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Fatty Acid Profile of Muscles of Freshwater Fish from Olsztyn Markets

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The aim of the present study was to determine the profile of fatty acids in muscle lipids of freshwater fish: carp (*Cyprinus carpio* L.), rainbow trout (*Oncorhynchus mykiss* Walb.), bream (*Abramis brama* L.) and tench (*Tinca tinca* L.) from markets of Olsztyn (north-eastern Poland). Separation and identification of fatty acids were made using gas chromatography. The content of some fatty acids varied among species. Generally, palmitic acid (15.6%-19.5%) was the most abundant saturated fatty acid (SFA). The main monounsaturated fatty acid (MUFA) was oleic acid (20.7%-42.7%). Linoleic (4.6%-8.0%), arachidonic (AA) (0.8%-6.6%), docosahexaenoic (DHA) (1.7%-15.9%) and eicosapentaenoic (EPA) (1.2%-8.8%) acids were the predominant polyunsaturated fatty acids (PUFA). There were no significant differences ($p>0.05$) in the content of SFA (24.3%-28.7%) among the fish species. The content of MUFA in muscle lipids of carp (55.0%) was significantly higher ($p\leq0.01$) than in the lipids of the other fish examined (35.8%-38.3%). Bream and rainbow trout (26.6% and 27.7%, respectively) contained significant more *n*-3 PUFA than carp (4.7%) and tench (20.5%) ($p\leq0.01$). Total *n*-6 PUFA ranged between 8.4% (rainbow trout) and 12.9% (tench). Significant differences in *n*-6 PUFA content were only observed in the case of these fish ($p\leq0.01$). The differences in the *n*-3/*n*-6 ratio (rainbow trout (3.3) > bream (2.6) > tench (1.6) > carp (0.4)) were statistically significant ($p\leq0.01$).

INTRODUCTION

Lipids of fish, especially of the marine species, contain more PUFA, in particular *n*-3, than meat (beef, pork and veal) and vegetable oils [Givens & Gibbs, 2006; Abbas *et al*., 2009; DeFilippis *et al*., 2010]. EPA and DHA which are main representatives of *n-3* fatty acids are minimally synthesised in a human organism from a plant-derived precursor, *i.e.* α-linolenic acid (ALA, C18:3 *n*-3), and therefore must be supplied with food [Williams & Burdge, 2006; Lecerf, 2007]. AA can be metabolised from linoleic acid C18:2 *n*-6 (LA) by conversion through desaturation/elongation pathway [Holub & Holub, 2004]. Whereas the biosynthesis of ALA and LA takes place only in the vegetable kingdom and they cannot be produced by animal organisms [Cichon, 2003]. Steffens & Wirth [2005] have reported that freshwater fish are also an important source of *n*-3 fatty acids. According to Diraman & Dibeklioglu [2009], the fatty acid profiles (especially EPA and DHA) of freshwater fish are comparable to those of seawater fish, whereas Steffens [1997] found that freshwater fish can be even a better source of essential fatty acids than marine fish. The content of lipids and fatty acids in fish tissues depends on many factors both environmental ones as well as species or feeding type. Kołakowska *et al.* [2003] announced that the proportion of fatty acid group in the total lipid content from fish tissues differs and depends primarily on the content of total lipids and can be affected by numerous biological factors. Consequently, the objective of this study was to determine differences in the profile of fatty acids in muscle lipids of freshwater fish belonging to four species available on the local market.

MATERIALS AND METHODS

The samples of fresh carp (*Cyprinus carpio* L.), rainbow trout (*Oncorhynchus mykiss* Walb.), bream (*Abramis brama* L.) and tench (*Tinca tinca* L.) from Olsztyn markets were used in this study. The fish were collected on the same day (during the winter time) and were transported to the laboratory where body weight and total length of each fish were determined (Table 1). The dorsal part of muscles was taken for analyses. In the case of carp and rainbow trout, twelve specimens were examined, whereas in the case of bream and tench, five specimens. Each sample was prepared from individual specimens and kept in polypropylene bags at 248 K (-25°C) until analysis.

