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Lipid profile and health benefit of commonly consumed fresh water and sea water fish species in the population of Serbia

Lipidni profil i zdravstveni značaj najčešće konzumiranih rečnih i morskih riba u populaciji Srbije

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Abstract

Background/Aim. Dietary intake of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) is important in prevention and treatment of different diseases. In general population, the average intake of n-3 LC-PUFA is often significantly lower than recommended levels. Fish lipids are rich sources of these fatty acids, of which the most important are eicosapentaenoic (20:5 n-3, EPA) and docosahexaenoic (22:6 n-3, DHA) fatty acids. This study was designed to determine and compare fat, fatty acids and lipid quality indices in 10 commercial fish species available on the Serbian market, as well as relation between their price and nutritional value. **Methods.** Freshwater fish originated from the Danube River in the Belgrade Region, while seawater fish were mostly from the Adriatic Sea. A gas chromatography method was used to define fatty acids in 40 fish samples after lipid extraction. Cost-minimization analysis was conducted to assess the economic utility. **Results.** Seawater fish had a significantly higher value of flash lipid quality compared to the freshwater fish ($p < 0.05$). Value of hypercholesterolaemic fatty acids (OFA) for the freshwater group was 18.70 (17.40–21.30) while the seawater group had a similar range of values 18.90 (17.55–22.75). Hypocholesterolaemic fatty acids (DFA) also showed similar ranges for both groups: 68.80 (66–70.20) for freshwater and 68.40 (64.85–73.05) for seawater group. The ratio of DHA/EPA ranged from 1.8 for sardine samples and up to 10 for tuna samples, indicating that the amount of DHA in natural samples exceeds the amount of EPA in many cases. The values of atherogenic (AI) and thrombogenic index (TI) were lower than 1 for all analysed samples. **Conclusion.** Sardine and mackerel had the highest content of n-3 LC-PUFA and presented the least expensive sources of EPA and DHA. The low values of AI and TI obtained from studied fish indicate its benefits from a health point of view.

Key words:
economic factors; fatty acids, unsaturated; food; health; oceans and seas; rivers; serbia.

Apstrakt

Uvod/Cilj. Unos n-3 polinezasićenih masnih kiselina (PMK) je od velike važnosti u prevenciji i tretmanu različitih oboljenja. Generalno posmatrajući, prosečan unos n-3 PMK je obično značajno niži od utvrđenih preporuka. Lipidi riba sadrže masne kiseline n-3 serije od kojih su najvažnije eikozapentaenska (20:5 n-3, EPA) i dokozaheksaenska (20:6 n-3, DHA) masna kiselina. Cilj ove studije bio je odrediti i uporediti lipidni profil i lipidne indekse u 10 različitih vrsta riba dostupnih na tržištu Srbije. Takođe, određen je odnos cene i nutritivne vrednosti odabranih vrsta. **Metode.** Ispitivane su rečne ribe Dunava iz Beogradskog regiona, dok su morske ribe uglavnom vodile poreklo iz Jadranskog mora. Gasna hromatografija sa jonskim detektorom je korišćena za određivanje masnih kiselina u 40 uzoraka nakon lipidne ekstrakcije. *Cost-minimization* analiza je korišćena za procenu ekonomske koristi. **Rezultati.** Morske ribe su imale značajno veće vrednosti za parametar *flash lipid quality* u odnosu na rečne ribe ($p < 0,05$). Vrednosti hiperholesterolemijskih masnih kiselina za grupu rečnih riba [18,70 (17,40–21,30)] bile su slične vrednostima dobijenim za morske ribe [18,90 (17,55–22,75)]. Hipoholesterolemijske masne kiseline su takođe pokazale sličan raspon vrednosti za rečne [68.80 (66–70.20)] i morske ribe [68.40 (64.85–73.05)]. Odnos DHA/EPA kretao se od 1,8 za uzorke sardine, do 10 za uzorke tune, što potvrđuje činjenicu da DHA prevazilazi vrednosti za EPA u svim ispitivanim uzorcima. Vrednosti za aterogeni i trombogeni indeks su bile niže od 1 za sve analizirane uzorke. **Zaključak.** Sardine i skuša su imale najveći sadržaj n-3 PMK i predstavljale su najekonomičniji izvor EPA i DHA. Niske vrednosti za aterogeni i trombogeni indeks ukazuju na potencijalno povoljan zdravstveni efekat ispitivanih vrsta.

