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Analytical Methods

Determination of the seasonal changes on total fatty acid composition and ω3/ω6 ratios of carp (*Cyprinus carpio* L.) muscle lipids in Beysehir Lake (Turkey)

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Abstract

The muscle lipid and fatty acid composition of carp, *Cyprinus carpio* in Beysehir Lake the largest freshwater lake in Turkey, was determined. Polyunsaturated fatty acids (PUFA) of carp, the most abundant fish species in Beysehir Lake, were found to be higher than those of saturated fatty acids (SFA) in spring, summer and autumn and also the monounsaturated fatty acids (MUFA) in spring and summer. Palmitic acid was the major SFA (14.6–16.6%) in all seasons. Oleic acid was identified as the major MUFA (15.1–20.3%). Docosahexaenoic acid (DHA) was the major PUFA in summer and winter, whereas linoleic acid (LA) was the major PUFA in spring and autumn. The percentages of total ω3 fatty acid were higher than those of total ω6 fatty acid in the fatty acid composition of carp in winter. It was shown that the fatty acid composition in the muscle of carp was significantly influenced by feeding period and seasons.

Keywords: Carp; *Cyprinus carpio*; Fatty acid composition; Seasonal variations; Beysehir Lake

1. Introduction

Fish and shellfish provide an almost unlimited variety of fatty acids with beneficial roles in human health (Ackman, 2000). The overall net effect of the consumption of fish and fish oils appears to reduce the risk of coronary heart disease (Kris-Etherton, Harris, & Appel, 2002). The potential health benefits related to fish consumption are due to the presence of proteins, unsaturated essential fatty acids, minerals, and vitamins. Additional health benefits from the consumption of fish or fish lipids may be related with PUFAs especially ω3 PUFAs (Sidhu, 2003).

Long chain ω3 PUFA cannot be synthesised by humans and must be obtained from the diet (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002). If we attempt to maintain or enhance our present-day health we must take a proactive approach to ensure our sustained access to essential fatty acids and, in particular, to eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA) (Arts, Ackman, & Holub, 2001). AA, EPA and DHA are important structural components of cell membranes (Bezard, Blond, Bernard, & Clouet, 1994; Innis, 1991) and EPA and DHA, found only in fish and seafoods, have extremely beneficial properties for, in particular, the prevention of human coronary artery disease (Leaf & Weber, 1988).

The valuable ω3 fatty acids are always present in fish flesh, even in lean fish (Ackman, 2002). All fish contain EPA and DHA; however, the quantities vary among species and within a species according to environmental variables such as diet and whether fish are wild or farm-raised (Kris-Etherton et al., 2002). Omega-3 fatty acids, from both plant and marine sources, have been shown to

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reduce the incidence of coronary heart disease in both men and women (Kris-Etherton et al., 2002; UK SACN – U.K. Scientific Advisory Committee on Nutrition., 2004). The UK Fish Intercommittee Subgroup, a collaboration of the Scientific Advisory Committee on Nutrition (SACN) and Committee on Toxicology (COT) concluded that with reference to data on dioxin and dioxin-like polychlorinated biphenyl (PCB) levels in fish in the UK, about two portions of oily fish per week could be consumed without appreciable exceedance of the tolerable daily intake (TDI) of 2 pg/kg/day (UK SACN, 2004).

It has been reported that the type and amount of fatty acids in fish tissues vary mainly with feeding of the fish, but other factors may also influence their fatty acid composition. For example, size or age, reproductive status, geographic location, and season all influence fat content and composition of fish muscle (Ackman, 1989; Alasalvar et al., 2002; Henderson and Tocher, 1987; Saito, Yamashiro, Alasalvar, & Konno, 1999). The fatty acid profile of fish is certainly influenced by temperature (Leger, Bergot, Lekuet, Flanzy, & Meurot, 1977). Therefore, it is known that the biochemical contents of marine organisms undergo changes due to seasonal changes (Ackman, 1995).