The fat content was determined according to the Schmidt-Bondzynski-Ratzlaff's procedure. Similarly in the case of fatty acids analysis, lipids were extracted from muscles (without skin) with the use of this method [Berg & Nilsson, 1997]. BHA (2-tert-Butyl-4-hydroxyanisole) was added to all samples during preparation. The fatty acid methyl esters were prepared following the Peisker method with a mixture of chlo-

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roform: methanol: sulphuric acid (100:100:1 v/v) [Żegarska *et al*., 1991]. The fatty acids profile was determined in an Agilent Gas Chromatograph 6890N with a flame-ionisation detector (FID) under the following conditions:

- capillary column (dimension 30 m x 0.25 μm with a 0.32 mm internal diameter, liquid phase Supelcowax 10),
- temperature: injector - 225°C, flame-ionisation detector - 250° C and the column - 180°C,
- carrier gas - helium, flow rate 1 mL/min.

Individual fatty acids were identified by comparing the relative retention time of peaks to known standards of Supelco.

Significant differences in the contents of fatty acids, *n*-3/*n*-6 ratio and DHA+EPA between species were calculated using the one-way analysis of variance ANOVA (Duncan's test). The significance levels of $p \leq 0.05$ and $p \leq 0.01$ were estimated.

RESULTS AND DISCUSSION

The results (mean±standard deviation) are given in Table 1, whereas the percentage share of the sum of fatty acids in muscles of fish examined is shown in Figure 1. Significant differences ($p \leq 0.05$ and $p \leq 0.01$) in the composition of some fatty acids were found between the fish species (Table 1). The lipid content varied from 2.4% to 5.1%. The muscles of rainbow trout contained more lipids than these of the other fish. The percentage composition of SFA ranged from 24.3% (rainbow trout) to 28.7% (carp), but there were no significant differences ($p > 0.05$) between the species. The total SFA in muscles of bream (29.90%) measured by Łuczyńska *et al.* [2008] was higher than in bream examined in this study. In turn, Jankowska *et al.* [2006] found similar values for SFA in tench reared on natural feed (25.18%). Kołakowska *et al.* [2000] observed a higher content of SFA for rainbow trout (31.06%) and a lower one for carp (24.73%) to those noted in the present study. According to Kandemir & Polat [2007], the total lipids and fatty acids in the muscle of rainbow trout varied by seasons ($p < 0.05$). The same authors found a higher content of total fatty acids in summer, autumn and winter than in the spring.

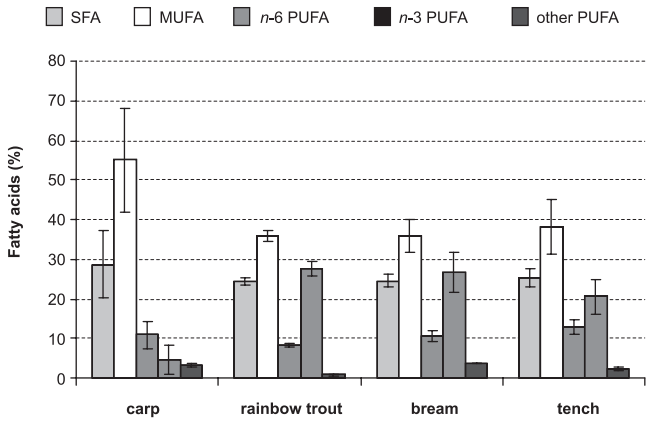

FIGURE 1. Sum of the fatty acids groups (percentage) in the muscle triacylglycerols of freshwater fish examined.

Generally, palmitic acid was the predominating SFA (15.6–19.5%), ($p > 0.05$). These findings are in agreement with literature data for Arctic char (*Salvelinus alpinus*), brook trout (*Salmo trutta fario*) and rainbow trout [Haliloğlu *et al*., 2002]. A similar observation was also made by Kmínková *et al.* [2001].

