c</td>
<td>0.890 ± 0.008 b</td>
<td>0.388 ± 0.007 d</td>
<td>0.327 ± 0.008 d</td>
<td>0.353 ± 0.004 d</td>
<td>0.560 ± 0.092 c</td>
<td></td>
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</tr>
<tr>
<td>C20:1</td>
<td>1.138 ± 0.030 a</td>
<td>0.391 ± 0.003 e</td>
<td>0.462 ± 0.003 d</td>
<td>0.582 ± 0.006 c</td>
<td>0.859 ± 0.005 b</td>
<td>0.312 ± 0.003 f</td>
<td>0.306 ± 0.007 f</td>
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</tr>
<tr>
<td>C20:2</td>
<td>0.703 ± 0.005 d</td>
<td>0.558 ± 0.010 e</td>
<td>0.767 ± 0.002 c</td>
<td>1.255 ± 0.010 b</td>
<td>1.396 ± 0.009 a</td>
<td>0.531 ± 0.009 e</td>
<td>0.666 ± 0.031 d</td>
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<tr>
<td>C22:0</td>
<td>2.446 ± 0.025 a</td>
<td>0.174 ± 0.002 b</td>
<td>-</td>
<td>0.140 ± 0.000 b</td>
<td>-</td>
<td>0.125 ± 0.001 b</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td>C20:5</td>
<td>8.935 ± 0.075 a</td>
<td>0.526 ± 0.009 c</td>
<td>0.880 ± 0.006 b</td>
<td>0.520 ± 0.004 c</td>
<td>0.769 ± 0.008 b</td>
<td>0.400 ± 0.000 c</td>
<td>0.706 ± 0.099 b</td>
<td></td>
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</tr>
<tr>
<td>C22:2</td>
<td>-</td>
<td>0.346 ± 0.012 a</td>
<td>-</td>
<td>0.209 ± 0.001 b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C24:0</td>
<td>2.269 ± 0.009 a</td>
<td>0.354 ± 0.008 f</td>
<td>1.125 ± 0.009 b</td>
<td>0.352 ± 0.003 f</td>
<td>0.879 ± 0.010 c</td>
<td>0.447 ± 0.005 e</td>
<td>0.752 ± 0.103 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C24:1</td>
<td>2.109 ± 0.011 a</td>
<td>0.997 ± 0.015 b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td>C22:6</td>
<td>10.332 ± 0.120 a</td>
<td>1.078 ± 0.018 d</td>
<td>3.737 ± 0.011 b</td>
<td>2.099 ± 0.090 c</td>
<td>3.417 ± 0.082 b</td>
<td>2.035 ± 0.009 c</td>
<td>3.109 ± 0.404 b</td>
<td></td>
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</tbody>
</table>

Values are means and S.D; Means with the same letter within a row were not significantly different at P < 0.05 level
This could be due to difference in initial fish lipid content, thickness of fillet and duration of frying. Pan-frying is a method that frequently applied and gives flavour characteristics to the food that are highly appreciated by the consumers (Sioen et al., 2006). Differences in consumer sense in juicier or crispier the fried fish, could affect in fatty acid composition. There are also differences in frying oils as Varela (1988) indicated that olive oil forms a crust that protects the food against absorption of oils. Current study showed that frying the fish with canola oil and especially sunflower oil increased the content of ω-6 fatty acids content in fish slices. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio, as is found in today’s Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6/omega-3 ratio) exert suppressive effects (Simopoulos, 2002). As fish and seafood are good sources of ω-3 fatty acids, they generally suggest balancing between ω-6 and ω-3 fatty acids and reducing the ω-6/ω-3 ratio. Due to effects of frying oil, generally frying method has the greater negative effect in compare with other common methods of fish cooking with regarding to health benefits (García-Arias et al., 2003; Bakar et al., 2008; Weber et al., 2008; Larsen et al., 2010). However, consumption of fried fish in comparison to other cooked fish showed absence of lower risk on cardiac benefits (Mozaffarian et al., 2003). Fried fish intake was also associated with structural abnormalities indicative of systolic dysfunction and potential coronary atherosclerosis (Mozaffarian et al., 2006).

All frying oil evaluated in this study, changed the fatty acid profile of silver carp slices. Changes in fatty acid composition and ω-6/ω-3 ratio were more prominent in sunflower oil fried samples. From a public health point of view, frying the fish by different oils reduce its benefits. As the deep fat frying and also shallow fat frying accepted by consumers due to unique flavor- texture combination and also flavor characteristics, more study should be done to reduce the negative effects of frying by choosing the better frying oil, better fish regarding to initial lipid content, better size and thickness of fillet and slices, pre-frying preparation.
(whole fish, fillet with skin, coating) and better time and temperature of frying.

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تأثیرات سرخ کردن با روغن های سرخ کردنی متفاوت بر ترکیب اسید چرب ماهی فیتوفاگ (Hypophthalmichthys molitrix)

اسحاق زکی بور رحیم آبادی*; سمیرا داد

چکیده

این مطالعه جهت بررسی تاثیرات سرخ کردن (در ماهی تایه معمولی و ماهی تایه گود) با روغن زیتون، کلزا و آفتابگردان بر ترکیب اسیدهای چرب ماهی فیتوفاگ انجام گردید. سرخ کردن با روغن زیتون و روغن کلزا بطور معنی داری (P<0.05) محتوای اسیدهای چرب تک غیر اشجعی (MUFA) کاهش داد که این امر سبب کاهش محتوای اسیدهای چرب اشجعی (SFA) گردید. سرخ کردن با روغن آفتابگردان بطور معنی داری (P<0.05) سبب افزایش محتوای اسیدهای چرب PUFA و کاهش MUFA و SFA گردید. نسبت اسیدهای چرب امگا-6 به امگا-3 (P<0.05) در سرخ کردن، نسبت اسیدهای چرب امگا-6 به امگا-3 ماهی فیتوفاگ کاهش یافت.

واژگان کلیدی: سرخ کردن، ترکیب اسید چرب، نسبت اسیدهای چرب امگا-6 به امگا-3 ماهی فیتوفاگ.

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