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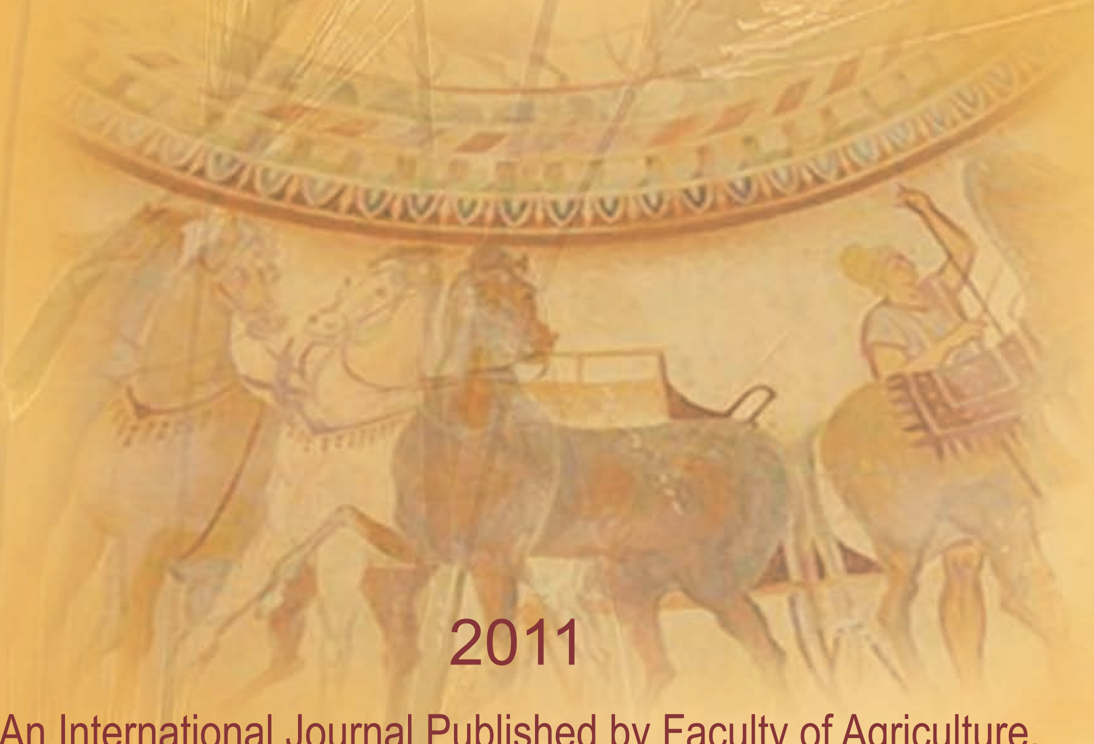

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Student's campus, 6000 Stara Zagora
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Fatty acid composition of common carp, rainbow trout and grey mullet fish species

M. Stancheva, A. Merdzhanova*

Department of Chemistry, Faculty of Pharmacy, Medical University, 55 Marin Drinov, 9000 Varna, Bulgaria

Abstract. *The aim of the present study was to determinate the fatty acid composition of two commercially important freshwater fish species - rainbow trout (Oncorhynchus mykiss) and carp (Cyprinus carpio L.) and one Black Sea fish – grey mullet (Mugil cephalus). Lipid extraction was done according to the Bligh and Dyer method. Methyl esters were prepared according to method EN ISO 5508:2000. The fatty acid (FA) composition was analyzed by GC-MS. The total lipid content in rainbow trout was 11.50 g/100g raw weight (r.w.), in carp – 12.74g/100 g r.w. while grey mullet showed a value of 3.80g/100g r.w. In comparison with other groups, the polyunsaturated FA (PUFA) showed the highest level in trout – 43.13 % including ω3 such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids followed by grey mullet –29.1%, whereas the carp presented lowest level – 17.55%. The amounts of total ω6 PUFAs were higher than the total ω3 PUFAs in all analyzed fish samples. A ω3 /ω6 and PUFA/SFA ratios were determined in all three fish species.*

