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Seasonal changes in the total fatty acid composition of Vimba, *Vimba vimba tenella* (Nordmann, 1840) in Eğirdir Lake, Turkey

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ABSTRACT

Total fatty acid compositions and its seasonal variations in Vimba, *Vimba vimba tenella* (Nordmann, 1840) in Eğirdir Lake, which is the second largest freshwater lake in Turkey, were investigated by a gas chromatographic method. Twenty seven different fatty acids were determined in the composition of *Vimba vimba tenella.* Monounsaturated fatty acids (MUFAs) were found to be in higher amounts than saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) in all seasons. Oleic acid (C18:1 n9) was the major MUFA in all seasons. Palmitic acid (C16:0) was identified as the major SFA in all four seasons. Arachidonic acid (C20:4 n6), docosahexaenoic acid (C22:6 n3), linoleic acid (C18:2 n6), and eicosapentaenoic acid (C20:5 n3) were at the highest levels among the PUFAs. In the present study, n-3/n-6 ratios were found to be 1.4,1.5,1.2 and 1.4 in spring, summer, autumn and winter, respectively. *Vimba vimba tenella* may be a valuable food for human consumption in terms of fatty acids.

1. Introduction

During recent years, fish lipids have been focused on as being beneficial for human health. Today, it is known that $n-3$ fatty acids or a balanced *n*-3/*n*-6 ratio in the diet are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, diabetes, hypertension and cancer. They also affect neurodevelopment in infants, fat glycemic control, learning ability and visual functions (Kinsella, Lokesh, & Stone, 1990). Recent studies have shown that an increased intake of long chain polyunsaturated fatty acids during pregnancy increases the length of gestation and birth size. This suggests that maternal long chain polyunsaturated fatty acid status may be critical in the development of the fetus (Muskiet et al., 2006). Major depression is associated with lowered $n-3$ PUFA levels (Hibbeln, 1998; Maes et al., 1999).

Fish lipids are well known to be rich in long chain $n-3$ polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids play vital roles in human nutrition, disease prevention and health promotion. Long chain $n-3$ PUFAs cannot be synthesised by humans and must be obtained through the diet (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002).

The n-3 fatty acids are always present in fish flesh, even in lean fish (Ackman, 2002). The n-3 and n-6 PUFAs are also considered essential for the growth and development of children and they are precursors of composite hormones known as eicosanoids, involved in several metabolic processes of great importance for the human body, mainly related to cardiovascular activity (Eder, 1995; Inhamuns & Franco, 2008).

The fat content and the fatty acid composition of fish are not constant (Zlatanos & Laskaridis, 2007). The amount of longer-chain n-3 PUFAs differs among species and can be influenced by a number of factors. The fatty acid composition of fish tissue can be affected by diet, size, age, reproductive cycle, salinity, temperature, season and geographical location (Henderson & Tocher, 1987; Inhamuns & Franco, 2008).

Research indicates that fresh water fish generally have lower levels of $n-3$ PUFA than have marine fish. Fish need PUFA to provide the lower water temperature adaptation. Fatty acids of cold and deep sea fish are abundant and the melting temperatures of $n-3$ fatty acids are lower than are the $n-6$ fatty acids (Celik, Diler, & Kucukgulmez, 2005; Rahman, Huah, Hassan, & Daud, 1995). Some fish require a mixture of $n-3$ and $n-6$ fatty acids, or, as in the case of *Tilapia zilli*, $n-6$ fatty acids only (Kanazawa, Tanaka, Teshima, & Kashiwada, 1971). A possible reason that fish, especially cold water species, require $n-3$ instead of $n-6$ fatty acids is that the $n-3$ structure permits a greater degree of unsaturation, which is necessary in the membrane phospholipids to maintain flexibility and permeability characteristics at low temperatures (Lovell, 1991). Generally, a decrease in temperature results in an increase in the degree of unsaturation (Henderson and Tocher,1987).

Vimba vimba tenella is a common fish species in western Turkey. Its diet includes copepods, crustaceans and benthic worms. The

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vimba breeds between May and July and temperature may be an influential factor in its growth (Geldiay & Balik, 1996).

There is no previous study of the fatty acids of *Vimba vimba tenella* in Turkey. Thus, the fatty acid dynamics of this species are not yet well known. The main objective of this study was to determine the seasonal variations of the total fatty acid composition in the muscles, and n-3/n-6 fatty acids ratio of *Vimba vimba tenella*.