The content of MUFA in muscle lipids of carp (55.0%) was significantly higher ($p \leq 0.01$) than in the other fish examined. The lower values of MUFA were 35.8% (bream), 35.9% (rainbow trout) and 38.3% (tench), respectively. In all fish species, the most abundant fatty acids groups were MUFA. These results were not consistent with those of Passi *et al.* [2002] for Mediterranean marine fish species. Kujawa *et al.* [2005] found that the MUFA were predominant in the muscle lipids of bream, but the most abundant fatty acids group in muscles of pike (*Esox lucius* L.) and asp (*Aspius aspius* L.) were PUFAs. Özogul *et al.* [2007] also showed higher contents of MUFA for carp (13.8%) than tench (10.7%). These results were lower than data of the current findings. Bieniarz *et al*. [2001] observed that MUFA in muscle with skin of carp which was cultured in Experimental Fisheries Station in Zator ranged from 54.2 to 61.0%. According to Jankowska *et al.* [2006] muscle lipids of tench fed formulated feed and pond-reared tench contained 42.15% and 27.90% of MUFA, respectively. Przygoda *et al.* [2003] measured that the muscle lipids of rainbow trout contained 42.45% of MUFA. These values were higher than those observed in the present study, whereas lower levels in muscles of rainbow trout (30.81%) were found by Haliloğlu *et al.* [2002]. Similarly, Łuczyńska *et al.* [2008] showed lower values of MUFA in bream (33.44%) from the Mazurian Great Lakes than in the bream examined.

In the case of all freshwater fish, the major fatty acid among MUFA group was oleic acid (20.7–42.7%). This is in accordance with the results of Ugoala *et al.* [2008] for other fish species. These results confirm also findings of Kołakowska *et al*. [2000] and Kmínková *et al.* [2001]. The content of oleic acid was significantly higher in carp than in other fish species ($p \leq 0.01$), (Table 1).

The differences in Σn-3 PUFA (26.6% and 27.7%, respectively) were not significant between bream and rainbow trout ($p > 0.05$). The same fish had a significantly higher content of this group of fatty acids than carp (4.7%) and tench (20.5%) ($p \leq 0.01$). Carp contained significantly more *n*-3 PUFA than bream ($p \leq 0.01$). Low content of fatty acids of this group were reported by Kołakowska *et al.* [2000]. The muscles of rainbow trout studied by the above authors were characterised by a lower content of Σn-3 PUFA (24.02%) than these of the fish analysed in this study. The results noted for rainbow trout were higher than those found by Haliloğlu *et al.* [2002]. The muscles of tench examined had more *n*-3 PUFA than the muscle tissue of the same fish species observed by Steffens & Wirth [2007], whereas in the case of dorsal muscle of carp the content of those acids was lower. Jankowska *et al.* [2006] determined higher contents of *n*-3 PUFA for tench. In the case of rainbow trout and bream, the contents of *n*-3 PUFA were higher than in Norwegian salmon (*Salmo salar* L.) oil observed by Usydus *et al.* [2007], and close to those noted for tench. In turn, Łuczyńska *et al.* [2008] reported on a lower content

TABLE 1. Fat content, the composition of fatty acids (% of total fatty acids) and the biometric data in freshwater fish species