Keywords: fatty acid composition, common carp, grey mullet, rainbow trout, GC-MS

Abbreviations: FA – fatty acid, PUFA – polyunsaturated fatty acid, MUFA – monounsaturated fatty acids, TL – total lipids, SFA – saturated fatty acids, EPA – eicosapentaenois, DHE – docosahexaenoie, ALA – Aa-alphalinoleic, ARA – arahidonic

Introduction

The polyunsaturated fatty acids (PUFAs) are considered as essential for the normal human growth, development, and they play an important role in the prevention and treatment of coronary heart disease, hypertension, diabetes, arthritis, inflammatory and autoimmune disorders and cancer. Fishes are the main food source of omega 3 (ω 3) and omega 6 (ω 6) PUFAs in the human diet. The fatty acid composition of fish lipids, (especially PUFA) varies in response to their habitats as water temperature change, salinity and dietary lipids (Tocher, 2003; Henderson et al.,1987). The Black Sea appears to be one of the important fish basins influencing greatly the economy of all countries around the basin and grey mullet (*Mugil cephalus*) is one of the commercially important species. Due to its economic importance, two of the most widely farmed fish species in our country are the rainbowtrout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio L.).* These freshwater fishes are the preferred fish species for breeding and consumption because of ther rapid growth and rich and diverse composition of the meat (Videv et al., 2009).

The lipid FA profiles data for different marine and freshwater fish species especially originating from Canada, Norway and Japan are available in literature. However, information about the fatty acids composition of Bulgarian Black Sea and freshwaters fish species is lacking. Two reports were encounted in the literature, in witch FA content in carp and trout were mentioned (Ribanova et al., 2003; Hadjinikolova, 2005).

The purpose of the present study was to compare the level of the fatty acid composition of two commercially important freshwater fish species - rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio)* and one Black Sea fish – grey mullet (*Mugil cephalus).*

* e-mail: a.merdzhanova@gmail.com

Material and methods

Sampling

Grey mullet fish species was purchased from the Varna local fishery market (2010). Common carp was obtained from Pjasachnik Dam Lake (early spring 2010, Plovdiv region) and rainbow trout – from Dospat Dam Lake (spring 2010, Smolyan region). Three specimen of each species were used for lipid and fatty acid analysis. Table 1 presented biometric and biologic characteristics of the observed fish species.

Standards and reagents

For fatty acids analysis Fatty Acid Methyl Esters (F.A.M.E.) mix standard (SUPELCO F.A.M.E. Mix C4-C24), nonadecanoic acid and methyl ester nonadecanoic acid standards were purchased from Sigma – Aldrich ™. All chemicals used were of analytical and GC grade (Sharlau, Spain).

Sample preparation

Lipid extraction. To define total lipids the tissue was finely cut and three samples were taken in parallel, each of which weighed 5g. Total lipids (TL) were extracted with chloroform/methanol (2:1 v/v) according to Bligh and Dyer method (1959). After phase separation, the chloroform extracts were evaporated until dryness and were quantified by weight. The total lipid content was measured in triplicate by gravimetry.

Fatty acids methyl esters (FAME) analysis.

Fatty acid methyl esters (FAME) were prepared by base-catalysed transmethylation with 2M KOH in methanol using EN ISO

Table 1. Biometric and biologic characteristics of analysed Bulgarian fish species

Fish species	Mean total weight (g) ± SD	Mean stand length (cm) ± SD	Habitat		Food habits	
			Pelagic	Demersal	Herby vorous	Omni vorous
Carp (*Cyprinus carpio L.) (n=3)*	1460.0 ± 160.0	55.0 ± 5.0	-	+	-	+
Rainbow trout (*Oncorhynchus mykiss*) *(n=3)*	342.0 ± 25.0	28.0 ± 2.0	+	-	-	+
Grey mullet (*Mugil cephalus) (n=3)*	290.0 ± 10.0	35.0 ± 2.5	+	-	+	-

Weight and length data are presents as mean ±SD

5509:2000. After 10 minutes centrifugation (3500 g), the upper hexane layer (a sample of 1 μl) was analysed by GS/MS.

