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DETERMINATION OF FATTY ACID COMPOSITIONS AND CHOLESTEROL LEVELS OF SOME FRESHWATER FISH LIVING IN PORSUK DAM, TURKEY

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*We determined the oil content, fatty acid composition, and cholesterol content of common carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*), chub (*Leusiscus cephalus*), and tench (*Tinca tinca*) by GLC. The saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) levels were found to be 36.49%, 31.92%, 31.59% in common carp; 32.92%, 32.21%, and 34.87% in crucian carp; 36.19%, 32.91%, and 30.90% in chub; and 32.86%, 30.77%, and 36.37% in tench, respectively. The cholesterol (mg/100 g oil) levels of common carp, crucian carp, chub, and tench were determined by GLC methods as 119±2.64 mg, 170.37±2.36 mg, 94.68±3.13 mg, and 179.84±6.75 mg, respectively. Thus, the cholesterol contents of the analyzed freshwater fish species were low but their PUFA contents and nutritional values were high.*

Key words: fatty acids, cholesterol, common carp, crucian carp, chub, tench.

Polyunsaturated fatty acids (PUFA) are considered essential fatty acids. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found only in fishes and seafood and have extremely beneficial properties for the prevention of human coronary artery disease, arterial hypertension, human breast cancer growth, inflammatory disease, asthma, and disorders of the immune system [1, 2]. All of the risks mentioned above, especially cardiovascular diseases, are reduced by increased consumption of fish or fish products which are rich in PUFA of the n-3 family and poor in PUFA of the n-6 family. The ratio of n-6 PUFA to n-3 PUFA (derived from fish oils) in the diet should be reduced from the current levels of from 10 to 20:1 to the traditional range of around 1 to 2:1 [3, 4].

According to the World Health Organization [5] the maximum cholesterol should be 300 mg/day. There is a linear relationship between dietary cholesterol and blood cholesterol [6].

The aim of this study was to determine the fatty acid compositions and cholesterol amounts in fish species living in Porsuk Dam in Kutahya.

Table 1 shows oil (%) and cholesterol contents of four fish species. According to Table 1, the total fat content of *Cyprinus carpio* was a little bit higher than that of other fish species. The findings of previous studies are similar to those of this study.

The cholesterol contents of the fish species analyzed ranged from 94.68±3.13 to 179.84±6.75 mg/100g. The fat and cholesterol content of fish depend on what they eat, as well as on age, sex, spawning cycle, season, and geography. Fish caught during the spawning season or in waters having sparse food supplies have a lower fat and cholesterol content than usual, and decreasing PUFA in diet increases the cholesterol level [7]. In five commercial fish species in Brazil, Luzia et al. [6] found that cholesterol levels were between 66.8 mg/100g and 165 mg/100g. Table 1 shows that our findings are similar to these results.

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TABLE 1. % Oil and Cholesterol Contents of the Fish Species

Species	Oil, %	Cholesterol (mg/100 g oil)
Cyprinus carpio	5±0.26	119±2.64
Carassius carassius	4.5±0.20	170.37±2.36
Leusiscus cephalus	3.5±0.36	94.68±3.13
Tinca tinca	4±0.26	179.84±6.75
Mean	4.25±0.27	140.97±3.72

TABLE 2. Fatty Acid Compositions of the Fish Species, %

Fatty acids	*Cyprinus carpio*	*Carassius carassius*	*Leusiscus cephalus*	*Tinca tinca*	Mean
14:0	3.56±0.17	2.05±0.05	3.18±0.21	3.10±0.11	2.97±0.14
15:0	0.52±0.02	0.45±0.02	0.60±0.01	0.64±0.01	0.55±0.02
16:0	21.77±0.76	21.49±0.70	23.74±1.50	20.87±1.24	21.96±1.05
17:0	0.85±0.01	0.57±0.01	0.92±0.04	0.80±0.07	0.79±0.03
18:0	6.94±0.34	6.14±0.15	5.48±0.65	4.90±0.17	5.87±0.33
20:0	1.89±0.05	0.88±0.01	1.05±0.05	1.31±0.06	1.28±0.05
22:0	0.36±0.02	0.78±0.02	0.47±0.03	0.60±0.01	0.55±0.02
24:0	0.59±0.01	0.55±0.03	0.75±0.05	0.63±0.02	0.63±0.03
$\Sigma_{Sat.}$	36.49	32.92	36.19	32.86	34.62
16:1	7.98±0.28	6.82±0.23	9.05±0.41	9.64±0.73	16.72±0.41
18:1	23.61±0.97	24.68±0.42	23.29±1.77	20.39±0.84	22.99±1.00
20:1	0.20±0.01	0.47±0.01	0.41±0.01	0.52±0.04	0.40±0.02
22:1	0.12±0.01	0.23±0.02	0.17±0.01	0.22±0.01	0.37±0.01
$\Sigma_{Monounsat.}$	31.92	32.21	32.91	30.77	31.96
18:2 *trans* n-6	5.10±0.13	7.03±0.10	4.26±0.30	4.14±0.27	5.13±0.20
18:2 *cis* n-6	5.07±0.08	6.01±0.01	3.99±0.27	5.45±0.56	5.13±0.23
18:3 n-3	2.53±0.02	3.69±0.20	3.53±0.15	4.90±0.18	3.66±0.18
18:4 n-3	1.05±0.08	0.62±0.03	1.06±0.06	1.33±0.06	1.02±0.06
20:3 n-3	0.20±0.02	0.51±0.01	N.d.	0.14±0.02	0.21±0.02
20:4 n-3	0.26±0.01	0.22±0.01	0.11±0.03	0.22±0.01	0.20±0.01
20:5 n-3	5.32±0.26	6.08±0.17	6.22±0.29	7.61±0.36	6.31±0.27
22:5 n-3	2.42±0.03	2.72±0.44	2.68±0.21	3.34±0.29	2.79±0.24
22:6 n-3	9.64±0.65	8.00±0.23	9.05±0.24	9.24±0.65	8.98±0.44
$\Sigma_{Polyunsat.}$	31.59	34.87	30.90	36.37	33.43
EPA+DHA	14.96	14.08	15.27	16.85	15.29
ω3/ω6	2.11	1.67	2.73	2.80	2.33

N.d.: not detected.