2. Materials and methods

Vimba vimba tenella, used in this study, were obtained from Eğirdir Lake, Turkey. Eğirdir Lake is the second largest lake in Turkey. *V. v. tenella* feeds on copepods, crustaceans and benthic worms (Geldiay & Balik, 1996). In the present study, the four seasons have been chosen for analysis. The samples were collected in the middle month of each season during 2006. After being caught, they were transported in ice to the laboratories, filleted and frozen. At the beginning of each analysis, the samples were allowed to equilibrate to room temperature, ground and homogenized in a chloroform/methanol mixture (2/1, v/v).

The total lipids obtained were saponified by refluxing with methanol (50%) containing 6% KOH for 1 h. Samples of fillets were extracted by the Folch, Lees, and Sloane Stanley (1957) method and were transesterified with BF_3/methanol (Moss, Lambert, & Merwin, 1974). The saponifiable lipids were converted to their methyl esters by using the standard boron tri-fluoride-methanol (BF_3) method. Fatty acids methyl esters were analyzed on a HP Agilent 6890 N model gas chromatograph, equipped with a flame ionization detector 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 programme 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 retention times with those obtained for Alltech standards. Results were expressed as FID response area in relative percentages. Each reported result is the average of three GC analyses. The results are recorded as means ± SD. The results were submitted to analysis of variance at 0.05 significant level, using LSD.

3. Results and discussion

Seasonal variations of total fatty acid composition of *Vimba vimba tenella* are presented in Table 1. We found 27 fatty acids in muscle lipids of Vimba. The highest fatty acids in the fish in all seasons were found to be 18:1n-9, 16:0, 16:1n-7, 22: 6 n-3, 20:5 n-3 and 20:4 n-6.

Oleic acid (C18:1 n9) was identified as a primary monounsaturated fatty acid (MUFA) in the *Vimba vimba tenella* for all seasons. This fatty acid in muscle tissue of vimba was found to be at levels of 22.4%, 25.4%, 26.2% and 28.4% in spring, summer, autumn and winter, respectively. The highest level of oleic acid was in winter. Similarly Guler, Aktumsek, Citil, Arslan, and Torlak (2007) found that C18:1 n9 was the major MUFA in muscle in tissue of zander, *Sander lucioperca* living in freshwater in Turkey. According to Akpinar, Görgün, and Akpinar (2009), oleic acid was the major monounsaturated fatty acid in liver (15.6–17.6%) and muscle (22.4–22.1%) of male and female *Salmo trutta macrostigma*. The high levels of oleic, palmitoleic, and arachidonic acids had been reported as a characteristic property of freshwater fish oils (Andrade, Rubira, Matsushia, & Souza, 1995). In the present study, these fatty acids were found to be at higher levels than linoleic and linolenic acids. Palmitoleic acid C16:1 n-7 was the second major MUFA (11.5–12.3%) in the present study. There were no differences between spring, summer, autumn and winter in terms of C16:1 n-7 ($p < 0.05$). Although vimba had the most stable fatty acid composition, there were quantitative differences. C14:1 n-5 and C15:1 n-6 were found to be low in the MUFA fractions of the muscle investigated. On the other hand, MUFA contents were higher than the SFAs and PUFAs in spring, summer, winter and autumn. In winter, a high ratio of C18:1 n-9 (28.4%) increased the MUFA content and a high ratio of C20:4 and C22:6 increased the PUFA content in spring. Variations in the fatty acid composition might be related to the changes in nutritional habits of the fish (Norrobin, Olsen, & Tande, 1990). In the present study, variations in MUFA and PUFA contents in the vimba may be attributed to the season. Apart from that, size, age, reproductive status of fish, environmental conditions, and especially water temperature, influence lipid content and fatty acid composition of fish muscle to a certain extent (Ackman, 1989).

Saturated fatty acids were lower than total monounsaturated fatty acids. The ratio of total SFAs ranged from 24.2 to 29.3%. Palmitic acid was the primary saturated fatty acid, 20.0–16.2% for *V. vimba. tenella* in all seasons. Similar results for other fish species have also been reported in the literature (Celik et al., 2005; Guler, Kiztanir, Aktumsek, Citil, & Ozparlak, 2008; Rahman et al., 1995). Zlatanos and Laskaridis (2007) determined seasonal variation in the fatty acid composition of anchovy and picarel. C16:0 was found to be the predominant fatty acid for anchovy and picarel in four and five sampling months, respectively. Picarel had the highest C18:1n-9 content over the whole year among the other species. Because of its high C18:1 n-9 content, the picarel exhibited the high-

Table 1
Seasonal variations in total fatty acid composition of fillets of Vimba (*Vimba vimba tenella*) from Eğirdir Lake* (% of total FA).