GC-MS analysis

Gas chromatography was performed by a model FOCUS Gas Chromatograph with autosampler A 3000, equipped with Polaris Q MS detector (Thermo Scientific, USA). The capillary column used was a TR-5 MS (Thermo Scientific, USA) universal column 30 m length and 0.25mm i.d.. Helium was used as a carrier gas at flow rate 1 ml/min. Peaks were identified according to two parameters: Retention Time (RT) based on available fatty acid methyl esters (FAME) mix standard and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA).

Statistical analysis

Total lipid content of edible tissue was determined for each group (n=5) and the results were presented as g.100g^{-1} raw tissue (g.100g^{-1}r.t.). Standard curves for FA were obtained using five different concentrations of standard solutions. For the determination of the recoveries, samples of homogenized fish tissue were spike with a methanolic solution containing nonadecanoic acid (1mg/ml).

Three replicate GC analyses were performed and the results were expressed in GC area wt percentage as a mean value and ± standard deviation (SD). The significant differences of total lipids ($P<0.0001$) were observed. The significant variations of results for FA analyses were considered at $P<0.0001$. Data were analysed using Graph Pad Prism 5 program.

Results and discussion

Total lipid content

The lowest total lipid (TL) content was found in grey mullet (3.800.07g.100^{-1}g r.t.) followed by the rainbow trout (11.500.55g.100^{-1}g r.t.), whereas the carp (12.74 0.75g.100^{-1}g r.t.) was the fish with the highest content of TL. Sengor et al. (2003); Pirestani et al., (2010) and Bayir et al. (2006) determined higher TL values for grey mullet (4.15 to 5.15 g.100^{-1}g r.t.) compared with our result. Atanasov et. al. (2009) reported 10.5 -12.5 g.100^{-1}g r.t. TL for carp and 4.4 up to 5.0 g.100^{-1}g r.t. TL for rainbow trout witch are similar to our data. On the contrary, Ribanova et al. (2003) accounted twice as low TL content in carp (5.80 g. 100^{-1}g r.t.). Saglik et al. (2007) described for rainbow trout 7.2 g. 100^{-1}g r.t. TL, whereas Jabeen and Chaundhry (2010) found 14.0 g. 100^{-1}g r .t. TL for carp which confirm the hypothesis of Hadjinikolova (2005) that carp fish lipid levels a strongly depend of the type of diet.

Fatty acids composition

A lipid analysis enabled the classification and quantitative determination of FA as well as the sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA). FA contents in grey mullet followed a relative pattern with SFA > PUFA > MUFA. An exception to this was rainbow trout which contained a higher amount of PUFA compared to MUFA ($p<0.001$) and SFA ($p<0.001$) (e.g. PUFA>SFA > MUFA). The carp reveals significantly higher MUFA content compared to PUFA ($p<0.001$) (e.g. MUFA > SFA > PUFA). Figure 1 presented significant differences obtained when comparing the FA groups in each analyzed fish species.