Table 2 shows the fatty acid compositions of four fish species in summer. According to the table 2, the dominant saturated fatty acid (SFA) was 16:0 ($C_{16:0}$), which was found at the highest level in *Leusiscus cephalus* 23.74±1.50%. *Cyprinus carpio* had the highest total SFA (36.49%). Oleic acid ($C_{18:1}$) was the main monounsaturated fatty acid (MUFA), which was found at the highest level (24.68±0.42%) in *Carassius carassius*. The total polyunsaturated fatty acid (PUFA) content and EPA+DHA ratio of *Tinca tinca* were the highest, 36.37% and 16.85%, respectively. The other fish species approximately showed the same EPA + DHA ratios. Rahman et al. [8], Kinsella et al. [7], and Luzia et al. [6] have reported that EPA and DHA were the main omega-3 fatty acid in freshwater fish, and with respect to the omega-3 fatty acid content, there were no significant differences among these fish species. In the warm season, fishes in waters that are poor in nutrients and mineral salts have decreased PUFA content [2]. Also the fatty acid contents of these fishes are apparently affected by factors such as season, temperature, diet, age, size, and sex [2].

The omega-3 (n-3)/omega-6 (n-6) fatty acid ratio is a better index for comparing the relative nutritional value of fish oil for different species, which is suggested to be 1 to 2:1 in the diet [9].

The ratios of ω3/ω6 fatty acids in the oils extracted from common carp, crucian carp, chub, and tench were 2.11, 1.67, 2.73, and 2.80, respectively (Table 2). The ω3 fatty acid contents of these fishes were two or three times higher than the ω6 fatty acid contents.

While the level of fat was determined to be of medium level, whereas the cholesterol contents were found to be very low, EPA and DHA contents of omega-3 fatty acids were also determined as high. From the nutritional perspective, fat, fatty acids, and cholesterol are important criteria for human nutrition.

EXPERIMENTAL

Lipid Extraction. All fishes were obtained from the Porsuk Dam in October 2005 in Kutahya. Each of the four species were analyzed three times. The fish were first wrapped with two layers of polyethylene plastic and immediately iced. The tissue of the fish species was homogenized, and 10 g fish lipid was extracted with 60 mL methanol and 30 mL chloroform according to Kinsella et al. [7]. Chloroform was removed by evaporation under vacuum. The residual chloroform was removed by nitrogen and the oil was weighed.

Preparation of Fatty Acid Methyl Esters. Fatty acid methyl esters were prepared according to AOCS [10]. Fish oil (approximately 200 mg) was accurately weighed into a stoppered-glass centrifuge vial, then 2 mL of hexane was added, followed by 0.1 mL of 2 g/L methanolic KOH. Then the vial was closed and shaken well for 30 s, then centrifuged. Two drops of the upper layer were removed and diluted with 2.0 mL of hexane. The sample was injected into a capillary column for GC analysis using split injection.

Determination of Fatty Acid Compositions by Gas Chromatography. The instrumentation used for the analysis was as follows: a Hewlett-Packard GC (Model 6890) equipped with Supelco SP-2380 fused silica capillary column (60 m × 0.25 mm i.d., 0.2 mm film thickness; Supelco Inc., Bellefonte, PA USA), and a flame ionization detector. The injection volume was 2 μL. The temperature of the GC oven was programmed from 100 to 220°C at the rate of 4°C/min. The injector and detector temperatures were 300°C. Nitrogen was used as the carrier gas, and the flow rate was 1 mL/min. The split ratio was set at 1:100.

Determination of Cholesterol. Cholesterol was determined by the procedure described by Fletouris et al. [11]. For preparation of cholesterol standards, the stock solution (2 mg/mL) was prepared by dissolving 20 mg of reference standard (Sigma Chemical Company, St. Louis, MO, USA) with hexane in a 10 mL volumetric flask. Working solutions were prepared by appropriately diluting aliquots from the stock solution with hexane to obtain solutions in the range of 10–80 mg/mL. GC conditions used for analysis were as follows: Ultra1 fused silica capillary column (25 m × 0.32 mm i.d., 0.52 mm film thickness; Hewlett-Packard, USA). Oven temperature was set at 285°C, injection temperature at 300°C, and flame ionization detector temperature at 300°C. The flow rates were 2 mL/min for nitrogen, 30 mL/min for hydrogen, and 300 mL/min for air. The injection volume was 2 μL with a split ratio of 20:1. The concentration of cholesterol (C) in the analyzed samples was calculated according to the equation $C = M \times V \times 2:5$, where M is the computed mass (nanograms) of the analyst in the injected extract (1 mL) and V is the dilution factor, if any, that was applied.

Recovery. The percentage recovery was determined by adding a known concentration of the cholesterol standard to selected samples during extraction. The amounts added were roughly 50% of the actual concentration of the samples. The concentration of cholesterol standard in the mixture was then determined in the same way as the sample analysis. Without exception, recovery rates of >91% were achieved for the compound analyzed.

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