Ključne reči:
ekonomski faktori; masne kiseline, nezasićene; hrana; zdravlje; okeani i mora; reke; srbija.

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Introduction

Fish and seafood are the only significant sources of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) in the modern human diet. Alfa-linoleic acid (ALA) is also found in foods of plant origin, but the process of converting this fatty acid (FA) into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans is not effective enough. Due to the importance of fish as a source of n-3 LC-PUFA in the past decades, numerous investigations have been carried out on the health impact of their consumption. The Danish scientists established a link between the high intake of sea fish and the low incidence of cardiovascular diseases in the Greenland Inuit [1]. Further research has shown that the consumption of fish in everyday diet has numerous beneficial effects. A study including subjects from 36 countries found a significant correlation between fish consumption and a reduced risk of general mortality, as well as the mortality caused by ischemic heart disease and cerebrovascular disease [2]. The Hordaland Health Study among middle-aged men and women confirmed an inverse relation between total fish intake and parameters of metabolic syndrome [3]. Also, very recent review revealed results from interventional and prospective cohort studies, with mostly beneficial effects of fish consumption on cardiovascular diseases and metabolic syndrome [4]. In addition to the protective effect on the cardiovascular system, it is considered that the intake of fish can positively influence the development and proper functioning of the central nervous system, especially in children. Fish intake and n-3 LC-PUFA are associated with favourable effects in neurodegenerative and retinodegenerative diseases [5, 6]. In 2012, European Food Safety Authority (EFSA) established dietary recommendations for the intake of 250–500 mg/day of EPA + DHA based on cardiovascular risk for European adults [7]. Fish consumption of at least twice a week (recommended fatty fish – salmon, herring and mackerel) and the use of vegetable oils with ALA for preparing food (soybean oil) has been recommended by the American Heart Association (AHA). For the purpose of secondary preventions, the AHA recommends 1 g EPA + DHA/day [8]. According to other governing bodies, at least 2 servings of fish per week are set by Australian Dietary Guidelines [9], while 280–525 g of fish is the appropriate weekly intake recommended by Dietary Guidelines for Chinese Residents [10].

Intake of n-3 LC-PUFA in a population can vary significantly according to dietary and supplementation habits, age group, and gender, but in general, the average intake is often significantly lower than recommended levels [11–13]. For the Serbian population, there are still no published data on EPA and DHA intake, but it is deemed that dietary fish intake is low and infrequent, and our previous study showed that n-3 LC-PUFA status in middle-aged healthy individuals was inadequate, confirming low fish consumption [14, 15]. Additionally, patients with cardiometabolic risk factors in Serbia have abnormal n-3 LC-PUFA profile compared to healthy subjects [16].

When increased fish intake is recommended, attention should be paid to the fact that both the total fat and PUFA content vary considerably among species. The fat content and the fatty acid composition of fish can vary significantly due to species, variations in their diet, life cycle, temperature, location, gender and environmental conditions [17, 18]. Moreover, fish can be an important source of aquatic contaminants such as heavy metals and polychlorinated organic compounds with high persistence, bio-accumulative properties and potential harmful human health effects [19].

Thus, the aim of this study was to determine and compare the fat, fatty acids and lipid quality indices in common freshwater and seawater fish species available on the Serbian market and to evaluate the most economical fish species as sources of dietary long-chain n-3 fatty acids. In addition, the potential health risks due to the toxicity of contaminants present in fish were also taken into account.