Fatty acids	Spring	Summer	Autumn	Winter
C 8:0***	0.02 ± 0.02a**	–	0.01 ± 0.02a	–
C10:0	0.01 ± 0.01a	0.1 ± 0.10a	0.02 ± 0.02a	0.01 ± 0.03a
C 11:0	0.02 ± 0.02a	0.01 ± 0.01a	0.03 ± 0.04a	–
C 12:0	0.1 ± 0.01b	0.1 ± 0.02a	0.1 ± 0.03a	0.1 ± 0.02b
C 13:0	0.04 ± 0.01a	0.04 ± 0.01a	0.03 ± 0.01b	0.02 ± 0.01c
C 14:0	1.4 ± 0.03c	1.8 ± 0.05a	1.6 ± 0.15b	1.4 ± 0.10c
C 15:0	0.7 ± 0.01b	1.1 ± 0.30a	0.7 ± 0.11b	0.6 ± 0.04b
C 16:0	16.2 ± 0.22c	19.2 ± 0.50b	20.01 ± 0.87a	18.4 ± 0.56b
C 17:0	1.1 ± 0.12a	1.3 ± 0.11a	1.1 ± 0.24a	1.0 ± 0.24a
C 18:0	3.5 ± 0.16b	5.0 ± 0.14a	4.6 ± 0.63a	4.5 ± 0.97a
C 20:0	0.3 ± 0.02a	0.3 ± 0.07a	0.3 ± 0.09a	0.2 ± 0.03a
C 22:0	0.6 ± 0.08a	0.3 ± 0.02c	0.4 ± 0.05b	0.4 ± 0.02b
ΣSFA	24.2	29.3	28.9	26.6
C 14:1 n5	0.2 ± 0.01b	0.2 ± 0.01b	0.2 ± 0.04a	0.2 ± 0.01c
C 15:1 n6	0.8 ± 0.27ab	0.8 ± 0.03a	0.6 ± 0.10bc	0.5 ± 0.10bc
C 16:1 n7	12.3 ± 0.69a	11.6 ± 1.61a	12.2 ± 1.53a	11.5 ± 0.05a
C 17:1 n8	2.0 ± 0.16b	3.0 ± 0.80a	2.0 ± 0.22b	1.9 ± 0.34b
C 18:1 n9	22.4 ± 1.96c	25.4 ± 0.35b	26.2 ± 2.45b	28.4 ± 0.14a
C 20:1 n9	2.7 ± 0.37a	2.5 ± 0.37a	2.4 ± 0.62a	2.8 ± 0.64a
ΣMUFA	40.4	43.5	43.6	45.3
C 18:2n6	4.3 ± 0.09a	3.1 ± 0.15c	4.0 ± 0.40b	3.4 ± 0.06c
C 18:3n6	0.5 ± 0.05b	0.8 ± 0.16a	0.4 ± 0.11b	0.5 ± 0.22b
C 18:3n3	2.7 ± 0.05a	1.7 ± 0.10c	2.3 ± 0.62ab	1.8 ± 0.74bc
C 20:2 n6	1.4 ± 0.14a	1.1 ± 0.16b	1.0 ± 0.21b	1.0 ± 0.16b
C 20:4 n6	6.3 ± 0.82a	4.0 ± 0.35b	5.5 ± 0.26a	5.3 ± 0.91a
C 20:5 n3	5.3 ± 0.72b	6.7 ± 0.99a	4.8 ± 0.91b	4.3 ± 0.25b
C 22:4 n6	0.8 ± 018a	0.6 ± 0.23a	0.6 ± 0.17a	0.5 ± 0.25a
C 22:5 n3	2.7 ± 0.02a	1.9 ± 0.04b	1.7 ± 0.38b	2.6 ± 0.77a
C 22:6 n3	8.7 ± 1.31a	5.7 ± 0.53b	5.4 ± 2.10b	6.9 ± 0.27b
ΣPUFA	32.7	25.6	25.7	26.3
Unknown	3.1	1.8	2.0	2.0
Σn3	19.4	16.0	14.2	15.6
Σn6	14.1	10.4	12.1	11.2
n3/n6	1.4	1.5	1.2	1.4

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

est monounsaturated fatty acid content. The major fatty acid identified in the Vimba was 18:1 n-9, while C16:0 was the second fatty acid. C8:0 was only found in spring and autumn in low amounts (0.01–0.02%). C10:0, C11:0, C12:0, C13:0 were found to be low in the SFA fractions of the muscle investigated. Stearic acid (C18:0) was the second major SFA (3.6–5.0%). There were no differences between seasons in terms of C17:0 and C20:0 in SFA ($p < 0.05$).

