Chemistry of Natural Compounds, Vol. 47, No. 3, July, 2011 [Russian original No. 3, May–June, 2011]

COMPARISON OF FATTY ACID AMOUNTS AND RATIOS OF ω3 AND ω6 FATTY ACIDS IN MUSCLE OF SOME FRESHWATER FISH UNDER NATURAL EXTREME COLD CONDITIONS

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UDC 547.916

Fish oil is rich in long-chain ω3 polyunsaturated fatty acids (ω3 PUFAs), especially eicosapentaenoic and docosahexaenoic acids. Research has evidenced the role of ω3 PUFAs in the prevention of many diseases such as cardiovascular, cancer, alzheimer's, depression, inflammatory, and autoimmune diseases [1–6]. Nevertheless, the fatty acid composition of fish varies with many factor such as season, diet, size, and sex [7]. The most consistent biochemical response of fish to environmental cooling is an increase in fatty acid unsaturation of both membrane and depot lipids [8]. Since fish need polyunsaturated fatty acids (PUFAs) to provide tolerance to low water temperatures, it should be expected that fish in cold temperate waters accumulate high amounts of PUFAs. Therefore, fish in cold regions and seasons are more beneficial to human health than those in temperate conditions.

Lake Uluabat (or Apolyont) is a large lake in the vicinity of Bursa, Turkey. Crucian carp (*Carassius gibelio*), pike (*Esox lucius*), and rudd (*Scardinus erythrophthalmus*) are the most economically important fish in the lake. There are also important as a game fish in the region. Thus, the aim of the study is to evaluate the nutritional value of the fatty acids in fish and the proportions of ω3 PUFAs of the species in Lake Uluabat in natural extreme cold conditions.

The amounts of palmitic acid (16:0) and stearic acid (18:0) in 100 g total fatty acid of muscle were found to be 15.28 g and 5.38 g in crucian carp, 14.85 g and 3.97 g in pike, and 17.05 g and 3.73 g in rudd (Table 1). Palmitic and stearic acids were the most abundant saturated fatty acids in all species. Similar results concerning the ratios of these fatty acids also appeared for some fish in different habitats [9]. 100 g total fatty acid (TFAs) in muscle of crucian carp, pike, and rudd contained 24.84 g, 23.04 g, and 24.51 g saturated fatty acids (SFAs), respectively. While the feeding habits of these species are different, there was no difference among the amounts of SFAs of the species ($p > 0.05$).

Monounsaturated fatty acids MUFAs accounted for 22.15 g in crucian carp, 15.52 g in pike, and 24.04 g in rudd of the 100 g of TFA. The ratio of MUFAs in muscle of pike was remarkably lower than that of both crucian carp and rudd ($p < 0.05$).

The nutritional importance of fish is in great extent associated with the content of polyunsaturated fatty acids (PUFAs), particularly the ω3 fatty acids. The results indicated that a significant part of the fatty acids in the muscle of the species was polyunsaturated fatty acids (PUFA). In this study, PUFAs accounted for 39.73 g, 55.22 g, and 41.59 g of the 100 g of TFA in muscle of crucian carp, pike, and rudd, respectively. The contents of PUFAs and ω3 fatty acids were significantly higher ($p < 0.05$), while the contents of ω6 and ω9 fatty acids in muscle of pike were significantly lower compared to the those of crucian carp and rudd. These differences may arise from the differences of the species and their feeding habits, as indicated in the literature [10, 11]. Pike is from the Percidae family and a carnivorous species, whereas crucian carp and rudd are from the Cyprinidae family and an omnivorous species.

The ω3 and ω6 fatty acids are two biochemical families within the PUFAs, and they also have different biological effects [12]. In this study, the ω3 fatty acids comprised mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

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0009-3130/11/4703-0431

TABLE 1. Fatty Acid Compositions (g of 100 g total fatty acids) of the Species

Fatty acid	*C. gibelio* n = 12	*E. lucius* n = 5	*S. erythrophthalmus* n = 12	Fatty acid	*C. gibelio* n = 12	*E. lucius* n = 5	*S. erythrophthalmus* n = 12
12:0	0.06 ± 0.01	BDL	BDL	20:1	2.57 ± 0.18	1.46 ± 0.14	4.36 ± 0.6
13:0	0.01 ± 0.01	BDL	BDL	20:2	0.84 ± 0.05	0.32 ± 0.13	0.59 ± 0.02
14:0	0.9 ± 0.08	0.49 ± 0.05	0.89 ± 0.07	20:3 ω6	0.74 ± 0.03	BDL	0.51 ± 0.02
14:1	0.04 ± 0.01	BDL	0.02 ± 0.01	20:3 ω3	4.02 ± 0.14	3.02 ± 0.05	3.18 ± 0.17
15:0	0.44 ± 0.01	0.33 ± 0.02	0.46 ± 0.05	20:4 ω6	0.64 ± 0.05	0.14 ± 0.09	0.72 ± 0.06
15:1	0.04 ± 0.01	0.05 ± 0.03	BDL	20:5 ω3	7.86 ± 0.35	7.19 ± 0.36	7.84 ± 0.14
16:0	15.28 ± 0.27	14.85 ± 0.16	17.05 ± 0.28	21:0	0.28 ± 0.06	BDL	0.26 ± 0.05
16:1	3.53 ± 0.51	1.71 ± 0.13	5.8 ± 0.51	22:0	BDL	BDL	BDL
17:0	0.72 ± 0.03	0.41 ± 0.01	0.47 ± 0.03	22:1 ω9	0.44 ± 0.04	BDL	0.84 ± 0.09
17:1	0.51 ± 0.04	0.36 ± 0.09	0.28 ± 0.03	22:2 ω3	1.26 ± 0.04	0.19 ± 0.12	0.49 ± 0.03
18:0	5.38 ± 0.14	3.97 ± 0.16	3.73 ± 0.18	22:6 ω3	17.69 ± 1.01	41.97 ± 0.44	24.25 ± 1.12
18:1 ω9t	0.1 ± 0.02	BDL	0.18 ± 0.03	23:0	BDL	BDL	BDL
18:1 ω9c	8.77 ± 0.84	6.06 ± 0.3	8.35 ± 0.72	24:0	1.66 ± 0.11	2.98 ± 0.12	1.64 ± 0.2
18:2 ω6t	0.28 ± 0.02	BDL	0.17 ± 0.02	24:1	5.95 ± 0.18	5.88 ± 0.04	4.21 ± 0.06
18:2 ω6c	5.34 ± 0.47	2.3 ± 0.12	3.24 ± 0.17	Unknown	13.52 ± 1.13	6.22 ± 0.22	9.96 ± 0.54
18:3 ω6	0.12 ± 0.03	BDL	0.08 ± 0.03	ω3/ω6	4.81 ± 0.5	21.55 ± 0.52	7.8 ± 0.41
18:3 ω3	0.92 ± 0.03	0.1 ± 0.06	0.43 ± 0.07	EPA + DHA	25.55 ± 1.29	49.16 ± 0.12	32.08 ± 1.2
20:0	0.12 ± 0.03	BDL	0.01 ± 0.01				