Methods

Chemicals and reagents

All chemicals used were of analytical, high pressure liquid chromatography (HPLC or gass chromatography (GC grades (≥ 99.8%, puriss. pa., Sigma Aldrich). Fatty Acid Methyl Esters (FAME) mix standard (SupelcoTM FAME Mix C4-C24) was provided by Supelco (Bellefonte, PA, USA).

Sample preparation

Five seawater fish species commonly consumed in the Serbian diet were chosen and purchased from the local fish market (salmon, tuna, mackerel, sardines, and hake). The origin of salmon was the North Sea (Norway), while other four species originated from the Adriatic Sea around Montenegro and Croatia. On the other hand, freshwater fish species were caught on the Sava and Danube (rivers) in the vicinity of Belgrade. These are carp, catfish, perch and bream. Trout was the only freshwater cultivated species analyzed in this study. The season chosen for investigation was winter. Skin, head, gills, fins, and bones from each fish species were removed; meat was cut into pieces and homogenized. Three to five specimens from each species were used for the analyses.

Total fat content

The total lipids content from each homogenized sample was determined by the gravimetric method after Soxhlet extraction. Total fats were extracted according to Soxhlet method with chloroform as an organic solvent [20]. About 10 g of the homogenized sample was extracted for 4–5 hrs. Fat content per 100 g of sample was expressed in %.

Fatty acid methyl esters (FAMEs) preparation and determination

Approximately 0.2 g of extracted lipids from each specimen was transferred into glass cuvettes and 1.5 mL of 3 mol/L HCl/methanol was added for conversion of lipids to volatile FAMEs. The cuvettes were mixed, heated in the water bath at 85 °C for 45 min, and cooled. One mL of hexane was added for FAMEs extraction. After centrifugation for 10 minutes at 3,000 rpm, the hexane (upper layer) containing the FAMEs was transferred into vials and immediately analysed [21]. FAMEs were further analyzed using an Agilent Technologies 7890A Gas Chromatograph with a flame ionization detector (FID). Separation of the FAMEs was performed on a CP-Sil88 capillary column (100 m x 0.25 mm x 0.2 μm). The oven temperature program started at 80 °C, and increased by 4 °C/min up to 220 °C (hold time 5 min), then by 4 °C/min up to 240 °C and held at 240 °C for 10 min. Injector temperature was 250 °C and the detector temperature was 270 °C. The carrier gas was helium with a constant flow of 1 mL/min and makeup gas was nitrogen, with a flow of 25 mL/min. Fatty acids were identified by their retention time in comparison with reference fatty acids standard. The results were expressed as a percentage of individual fatty acid in total fatty acids content.

The lipid quality indices

From the data on the fatty acid composition, the atherogenic and thrombogenic indices were calculated using modified equations by Ulbricht and Southgate [22] and Garaffo et al [23]:

Index of atherogenicity (AI):

AI = [C12:0 + (4 × C14:0) + C16:0]/(n-3PUFA + n-6PUFA + MUFA)] (1)

Index of thrombogenicity (TI):

TI = [C14:0 + C16:0 + C18:0]/[(0.5 × C18:1) + (0.5 × sum of other MUFA) + (0.5 × n-6PUFA) + (3 × n-3PUFA) + n-3PUFA/n-6PUFA)] (2)

Myristic acid (C14:0) is considered to be 4-times more atherogenic than the other fatty acids; thus a coefficient of 4 was assigned to it. Monounsaturated fatty acids (MUFA) and n-6 PUFA were assigned coefficients of 0.5 since they are less antiatherogenic than n-3 PUFA, which was assigned a coefficient of 3 [24].