	Carp (n=12)	Rainbow trout (n=12)	Bream (n=5)	Tench (n=5)
Weight (g)	726±103	358±77	840±109	1054±244
Length (cm)	35.3±2.2	32.2±3.8	41.8±1.9	40.8±3.7
Total lipids (%)	4.9±3.7	5.1±1.9	2.4±1.3	2.6±1.5
		Fatty acids		
C12:0	0.0 ± 0.0^{B}	0.1 ± 0.0^{A}	0.1 ± 0.0^{A}	0.1 ± 0.0^{A}
C14:0	1.0 ± 0.3^{C}	4.2 ± 0.4^{A}	2.0 ± 0.3^{B}	2.0 ± 0.3^{B}
C15:0	0.2 ± 0.1^{C}	0.4 ± 0.0^{B}	0.5 ± 0.1^{B}	0.7 ± 0.1^{A}
C16:0	19.5 ± 5.3^{A}	15.6 ± 0.7^{A}	16.3 ± 0.8^{A}	17.2 ± 1.8^{A}
C17:0	0.2 ± 0.1^{C}	0.3 ± 0.0^{C}	0.7 ± 0.2^{B}	0.9 ± 0.1^{A}
C18:0	7.6 ± 2.7^{A}	3.4 ± 0.2^{B}	4.5 ± 0.9^{B}	3.8 ± 1.0^{B}
C20:0	$0.2\pm0.1^{c\,B}$	$0.2\pm0.0^{bc\,AB}$	$0.3\pm0.0^{a\,A}$	$0.2\pm0.0^{ab\,AB}$
C22:0	0.0 ± 0.0^{B}	0.1 ± 0.1^{A}	0.1 ± 0.1^{A}	0.1 ± 0.1^{A}
Σ SFA	28.7 ± 8.3^{a}	24.3 ± 1.0^{a}	24.6 ± 1.6^{a}	25.2 ± 2.2^{a}
C14:1	0.1 ± 0.1^{B}	0.2 ± 0.0^{A}	0.1 ± 0.0^{B}	0.1 ± 0.0^{B}
C16:1	$8.5\pm2.3^{b\,BC}$	$6.2\pm0.4^{c\,C}$	$10.4\pm1.4^{b\,B}$	$13.6\pm3.9^{a\,A}$
C17:1	0.3 ± 0.2^{D}	0.7 ± 0.1^{C}	0.9 ± 0.1^{B}	1.2 ± 0.2^{A}
C18:1	42.7 ± 15.4^{A}	23.6 ± 1.1^{B}	23.0 ± 3.0^{B}	20.7 ± 2.8^{B}
C20:1(*n*-7)	$0.1\pm0.1^{c\,C}$	$0.3\pm0.1^{b\,B}$	$0.2\pm0.0^{b\,BC}$	$0.6\pm0.2^{a\,A}$
C20:1(*n*-9)	2.6 ± 1.1^{A}	2.5 ± 0.2^{A}	0.5 ± 0.1^{B}	0.7 ± 0.2^{B}
C20:1(*n*-11)	$0.5\pm0.1^{b\,BC}$	$0.3\pm0.0^{c\,C}$	$0.6\pm0.3^{b\,B}$	$1.2\pm0.3^{a\,A}$
C22:1(*n*-9)	0.1 ± 0.2^{BC}	0.4 ± 0.0^{A}	$0,0\pm0.1^{C}$	0.3 ± 0.2^{AB}
C22:1(*n*-11)	0.0 ± 0.1^{B}	1.8 ± 0.2^{A}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}
Σ MUFA	55.0 ± 13.0^{A}	35.9 ± 1.4^{B}	35.8 ± 4.2^{B}	38.3 ± 7.0^{B}