BDL: below detection limit.

The ω6 fatty acids are also composed mainly of linoleic acid. The levels of linoleic acid, which was the main ω6 fatty acid, in 100 g total fatty acids, were 5.34 g in crucian carp, 2.30 g in pike, and 3.24 g in rudd. In the present study, it was observed that the DHA + EPA ratios in the muscle of crucian carp, pike, and rudd accounted for 25.55 g, 49.16 g, and 32.08 g of the 100 g of total fatty acids, respectively. The omega3 index (EPA + DHA as a percent of fatty acids in total red blood cell membranes) have been considered a new risk factor for death from coronary heart disease for humans. It has been shown that the desirable omega3 index in humans must be higher than 8 in order to reduce the incidence of coronary heart disease [3]. From the aspect of ensuring the omega3 index, it may be said that the investigated species are much a source of health food for humans.

In the present study, the ratio of ω3 to ω6 fatty acids was found to be 4.81 in crucian carp, 21.55 in pike, and 7.8 in rudd (Table 1). It was noted that the ratio of ω3 to ω6 fatty acids was usually 1–4 in freshwater fish and 5–14 in marine fish [13]. The present results indicate that the ratio of ω3 to ω6 fatty acids in muscle of pike is remarkably higher than that in marine fish. The ratios of ω3 to ω6 fatty acids in muscle of crucian carp and rudd were also higher than those in freshwater fish. Most poikilotherms like fish respond to thermal changes by changing the physical properties of their membranes, termed "homeoviscous adaptation." Docosahexaenoic acid (DHA) is an important constituent of ω3 fatty acids and plays a role in maintaining structural and functional integrity of membranes [14, 15]. This study was carried out in natural extreme cold conditions (winter). The high ratios of ω3 to ω6 fatty acids in this study may be attributed to homeoviscous adaptation of the fish. This adaptative property makes fish in cold conditions much healthier. It has been shown that the ratio of ω3 to ω6 fatty acids is a better index in comparing the nutritional value of fish and that the optimal ratio of ω3 to ω6 fatty acids must be 1:1. This ratio is also remarkably low in the western diet. Therefore, it is recommended that the ratio of ω3 to ω6 fatty acids in human diets be increased for health. It might be said that crucian carp, rudd, and especially pike in cold conditions are healthiest food for humans with respect to the ratio of ω3 to ω6 fatty acids.

Determination of Fatty Acids. The AOAC official method was carried out to obtain total lipid and fatty acid methyl esters [16]. Ten grams muscle tissue of each fish was homogenized, and lipid was extracted with 100 mL chloroform–methanol (2:1) mixture. The solvent was removed by evaporation under vacuum. The residual chloroform was then removed by nitrogen. The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% BF_3 (w/v) in methanol. Routine analysis of fatty acid methyl ester samples were carried out with an HP Agilent 7890 model gas chromatography equipped with a flame ionization detector (FID) using a capillary column (100 m length, 0.25 mm internal diameter, and 0.20 μm film thickness; HP 88). The oven temperature was programmed at an initial temperature of 170°C, increased at a rate of 1°C per min to 208°C, and further increased at a rate of 2°C per min to 240°C, then held at that temperature for 5 min. The injector and flame ionization detector were set at 250°C. Nitrogen was

used as a carrier gas. The injection volume was 1 μL with a split ratio of 1:50. Fatty acid peaks were identified by comparison of their retention times with the appropriate fatty acid methyl ester standards. Individual fatty acid concentrations were expressed as percentages of the total content.

Statistical Analyses. The results are presented as means ± SD (standard deviation). The statistical differences between major fatty acid groups (total saturated fatty acids, total monounsaturated fatty acids, total polyunsaturated fatty acids, total ω3 polyunsaturated fatty acids, and total ω6 polyunsaturated fatty acids) among the species were analyzed using multiple comparison tests (SPSS package program-one-way ANOVA). Results were considered significant at $p < 0.05$.

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