Hadjinikolova (2005) and Ribanova et al. (2003) presented for carp the FA followed relative pattern with MUFA > SFA > PUFA which are similar to our data. Jabeen and Chaudhry (2010) showed a different pattern of FA profile of carp fish species from Indus River, e.g. SFA > MUFA > PUFA wich is a response to specificity of the local aquatic ecosystem. Our studied of FA composition on the rainbow trout are different to the information presented by Ribanova et al. (2003) and Saglik (2007), but are close to the data shown by Haliloglu et al. (2002) and other of our earlier studies on FA composition on rainbow trout (e.g. PUFA > SFA > MUFA) (Stancheva et al., 2010). Grey mullet as high migratory fish species presented different FA pattern in our investigation in contrast to other publication (Sengor et al., 2003; Bayir et al., 2006; Guler et al., 2008; Pirestani et al., 2010). Table 2 presents the fatty acid composition of analysed fish species. The FA composition of tissue lipids in fish is influenced by the FA in their dietary lipids (Tocher, 2003). The data showed that the amount of FA varied widely among the species but in most studies the palmitic (C 16:0) and stearic (C 18:0) acids are the predominant SFA (Fajmonova et al., 2003; Tocher, 2003; Saglik and Guven, 2007; Ozogul and Ozogul, 2007). In our analysis palmityc acid was the primary SFA followed by stearic and myristic acids but we observed also significant levels of nervonic acid (C24:0) for trout and grey mullet species. In our study the highest level for palmitic acid was observed in grey mullet (30.40% of total FA) whereas the trout was the fish with the lowest level (12.95% of total FA) therefore large differences in SFA groups in this species were observed. On the contary, Haliloglu et al. (2002) described that the several trout species have the higher levels of palmitic acid ranging from 18.30 to 23.00% of the total FA. Sengor et al., (2003) reported that Aegean grey mullet have lower level of palmitic acid (20.30%) compared to our data. Kalionchu et al. (2010) found that palmitic acid has lower level (19.50%) in the Izmir Dam Lake carp that our result.

The major classes of unsaturated fatty acids in nature are omega-9, omega-6 and omega-3, represented by oleic, linoleic and α-linolenic acids, respectively (Henderson and Tocher, 1987; Tocher, 2003). The amounts of unsaturated FA as MUFAs vary especially in wild fish (Ozogul and Ozogul, 2007; Saglik and Guven,

Table 2. FA composition (% of total FA) of trout, carp and grey mullet

Fatty Acid	Rainbow trout	Common carp	Grey mullet
C 12:0	0.40±0.05	0.17±0.05	1.10±0.65
C 14:0	3.35±0.20	1.23±0.10	2.50±0.55
C 16:0	12.95±1.05	29.03±2.10	30.40±1.65
C 17:0	0.51±0.08	0.10±0.01	0.75±0.20
C 18:0	3.35±0.20	2.97±0.50	2.65±0.45
C 20:0	2.68±0.31	0.19±0.04	2.15±0.30
C 21:0	0.00	0.00	0.48±0.05
C 22:0	1.95±0.14	0.21±0.07	2.50±0.15
C 23:0	0.00	0.12±0.01	0.60±0.05
C 24:0	3.20±0.18	0.21±0.06	2.50±0.30
Σ SFA	28.90	34.22	45.63
C 14:1	3.16±0.35	0.15±0.02	0.31±0.05
C 16:1	4.15±0.15	10.40±1.65	4.96±0.40
C 17:1	0.50±0.10	0.15±0.01	1.61±0.08
C 18:1 ω 9	11.50±1.02	36.53±2.61	8.56±0.85
C 20:1	2.03±0.20	0.35±0.07	1.30±0.25
C 22:1 ω 9	2.74±0.50	0.40±0.04	1.65±0.05
C 24:1	1.30±0.15	0.00	1.26±0.10
Σ MUFA	25.39	47.98	22.75
C 18:3 ω 6	3.19±0.95	0.36±0.04	1.45±0.09
C 18:2 ω 6	13.56±0.90	11.34±1.14	6.40±0.72
C 18:3 ω 3	6.16±0.80	1.80±0.05	1.75±0.14
C 20:5 ω 3	1.56±0.20	0.85±0.03	1.90±0.05
C 20:4 ω 6	3.73±0.08	0.75±0.03	3.84±0.12
C 20:2	1.46±0.10	0.36±0.04	3.37±0.50
C 20:3 ω 3	0.00	0.45±0.02	1.50±0.15
C 20:3 ω 6	1.98±0.23	0.47±0.03	1.78±0.09
C 22:6 ω 3	11.23±0.75	1.63±0.08	4.56±0.24
C 22:2	1.90±0.45	0.50±0.06	1.80±0.35
Σ PUFA	44.47	17.80	30.35

SFA – saturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA. In the table are no presented FAs with values lover than 0.05 % of total FA.