C18:2(*n*-6)	$8.0\pm2.6^{a\,A}$	$7.1\pm0.5^{a\,AB}$	$4.6\pm1.9^{b\,B}$	$4.8\pm0.3^{b\,B}$
C20:3(*n*-6)	$0.5\pm0.2^{ab\,AB}$	$0.3\pm0.1^{b\,B}$	$0.4\pm0.1^{b\,AB}$	$0.6\pm0.0^{a\,A}$
C20:4(*n*-6)	2.0 ± 1.5^{C}	0.8 ± 0.0^{C}	4.8 ± 1.3^{B}	6.6 ± 1.8^{A}
C22:5(*n*-6)	$0.4\pm0.2^{c\,B}$	$0.3\pm0.0^{c\,B}$	$0.7\pm0.1^{b\,A}$	$0.9\pm0.3^{a\,A}$
Σ PUFA *n*-6	10.8 ± 3.5^{AB}	8.4 ± 0.5^{B}	10.5 ± 1.4^{AB}	12.9 ± 1.8^{A}
C18:3(*n*-3)	1.1 ± 0.6^{B}	2.0 ± 0.1^{B}	4.8 ± 3.3^{A}	4.2 ± 1.9^{A}
C20:3(*n*-3)	$0.1\pm0.1^{d\,C}$	$0.1\pm0.0^{c\,C}$	$0.5\pm0.1^{a\,A}$	$0.4\pm0.1^{b\,B}$
C20:4(*n*-3)	0.1 ± 0.1^{D}	0.9 ± 0.1^{B}	1.1 ± 0.2^{A}	0.6 ± 0.1^{C}
C20:5(*n*-3)EPA	1.2 ± 1.0^{C}	6.4 ± 0.4^{B}	8.8 ± 1.3^{A}	5.5 ± 0.5^{B}
C22:5(*n*-3)	0.6 ± 0.5^{B}	2.3 ± 0.1^{A}	2.2 ± 0.4^{A}	2.3 ± 0.5^{A}
C22:6(*n*-3)DHA	1.7 ± 1.7^{C}	15.9 ± 1.7^{A}	9.1 ± 1.7^{B}	7.4 ± 3.0^{B}
Σ PUFA *n*-3	4.7 ± 3.7^{C}	27.7 ± 1.8^{A}	26.6 ± 5.0^{A}	20.5 ± 4.5^{B}
C16:4	0.0 ± 0.0^{B}	0.4 ± 0.1^{A}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}
C16:2	0.1 ± 0.1^{C}	0.7 ± 0.1^{A}	0.4 ± 0.1^{B}	0.5 ± 0.1^{B}
C18:3(*n*-4)	$0.2\pm0.1^{c\,C}$	$0.5\pm0.0^{b\,AB}$	$0.4\pm0.1^{b\,B}$	$0.6\pm0.1^{a\,A}$
C18:4	0.1 ± 0.1^{D}	1.3 ± 0.1^{A}	0.9 ± 0.1^{B}	0.4 ± 0.1^{C}
C21:5	$0.0\pm0.0^{b\,C}$	$0.5\pm0.2^{a\,A}$	$0.1\pm0.0^{b\,BC}$	$0.3\pm0.1^{a\,AB}$
C20:2	0.3 ± 0.1^{C}	0.4 ± 0.0^{C}	0.7 ± 0.2^{B}	1.3 ± 0.3^{A}
Σ other PUFA	0.7 ± 0.3^{D}	3.7 ± 0.3^{A}	2.5 ± 0.2^{C}	3.1 ± 0.5^{B}
Σ PUFA	16.2 ± 7.0^{B}	39.8 ± 1.5^{A}	39.6 ± 0.1^{A}	36.5 ± 6.2^{A}
n-3/*n*-6	0.4 ± 0.2^{D}	3.3 ± 0.4^{A}	2.6 ± 0.8^{B}	1.6 ± 0.2^{C}
EPA+DHA	2.9 ± 2.7^{D}	22.3 ± 1.9^{A}	17.9 ± 3.0^{B}	13.0 ± 3.4^{C}