The *n*-3/*n*-6 ratio is a good index for comparing relative nutritional value of fish oils (Pigott & Tucker, 1990). In this study, data show that the n-3/n-6 ratio was 1.4 in spring, 1.5 in summer, 1.2 in autumn and 1.4 in winter. An increase in the human dietary n-3/n-6 fatty acid ratio is essential in the diet to help prevent coronary heart disease by reducing plasma lipids and to reduce cancer risk (Kinsella et al., 1990). Studies from Scandinavia, The Netherlands and Japan showed that people who ate fish about twice a week (240 g total weekly intake) had lower risks of heart attacks than had people who rarely ate fish (Wardlaw, Insel, & Seigler, 1992). According to Guler et al. (2007) the ratio of n-3/n-6 fatty acids was 1.49 in spring, 1.45 in autumn, 1.22 in winter and the lowest value (0.72) was in summer in *Sander lucioperca*. A high level of n6 fatty acids lowered the n-3/n-6 ratio in summer in *Sander lucioperca*, which is a freshwater fish. Our study has revealed that *V. v. tenella* is a freshwater fish species with a high nutritional value for human consumption due to its high n-3/n-6 ratio.

The present data has shown that DHA (22:6n3) was the predominant fatty acid in muscle lipids of *V. v. tenella*. In spring, a high ratio of DHA (8.7%) increased the PUFA content. Sargent (1996) has reported that n3 PUFAs, principally DHA, have a role in maintaining the structure and functional integrity of fish cells. The percentages of EPA and DHA were 4.3–6.7% and 5.4–8.7% in all seasons, respectively. Thus, among the 3 series the vimba are good sources of EPA and DHA. The percentages of PUFA, such as EPA and DHA, in fish muscle, depend on diet (Sargent, 1997). Nutritionists believe that the ratio n-6:n-3 should be 5:1 and that the addition of n3 PUFAs to food could improve the nutritional picture and help to prevent diseases. The amounts of EPA and DHA for daily ingestion have been suggested to be in the range 200–1000 mg (Inhamuns & Franco, 2008; Simopoulos, 1991). Inhamuns and Franco (2008) have determined EPA and DHA in two species of freshwater fish from central Amazonia (*Hypophthalmus* sp. and *Cichla* sp.). The authors reported that relatively high amounts of DHA have been found in freshwater species. Gokce, Tasbozan, Celik, and Tabakoglu (2004) reported that the percentages of EPA and DHA, which have a vital role in human nutrition, are 3.36–4.26 and 16.8–20.2, respectively, in the *Solea solea*.

Freshwater fish normally contain $n-6$ PUFAs, whereas marine fish are rich in $n-3$ fatty acids, especially DHA and EPA (Wang, Miller, Peren, & Addis, 1990). In our study, PUFA levels were found to be 32.7 and 25.6%; α- linolenic acid level was found to be low (1.7–2.7%). However, the levels of γ-linolenic acid (0.4–0.8%), arachidonic acid (4.0–6.3%), and linoleic acid (3.1–4.3%) were found to be different from season to season. In these fatty acids, arachidonic acid level is quite important. It was found to be the fourth highest fatty acid in autumn (5.5%). Guler et al. (2008) have found that carp have higher contents of arachidonic acid in spring, summer, autumn and winter, 5.38%, 6.99%, 5.57%, 4.38%, respectively. Arachidonic acid is a precursor for prostaglandin and thromboxane, which influence blood clot formation and its attachment to the endothelial tissue during wound healing (Bowman & Rand, 1980). C18:3 n-6 and C20:2 n-6 were found to be low in the n-6 PUFA fractions of tissues investigated. It has also been found that C22:4 n-6 (0.5–0.8%) was quite low. There were no significant differences between spring, summer, autumn and winter in terms of C22:4 n6 ($p < 0.05$).

This study has shown that vimba from the Egirdir Lake of Turkey is a desirable item in the human diet when its levels of EPA, DHA and n-3/n-6 ratio are considered. The fish was found to be good source of n-3 fatty acids.

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