Cyprinus carpio, carp, as a freshwater fish species, has been one of the most widely cultured species all over the world due to its fast growth rate and easy cultivation, and *C. carpio* is one of the most abundant freshwater fish in Beysehir Lake, which is the largest freshwater lake and important bird nesting and visiting areas in Turkey. People who live in Beysehir and near this location consume the carp abundantly. No reports have yet been published about the effects of seasonal variations on the fatty acid composition of this important species in Beysehir Lake. In view of these facts, it seemed necessary to carry out a study on lipid profile of highly consumed fish, *C. carpio*, in this location. The objective of this work is therefore to characterize carp in terms of their lipid and fatty acid in different seasons of the year.

2. Materials and methods

2.1. Materials

C. carpio, used in this study, was obtained from Beysehir Lake seasonally. Beysehir Lake is located in central Anatolia and 90 km from the city of Konya (37° 45′ North–31° 30′ East). Its average depth is 7–8 m, length about 45 km and width is 14–26 km and it is 651 km^2. The water of the lake is used for irrigation and drinking purposes. Carp is one of the most abundant fish in all seasons and has a great commercial importance for Central Anatolian peoples.

The seasons chosen for analysis were summer, winter, spring, and autumn. The samples were collected in middle month of each season during the year. All representative fishes ($n = 3$ at each determination) used in the experiments were of almost the same size (average weight 1.250 kg) and age (over 2 years old). Gender differences were not threated. Reproductive period of carp in the Beysehir Lake is between in the middle of May and July (Erdem, 1983). After being caught, they were transported on ice to the laboratories, filleted, and these were frozen. At the beginning of each analysis, the samples were allowed to equilibrate to room temperature, ground, and homogenized in chloroform/methanol mixture (2/1, v/v).

2.2. Fatty acid analysis

Samples of fillets were extracted by the Folch, Lees, and Sloane Stanley (1957) method and fatty acids were transesterified with BF_3-methanol (Moss, Lambert, & Merwin, 1974).

Fatty acid methyl esters (FAME) were analyzed on a HP (Hewlett Packard) Agilent 6890N model gas chromatograph (GC), equipped with a flame ionization detector (FID) and fitted with a DB-23 capillary column (60 m, 0.25 mm i.d. and 0.25 μm). Injector and detector temperatures were 270 and 280 °C, respectively. Column temperature program was 190 °C for 35 min then increasing at 30 °C/min up to 220 °C where it was maintained for 5 min. Carrier gas was helium (2 ml/min) and split ratio was 30:1.

Identification of normal fatty acids was carried out by comparing sample FAME peak relative retantion times with those obtained for Alltech standards. Results were expressed as FID response area relative percentages.

2.3. Statistical analysis

Nine item carp samples were analysed each season and each sample were analysed in triplicate. The average results of peak area are offered as means ± SD. FID responses are corrected to weight percent automatically. The results were submitted to analysis of variance (ANOVA), at 0.05 significance level, using SPSS 10.0. The mean values were compared by Tukey's test.

3. Results and discussion

The total lipid content determined in the carp fillets throughout the four seasons are presented in Table 1. For this species total lipid contents in winter were determined to be higher than in other seasons. The differences can be explained by the known seasonal variation of the fat content in fish (Rahman, Huah, Hassan, & Daud,

Table 1
Lipid contents determined in spring, summer, autumn, and winter for the fillets of carps investigated

Seasons	Lipids (% wet weight basis)
Spring	2.94
Summer	1.09
Autumn	1.31
Winter	4.45

1995). Fat content is influenced by species, season, geographical regions, age and maturity (Piggot & Tucker, 1990). Similarly, Rasoarahona, Barnathan, Bianchini, and Gaydou (2004) found that lipid content of wet muscle of carp was at a low level (0.91%) during the hot season and a higher level (1.73%) during the cold season.