Flesh-lipid quality (FLQ) was calculated following the formulae by Abrami et al. [25] and Senso et al. [26]:

FLQ = 100 × (EPA + DHA)/(% of total fatty acids) (3)

Hypercholesterolaemic fatty acids (OFA):

OFA = C12:0 + C14:0 + C16:0 (4)

Hypocholesterolaemic fatty acids (DFA):

DFA = C18:0 + UFA (unsaturated fatty acids) (5)

Statistical analysis

All descriptive variables were shown as mean ± standard error (SE). The Shapiro-Wilk test was used for testing the distribution. Asymmetrically distributed variables were shown as median (interquartile range). Continuous variables were compared by the Mann-Whitney *U*-test. All data were analyzed using IBM® SPSS® Statistics version 22 software. A *p*-value less than 0.05 was considered statistically significant. Cost-minimization analysis was conducted to assess the economic utility.

Results

Table 1 shows the total lipid content of homogenized tissues of freshwater and seawater species. Among freshwater fish, it was observed that fat content varied the most in common carp (2.2–6.5%) and bream (1–4.2%), while perch (0.8–2.1%) and catfish (0.4–1%) showed less variability, comparatively. There were no significant differences in fat content between mackerel, sardine and salmon ($p = 0.183$). After the analysis of fatty acids composition in the lipid extracts, the proportional content of each FA was expressed as a percentage of the total amount of FA (data not shown). The most abundant saturated and monounsaturated fatty acids (SFA and MUFA) in freshwater

Table 1

Lipid content of fish species

Fish species	Total lipids (%), mean ± standard error
Freshwater fish	
carp (*Cyprinus carpio*), n = 5	3.76 ± 0.74
catfish (*Siluris glanis*), n = 5	0.63 ± 0.12
perch (*Sander lucioperca*) n = 5	1.51 ± 0.24
bream (*Abramis brama*) , n = 5	2.91 ± 0.25
trout (*Salmo irideus*), n = 3	7.69 ± 1.56
Seawater fish	
mackerel (*Scomber scombrus*), n = 3	13.20 ± 0.21
sardines (*Sardina pilchardus*), n = 3	11.03 ± 2.21
tuna (*Thunnus thynnus*), n = 3	1.17 ± 0.14
hake (*Merluccius merluccius*), n = 3	1.50 ± 0.35
salmon (*Salmo salar*), n = 5	10.60 ± 0.65

and seawater fish were palmitic (16:0) and oleic acid (18:1 n-9). Palmitic acid was most present in carp (16%) and in sardines (21.5%), while oleic acid predominated in salmon (22.1%) and carp (18%). Essential linoleic acids (18:2 n-6) were found in the highest percentage in carp and salmon samples (14.9 and 8.25%, respectively), while the content of arachidonic acid (20:4 n-6) was highest in mackerel (12%). EPA and DHA were the most abundant LC-PUFA ranging from only 3.2% in tuna to 14.7% in sardine samples for EPA and from 12.9% in carp up to 36.8% in hake samples for DHA. Figure 1 shows a relationship between SFA and UFA in the analyzed fish samples. In all freshwater fish species, the ratio was about 75% for unsaturated and 25% for saturated fatty acids. A similar ratio was observed in seawater fish. The content of PUFA was especially uniform, except for salmon which had a lower percentage of SFA (15%) and a higher percentage of MUFA (32%). No difference was found in terms of PUFA/SFA ratio between freshwater and seawater fish ($p > 0.05$).

Fatty acid content per 100 g of edible fish was calculated using the analyzed lipid content of each fish species with the equation: FA in g per 100 g muscle = (% FA of total FA/100) × (Muscle fat % × 0.9). The results are shown in Table 2 in relation to the total content of saturated, monounsaturated, polyunsaturated n-6 and n-3 fatty acids, as well as to the content of the most important long-chain n-3 fatty acids in fish meat. Among the freshwater and seawater fish species studied, the highest average content of SFA was found in sardine and mackerel samples. Salmon had the highest content of MUFA, whereas mackerel was with the highest content of PUFA. The highest values of total n-3 fatty acids and EPA were found in mackerel and sardines samples. Mackerel, sardines, and salmon had the highest content of DHA among seawater species, as well as trout among freshwater species. The ratio of DHA/EPA ranged from 1.8 for sardine samples and up to 10 for tuna samples, indicating that the amount of DHA in natural samples exceeds the amount of EPA in many cases. The content of n-3 fatty acids, EPA, and DHA was significantly higher in seawater fish compared to freshwater fish group (Table 3).