a, b, c, d - significant difference ($p\leq0.05$); A, B, C, D - significant difference ($p\leq0.01$). The same letter indicates the lack of significant differences between the muscles fish species ($p>0.05$); "0.0" - indicates that the contents are less than 0.05%.

of Σ *n*-3 PUFA in muscles of bream (24.66%) compared to data published in the present studies for bream.

The main fatty acids of *n*-3 PUFA in muscle lipids of fish were DHA (1.7–15.9%) and EPA (1.2–8.8%). These results are in accordance with literature data for marine fish [Njinkoué *et al*., 2002; Visentainer *et al*., 2007] and freshwater fish [Kujawa *et al*., 2005; Łuczyńska *et al*., 2008]. A higher value of DHA was observed in rainbow trout compared to the other fish studied ($p \leq 0.01$), while a higher content of EPA was found in bream ($p \leq 0.01$) (Table 1).

Total *n*-6 PUFA varied from 8.4% (rainbow trout) to 12.9% (tench). Significant differences for *n*-6 PUFA were observed only in the case of these fish ($p \leq 0.01$). The percentage contents of *n*-6 PUFA in bream and carp were similar (10.5% and 10.8%, respectively) ($p > 0.05$). The differences observed in *n*-6 PUFA content between carp and tench studies were not significant ($p > 0.05$). Özogul *et al.* [2007] found that *n*-6 PUFA values in tench (16.8%) and carp (16.3%) were close. According to these authors, the seawater fish had lower levels of *n*-6 PUFA than the freshwater fish, but in the case of *n*-3 PUFA, the marine fish were better sources of those acids. The total content of *n*-6 PUFA in bream muscles measured by Łuczyńska *et al.* [2008] was 11.21%. In turn, Σ *n*-6 PUFA reported by Grela & Dudek [2007] for carp was 17.09%. According to these authors the content of this group of fatty acids in fish decreased as follows: carp > pike (*Esox lucius* L.) ≈ zander (*Sander lucioperca* L.) > cod (*Gadus morhua* L.) ≈ salmon. Haliloğlu *et al.* [2002] demonstrated that the levels of *n*-6 PUFA in asp (15.82%), brook trout (16.57%) and rainbow trout (14.47%) were not significantly different ($p > 0.05$). In the case of rainbow trout examined, lower contents were found for this group of fatty acids. Jankowska *et al.* [2006] and Steffens & Wirth [2007] showed that the content of *n*-3 and *n*-6 PUFA was dependent on the diet.

Linoleic (4.6–8.0%) and AA (0.8–6.6%) acids were the predominant *n*-6 PUFA. This is in accordance with the results of Łuczyńska *et al.* [2008] and Ugoala *et al.* [2008]. Muscle lipids of carp contained significantly more linoleic acid than the other fish examined (with the exception of rainbow trout) ($p \leq 0.01$). The content of AA was higher in muscle lipids of tench ($p \leq 0.01$) (Table 1).

The differences in the *n*-3/*n*-6 ratio and EPA+DHA in the muscle lipids were statistically significant ($p \leq 0.01$). The ratio of *n*-3/*n*-6 accounted for 3.3 (rainbow trout), 2.6 (bream), 1.6 (tench), and 0.4 (carp), respectively. The *n*-3/*n*-6 ratio was close to that noted by Łuczyńska *et al.* [2008] for bream. This is also consistent with the results of Żmijewski *et al.* [2006] and Kujawa *et al.* [2005]. A similar ratio of *n*-3/*n*-6 was also observed in the case of tench reared on the basis of natural food in a pond (1.0) and tench fed supplementary wheat in a pond (1.1), [Steffens & Wirth, 2007]. According to Kołakowska *et al.* [2000] the value in carp was 0.1, whereas in the case of rainbow trout the *n*-3/*n*-6 ratio was 4.9. The value in muscles of rainbow trout noted by the same authors was higher than those determined in the present study. In contrast, a lower *n*-3/*n*-6 ratio (1.58) in rainbow trout was reported by Haliloğlu *et al*. [2002]. The above authors did not found any significant differences ($p > 0.05$) between the *n*-3/*n*-6 ratio in rainbow trout, asp and brook trout. The ratio in marine fish in Malaysian waters varied from 1.50 to 4.31 [Osman *et al*., 2001]. According to the literature, the *n*-3/*n*-6 ratio in raw marine fish oils was 1.72–2.77 [Usydus *et al*., 2007]. The ratio of *n*-3/*n*-6 in the muscle lipids of freshwater fish examined (with the exception of carp) was similar as in marine fish.

CONCLUSIONS

The content of the sum of fatty acids (with the exception of SFA) varied between species. There were also differences in the fatty acids compositions among species. The *n*-3/*n*-6 ratio in the muscle lipids of fish examined (with the exception of carp) was above 1.0. All fish examined had higher amounts of DHA, EPA and other long-chain fatty acids important from the nutritional point of view than some marine fish and can be as good sources of *n*-3 and *n*-6 fatty acids as those fish.

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