Fatty acid composition of the carp which were captured in different seasons are given in Table 2. The major fatty acids identified in the carp were 18:1ω9 (oleic), 16:0 (palmitic), 16:1 (palmitoleic), 22:6ω3 (docosahexanoic acid, DHA), 18:2ω6 (linoleic), 20:4ω6 (arachidonic, AA), 18:0 (stearic) and 20:5ω3 (eicosapentaenoic acid, EPA), in all seasons, respectively.

Palmitic acid was the primary saturated fatty acid (SFA), 14.6–16.6% for carp in all seasons. Similar results for carp (Kolakowska, Szczygielski, Bienkiewicz, & Zienkowicz, 2000) and other fish species have also been reported in the literature (Çelik, Diler, & Küçükgülmez, 2005; Rahman et al., 1995). In general, fish are relatively low in SFA (<30%), except for certain species (Ackman 1989). Similar results were identified in this study for all seasons (26.6–29.6%).

Table 2
Seasonal variations on total fatty acid composition of fillets of carps (*Cyprinus carpio*) in Beysehir Lake*

Fatty acids	Spring	Summer	Autumn	Winter
C 6:0***	0.17±0.37a**	1.28±1.93a	0.32±0.31a	–
C 8:0	0.06±0.14b	0.02±0.03b	0.12±0.1b	0.58±0.52a
C 10:0	0.06±0.12a	0.13±0.2a	0.1±0.17a	0.09±0.14a
C 12:0	0.05±0.1a	0.06±0.09a	0.1±0.12a	–
C 13:0	–	0.27±0.4a	0.11±0.13a	0.04±0.09a
C 14:0	2.24±0.59a	1.5±0.09b	1.75±0.17a	2.09±0.3a
C 15:0	0.76±0.17a	1.05±0.38a	0.93±0.28a	1.09±0.44a
C 16:0	14.6±0.28b	16.6±1.04a	15.7±0.54a	15.8±0.85a
C 17:0	1.4±0.21a	1.99±1.18a	1.77±0.51a	1.41±0.38a
C 18:0	4±1.31b	5.2±0.77a	4.17±0.58b	4.29±0.38b
C 19:0	0.54±0.27a	–	0.49±0.44a	0.09±0.13b
C 20:0	1.58±0.43a	–	0.16±0.23b	2.4±1.21a
C 21:0	0.72±0.5a	0.52±0.43a	0.83±0.15a	0.42±0.18a
C 22:0	0.36±0.27a	0.3±0.28ab	0.09±0.14b	0.34±0.07a
C 24:0	–	–	0.15±0.15a	0.96±0.66b
∑ SFA	26.6	28.9	26.8	29.6
C 14:1ω5	1.22±0.35a	0.59±0.06b	0.65±0.14b	0.67±0.28b
C 15:1 ω6	1.19±0.5b	1.83±0.83a	0.86±0.15b	1.08±0.54b
C 16:1 ω7	11.2±3.4a	5.11±0.84c	8.54±1.7b	13.2±1.6a
C 17:1 ω8	1.36±0.48a	1.72±0.51a	1.61±0.59a	1.53±0.97a
C 18:1 ω9	15.1±3.46b	15.4±0.95b	20.3±2.2a	19.6±3.02a
C 18:1 ω7	4.39±0.38a	1.72±0.01d	2.11±0.31c	3.52±0.55b
C 20:1 ω9	1.01±0.81b	1.88±0.36b	3.09±0.2a	1.48±1.09b
C 22:1 ω9	0.19±0.19a	–	0.10±0.10b	–
C 24:1 ω9	–	–	0.02±0.06a	–
∑ MUFA	35.7	28.3	37.3	41.1
C 16:2 ω4	2.77±0.98a	3.16±1a	1.64±0.2b	1.77±0.4b
C 18:2 ω6	7.83±2.44a	8.32±5.2a	10.5±0.49a	3.64±0.99b
C 18:3 ω6	4.38±1.93a	2.66±0.39b	3.52±1.41ab	1.57±0.37ab
C 18:3 ω3	1.09±0.72a	0.38±0.07b	0.66±0.29ab	0.68±0.36a
C 20:2 ω6	–	–	0.23±0.3a	0.15±0.23b
C 20:3 ω6	0.92±0.41ab	0.71±0.66b	1.31±0.28a	1.15±0.3ab
C 20:3 ω3	0.7±0.24a	0.39±0.31a	0.51±0.23a	0.57±0.34a
C 20:4 ω6	5.38±2.15ab	6.99±1.36b	5.57±1.84a	4.38±0.33a
C 20:5 ω3	5.69±0.56a	4.72±1.14b	4.10±0.61b	4.82±1.06b
C 22:3 ω3	–	–	–	0.13±0.19a
C 22:4 ω6	0.71±0.41b	0.33±0.25ab	0.72±0.55ab	0.95±0.51a
C 22:5 ω6	0.46±0.15b	1.79±0.97a	0.55±0.17b	0.98±0.26b
C 22:5 ω3	2.89±1.05a	2.33±0.32ab	2.23±0.35ab	2.11±0.09b
C 22:6 ω3	4.94±0.58b	11.0±3.07a	4.32±0.58b	6.4±1.74b
∑ PUFA	37.8	42.8	35.9	29.3
ω3	15.3	18.9	11.8	14.7
ω6	20.9	22.6	23.3	13.9
ω3/ω6	0.73	0.83	0.50	1.06