Results in Table 4 are related to the amount of weekly intake of fish in grams or portions necessary for achieving the mean value of the EFSA dietary recommendations of 400 mg EPA+DHA/day. The selected portion was similar to the

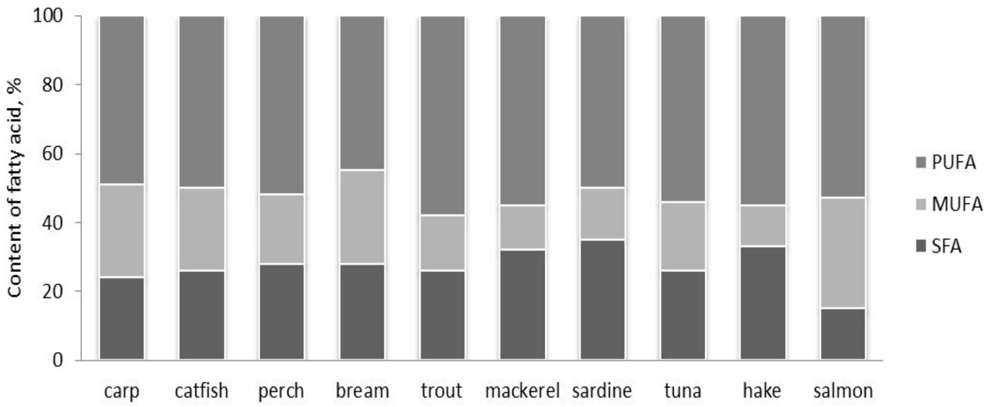


Fig. – 1. The average distribution of saturated and unsaturated fatty acids (MUFA and PUFA) in lipids of freshwater and seawater fish. For abbreviations see Table 2 below.

Table 2

Fatty acid content of each edible freshwater and seawater fish species

Fish species	Fatty acid content (g/100 g), mean ± standard error						
	SFA	MUFA	PUFA	n-3 FA	n-6 FA	EPA	DHA
Freshwater fish							
carp, n = 5	0.73 ± 0.15	0.79 ± 0.13	1.49 ± 0.33	0.74 ± 0.21	0.75 ± 0.15	0.14 ± 0.04	0.48 ± 0.17
catfish, n = 5	0.13 ± 0.02	0.14 ± 0.05	0.25 ± 0.04	0.18 ± 0.03	0.06 ± 0.01	0.05 ± 0.01	0.10 ± 0.02
perch, n = 5	0.35 ± 0.06	0.25 ± 0.04	0.66 ± 0.11	0.54 ± 0.09	0.12 ± 0.02	0.14 ± 0.02	0.33 ± 0.06
bream, n = 5	0.73 ± 0.20	0.77 ± 0.25	1.18 ± 0.36	0.90 ± 0.28	0.27 ± 0.07	0.27 ± 0.08	0.52 ± 0.18
trout, n = 3	1.70 ± 0.41	0.98 ± 0.10	3.79 ± 0.85	3.26 ± 0.85	0.54 ± 0.14	0.46 ± 0.14	2.57 ± 0.66
Seawater fish							
mackerel, n = 3	2.67 ± 0.16	1.51 ± 0.34	6.56 ± 0.30	4.87 ± 0.33	1.69 ± 0 09	1.18 ± 0.06	2.68 ± 0.46
sardines, n = 3	2.72 ± 0.61	1.18 ± 0.57	4.41 ± 1.04	4.01 ± 0.99	0.39 ± 0.05	1.34 ± 0.47	2.45 ± 1.05
tuna, n = 3	0.27 ± 0.04	0.14 ± 0.03	0.48 ± 0.06	0.40 ± 0.04	0.08 ± 0.01	0.04 ± 0.00	0.35 ± 0.03
hake, n = 3	0.39 ± 0.13	0.21 ± 0.05	0.66 ± 0.11	0.62 ± 0.11	0.04 ± 0.00	0.11 ± 0.01	0.48 ± 0.09
salmon, n = 5	2.04 ± 0.13	2.79 ± 0.23	4.47 ± 0.31	3.42 ± 0.28	1.04 ± 0.07	0.74 ± 0.08	2.15 ± 0.22

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; FA – fatty acids; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid.