2007; Kalionchu et al., 2010). In consequence, the percentage of this FA appeared did not similar in the observed fishes. The highest total MUFAs value was found for carp (47.98%), whereas the lowest - for grey mullet (22.75%). This was due to high concentration of oleic acid (C 18:1) (8.56 – 36.53%) which was the main MUFAs (especially in carp), followed by palmitoleic acid (C 16:1). Our results are in agreement with the data presented by Haliloglu et al. (2002), Sengor et al. (2003), Hadjinikolova (2005) and Kalionchu et al. (2010), for MUFA. In the many studies (Ozogul and Ozogul, 2007; Saglik and Guven, 2007; Guler et al., 2008) was reported that the oleic acid is the main MUFAs in freshwater fish species, but this fatty acid has exogenous origin and usually reflects the type of diet of the fish. This is particularly well observed in grey mullet which is migratory fish species and inhabits freshwater waters in spring and summer (Sengor et al., 2003). In contrast to some Mediterranean fish species as grey mullet (*Mugul cephalus*) and sardine (*Sardinella aurita*), the palmitoleic acid is presented at large amounts, respectively 17.00 % (mullet) and 12.00% (sardine) whereas oleic acids are only 4.50 % (Ozogul and Ozogul, 2007).

Fish is known to be a rich source of the unique PUFAs of the omega-3 family, including both EPA and DHA. Recent studies have suggested that the dietary consumption of fish may favourably influence a number of biological factors associated with cardiovascular disease (independent of blood-cholesterol lowering). In addition to cardiovascular disorders, there is evidence to suggest that the consumption of fish may offer benefits in the management of individuals with inflammatory disorders such as rheumatoid arthritis,psoriasis, etc. (Simopoulus and Cleland, 2003).

In our investigation the major FAs identified as PUFAs was linoleic acid (LA, C18:2 ω -6) in all analysed species. Important long – chain fatty acids such as eicosapentaenoic (EPA), docosahexaenoic (DHA), Aa-alpha-linoleic (ALA) and arahidonic (ARA) acids were also found in significant levels mainly in rainbow trout. The maximum value of DHA was obtained for rainbow trout (11.23% - which is 25% of total PUFAs), whereas the lowest - for carp (1.63 % - what is 9.15 % of total PUFAs).

Fish generally need PUFAs to tolerate low water temperatures; therefore, higher PUFA (especially DHA) concentrations are

Table 3. PUFA/SFA and n6/n3 ratios, total sum of ω 3 and ω 6 content in fish species

Fatty Acids	Rainbow trout	Common carp	Grey mullet
ω 3	18.95±0.60	4.73±0.32	9.71±0.40
ω 6	22.46±0.85	12.92±0.55	13.47±0.60
ω 3/ ω 6	0.84	0.40	0.74
PUFA/SFA	1.54	0.52	0.70

expected in fish that live in cold environments such as rainbow trout (Haliloglu et al., 2002). In our study - both species – rainbow trout and grey mullet EPA levels was found to be lower (1.56 % and 1.90 %) compared to ARA values and only in carp a lower level for ARA (0.75%) than EPA level was detected. Our results were similar with those presented by Haliloglu et al. (2002), Sengor et al. (2003) Hadjinikolova (2005) Ozogul and Ozogul (2007) for PUFA in different fish species and confirm high variability in this fatty acid group.

Freshwater fish normally contain ω 6 PUFAs, whereas marine fish are rich in ω 3 fatty acids (Henderson and Tocher,1987; Tocher, 2003). The competitive inhibition of chain elongation and desaturation of members from one series of fatty acids for members of another series is well established, with ω 3 > ω 6 being the usual order of potency for inhibition. Fish are able to synthesize the even-chain, saturated fatty acids can convert 16:0 to the 16:1 ω 7 monoene and 18:0 to the 18:1 ω 9 monoene. However they are unable to synthesize any fatty acids of the ω 6 and ω 3 series unless a precursor with this ω structure is present in the diet. The ability to elongate and desaturate fatty acids is not the same in all species of fish. (The composition of fish. FAO, 2005).