*Average of three lots analysed.
**Values reported are means ± SD.
***abc values for each sample with different letters in the same fraction are significantly different at $P < 0.05$.

Oleic acid was identified as a primary MUFA in the carp for all seasons (15.1–20.3%). Csengeri and Farkas (1993), Kim and Lee (1986), Kolakowska et al. (2000), Paaver, Kuusik, Gross, Mottus, and Tohvert (2002) found similar results in carp and Haliloğlu, Bayır, Sirkecioğlu, Aras, and Atamanalp (2004) also found similar results for other freshwater fish. Palmitoleic acid was the second most abundant MUFA (5.11–13.2%) in the present study. The high levels of oleic, palmitoleic, and arachidonic acids had been reported as a characteristic property of freshwater fish oils (Andrade, Rubira, Matsushita, & Souza, 1995).

PUFA contents in carp muscle have been reported to be in a very wide range: 11.6–15.7% of total fatty acids (Bieniarz, Koldras, Kaminski, & Mejza, 2000) to 32.3–34.5% (Geri, Poli, Gualtieri, Lupi, & Parisi, 1995). In this work, the PUFA contents were generally much higher than the SFA in spring, summer and autumn 37.8%, 42.8%, 35.9%, respectively. MUFA contents of carp fillets were higher than SFA in spring, autumn and winter, 35.7%, 37.3%, 41.1%, respectively. In summer, a high ratio of DHA (11.0%) increased the PUFA content and a low level of palmitoleic acid lowered the MUFA contents of carp fillets. In winter, a low level of C 18:2ω6 lowered the PUFA content in this species. The reason for the observed results is likely the feeding habits of the fish. The percentages of PUFA, such as EPA and DHA, in fish muscle are dependent on diet (Arts et al., 2001; Sargent, 1997). Variations in fatty acid composition might be related to the changes in nutritional habits of the fish (Norrobin, Olsen, & Tande, 1990). The low PUFA content (29.3%) in the carp in winter may be attributed to this reason.

Marketable carp from natural waters exhibited high concentrations of linoleic acid (LA), EPA and DHA while carp fed diets rich in carbohydrates showed a high level of oleic acid in muscles (Farkas, Csengeri, Majoros, & Olah, 1978). Kim and Lee (1986) reported that LAω6 (3.9%), EPA (6%), DHA (5%), LnAω3 (6%) and AA (3.5%) were the most obvious PUFA in carp. In our study, similarly, LAω6 (3.64–10.5%), EPA (4.10–5.69%), AA (4.38–6.99%) and DHA (4.94–11.0%) were the most obvious PUFA in the carp for all seasons. Similarly, Rasoarahona et al. (2004) found that oleic (17.0–21.5%), palmitic (13.1–16.1%) and linoleic (9.6–13.2%) acids are the dominant fatty acids and AA (2.9–5.9%), DHA (2.9–6.7%), EPA (1.9–3.4%) and docosapentaenoic acid (22:5 ω3, DPA, 1.6–4.3%) are also present.