Table 3

Comparison of fatty acid content between freshwater and seawater fish groups

Fish species	Fatty acid content (g/100 g), median (interquartile range)						
	SFA	MUFA	PUFA	n-3 FA	n-6 FA	EPA	DHA
Freshwater fish	0.47 (0.19–0.96)	0.39 (0.16–0.97)	0.87 (0.37–1.65)	0.65 (0.27–0.99)	0.18 (0.09–0.48)	0.15 (1.07–0.29)	0.32 (0.16–0.53)
Seawater fish	1.99 (0.34–2.60)	0.86 (0.20–2.37)	4.19 (0.64–5.76)	3.06 (0.56–4.28)	0.47 (0.07–1.19)	0.74 (0.09–1.02)	1.61 (0.46–2.41)
p-value	< 0.01	0.073	< 0.01	< 0.01	0.194	< 0.05	< 0.001

For abbreviations see Table 2 above.

Table 4

Weekly intake of fish necessary for achieving EFSA daily dietary recommendations and EPA + DHA weekly intake cost

Fish	Weekly intake (400 mg EPA + DHA/day), g (portions)	Mean cost of EPA + DHA weekly intake, €
Bream	300 (~2)	3.3
Catfish	1,570 (~10)	5.9
Carp	459 (~3)	4.2
Perch	600 (~4)	5.2
Hake	410 (~2.5)	1.7
Trout*	93 (~0.5)	0.46
Sardine*	67 (~0.5)	0.12
Mackerel*	75 (~0.5)	0.23
Salmon*	95 (~0.5)	1.4
Tuna	636 (~4)	11.8

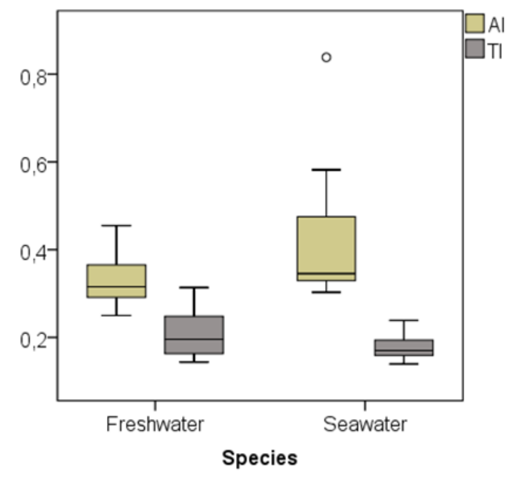
***Fish with high content of lipid.**
EFSA – European Food Safety Authority; for other abbreviations see Table 2 above.

middle portion of the fatty fish defined by the United Kingdom Scientific Advisory Committee on Nutrition, which is ~ 150 g. The evaluation of the costs of EPA + DHA using average prices of raw fish from the Serbian market was also presented.