The results shown in Table 3 indicate that all fish species analysed are characterized by high level of omega 6 FA series than omega 3 series. The total sum of omega 6 acids series of analysed fish samples were determined from 12.92% for carp up to 22.46% for rainbow trout (Table 3). Black Sea grey mullet was found to be the riches in EPA and 20:3 ω 3 and this fish species can also serve as a valuable source of essential fatty acids. These results agree with those obtained in other studies conducted with seawater fish species (Sengor et al., 2003).

The omega 3/omega 6 ratio has been sugested to be a useful indicator for comparing the relative nutritional value of different fish species. A ω 3 /ω 6 ratio of 1:1 is considered to be optimal for nutritional purposes but ratio within 1:5 – 1:0.68 would constitute a healthy human diet (Simopoulus and Cleland, 2003). The ratio of n3 /n6 in total lipids of freshwater fishes changes mostly between 0.55 and 5.60, whereas for marine fishes in 4.7-14.4 (Henderson and Tocher, 1987; Hearn et al., 1987). Hence, our findings are in accordance with those earlier studies. In all three fish species ratio within the recommended was found. The highest ω 3 /ω 6 ratios was found to be 0.84 for rainbow trout followed by 0.74 for grey mullet and 0.40 for carp. Levels higher than a maximum value recommended by the UK Department of Health are harmful to health and may promote cardiovascular diseases (Culyer, 1994). Other useful key factor for evaluation of fish nutrition quality is PUFA/SFA ratio. Values of PUFA/SFA ratio greater than 0.45 are recommended by HMSO (Culyer, 1994). Another results are in agreement with this requirement showing higher PUFA/SFA ratios for all studied fish species (Table 3). The highest PUFA/SFA ratio was observed in the rainbow trout, followed by grey mullet, whereas the lowest value was found for carp. The most balanced PUFA/SFA ratio was obtained for grey mullet

Conclusion

The total lipids and fatty acid composition of three fish species was determinated. Qualitative and quantitative determination of 30 fatty acids was done and these FA were classified into three groups – SFA, MUFAs and PUFAs. The dominant group of fatty acids in rainbow trout is PUFAs – about 44.47 % of all FA content, while in carp this group was MUFAs – 47.98 %. In grey mullet the SFA groups are presented in the highest level –45.63 %. The most important ω3 fatty acids in PUFAs group are DHA and EPA and these were found in significant concentration. The ω 3/ ω 6 ratios varies within the range of 0.40 up to 0.84. Regarding to the lipid contents, the ω 3/ ω 6 and PUFA/SFA ratio we can conclude that those Bulgarian fish species identified in this study were found to be good sources of unsaturated FA and have good nutritional quality. In conclusion it's of great interest to proceed with further investigation in that particular area especially expanding it with other local seawater and fresh water fish species from Bulgarian waters with their unique fatty acid compositions.

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The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods, Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

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Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows, IX th International Conference on Production Diseases in Farm Animals, Sept.11 – 14, Berlin, Germany, p. 302 (Abstr.).

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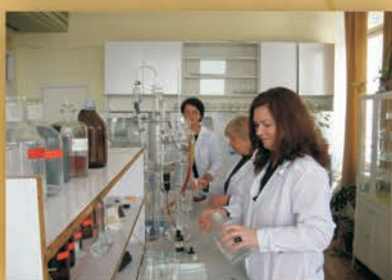

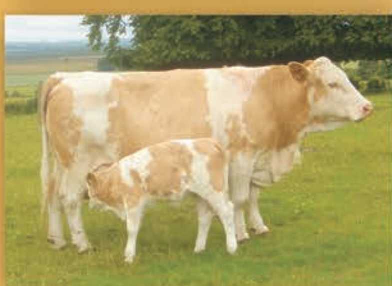

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