Bowman and Rand (1980) reported that AA is a precursor for prostaglandin and thromboxane which will influence blood clot formation and its attachment to the endothelial tissue during wound healing. Apart from that, the acid also plays a role in growth. In our study, the carp have higher contents of AA in spring, summer, autumn and winter, 5.38%, 6.99%, 5.57%, 4.38%, respectively.

EPA and DHA, found only in fish and seafoods, possess extremely beneficial properties for the prevention of human coronary artery disease (Leaf & Weber, 1988). Therefore, fish have been suggested as a key component for a healthy human diet (Rahman et al., 1995). Among the ω3 series, carp were good sources of EPA and DHA. The percentages of EPA + DHA in the carp were 15.9%, 11.2%, 10.6% and 8.42% in summer, winter, spring and autumn, respectively. Similarly, zander (*Sander lucioperca*) in the same lake also was good source of EPA + DHA with 29.2%, 28.3%, 24.3% and 21.3% in spring, autumn, winter and summer, respectively (Guler, Aktumsek, Citil, Arslan, & Torlak, 2007). Kim and Lee (1986) found that EPA + DHA were 11.1% and Csengeri and Farkas (1993) found them at 15.3% in carp. The amounts of EPA + DHA in the carp were found to be higher with respect to the results of Fajmonova, Zelenka, Komprda, Kladroba, and Sarmanova (2003) and reports of other freshwater fish species (Turon, Rwabwogo, Barea, Pina, & Graille, 2005).

The ratio of ω3 PUFAs/ω6 PUFAs in total lipids of freshwater fishes changes mostly between 0.5 and 3.8, whereas for marine fishes it is 4.7–14.4 (Henderson & Tocher 1987). The ω3/ω6 ratio has been suggested to be useful indicator for comparing relative nutritional values of fish oils (Piggot & Tucker, 1990). An increase in the human dietary ω3/ω6 fatty acid ratio is essential in the diet to help prevent coronary heart disease by reducing plasma lipids and to reduce cancer risk (Kinsella, Lokesh, & Stone, 1990). The increase in the dietary ω3/ω6 fatty acid ratio in favor of ω3 fatty acids also seems to be effective in preventing shock syndrome and cardiomyopathy (Bell, McVicar, Park, & Sargent, 1991). Muscles of common carp reared in warm water showed a higher ω3/ω6 PUFA ratio with 1.52 in comparison with carp of same age reared in water of natural temperature with 0.47 (Geri et al., 1995) and Fajmonova et al. (2003) found this ratio as 0.5 in the carp fillets. In our study, the average of the carp ratio is near 1 in winter, spring and summer and decreased until 0.5 in autumn. This is explained by a decreasing value of the ω3 PUFA from 18.9 to 11.8 in autumn. Rasoarahona, Barnathan, Bianchini, and Gaydou (2005) have found similar results in other freshwater fishes from Madagascar. Furthermore, in our study, the high amounts of 18:2ω6 decreased the ω3/ω6 ratio in the season. Similarly, Tokur, Ozkutuk, Atici, Ozyurt, and Ozyurt (2006) found that mirror carp fillets in Seyhan Dam Lake in Turkey had high amounts of 18:2ω6.

This study has shown that the carp is a desirable item in the human diet in the Beysehir Lake of Turkey when the levels of EPA, DHA and ω3/ω6 ratio are considered. This condition can be regarded as an explanation for the fact that the carp in Beysehir Lake are richer in ω3 fatty acids in winter, spring and summer, taking into consideration with the fatty acid profile of the fish. As a consequence, when human health is taken into account, the carp from Beysehir Lake appears to be quite nutritious in terms of fatty acid composition and ratio.

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