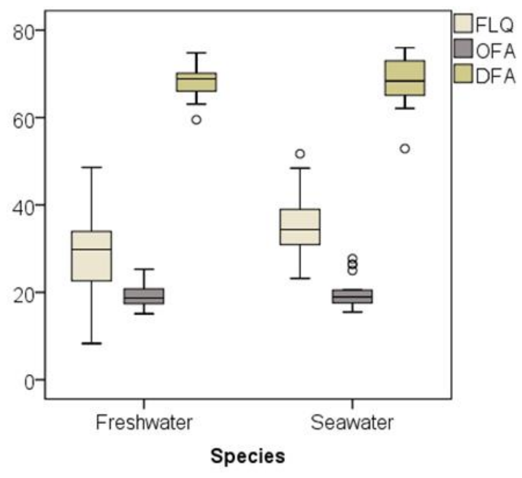
Median values (interquartile range) for lipid quality indices are shown in Figure 2. TI, OFA, and DFA within the freshwater and seawater fish groups did not differ significantly ($p > 0.05$). TI for freshwater and seawater fish groups were 0.20 (0.16–0.25) and 0.17 (0.15–0.19), respectively. OFA for the freshwater group was 18.70 (17.40–21.30) while the seawater group had a similar values [18.90 (17.55–22.75)]. DFA also showed similar ranges for both groups 68.80 (66–70.20) for freshwater and 68.40 (64.85–73.05) for seawater group. AI for the freshwater fish group was 0.32 (0.29–0.38) and 0.34 (0.33–0.49) for the seawater fish group ($p < 0.01$). Seawater fish had a significantly higher value for flash lipid quality 34.40 (30.25–39.95) compared to the freshwater fish [29.80 (22.60–34.60)], ($p < 0.05$).

Discussion

It was noted that all species of freshwater fish (except trout) had a similar content of lipids and the obtained values were very low compared to tested species of seawater fish.



a)



b)

Fig. – 2. Lipid quality indices: a) Atherogenic index (AI) and Thrombogenic index (TI); b) Flesh-lipid quality (FLQ); Hypercholesterolaemic fatty acids (OFA); Hypocholesterolaemic fatty acids (DFA).

Ljubojević et al. [27] reported high variability (6.3–15%) in lipid content of common carp in Serbia. On the other hand, Łuczyńska et al. [28] showed lower values of the fat content in common carp found on Polish market (0.21–1.47%). However, according to the Unated States Department of Agriculture (USDA) database [29], where data come from a variety of sources, the mean value for common carp lipid content is 5.6%. Ljubojević et al. [27] and Stancheva et al. [30] found about 4% of total lipid in catfish, while the USDA database records value of 2.8%. These results are higher compared to our results for catfish. Previous study on fat quality of marketable freshwater fish species in Serbia have indicated that total lipids obtained for perch ranged from 1.5 up to 2.2% [27], which is in agreement with our results as well as with the results given by the USDA database. Lipid content for bream obtained in this study is similar to previous data published by Łuczyńska et al. [31]. The muscles of trout contained more lipids than the other freshwater fish species. Our results are in accordance with the data obtained by Łuczynska et al. [31] and with the USDA database value (6.6%). In a recent study conducted by Bandarra et al. [32], similar results for sardine fat content were obtained as in our research (14 ± 2.9%), while in the study conducted by Zorica et al. [33], the fluctuations in fat content were also reported for the same species of sardine from the middle eastern Adriatic Sea Region. According to McCance and Widdowson's The Composition of Foods Integrated Dataset 2019 on the nutrient content of the United Kingdom food supply, fat content from tuna and salmon is similar to the results in this study, while results for mackerel are slightly higher (17.9 g/100 g of raw fish) [34]. The USDA database shows similar results for mackerel (12%) compared to ours. Ozyilmaz et al. [35] found the total lipid content of European hake from the northeastern Mediterranean of 1.21 ± 0.4%. According to Ackman [36], fish can be classified by its lipid content. He graded the fish in four categories based on lipid levels: lean (< 2%), low-fat (2–4%), medium-fat (4–8%), and high-fat (> 8%). Our results confirmed catfish, perch as lean, bream and carp as low-fat, trout as medium-fat freshwater fish, while within analysed sea fish only tuna and hake were lean fish, and mackerel, salmon, and sardines all belonged to the high-fat category. The fish classification into lean, low-fat, medium-fat, and high-fat can be significant for making tailored dietary programs depending on the dietary goals – either lower fat and energy intake or higher n-3 fatty acid intake.

Wood et al. [37] have suggested that the ratio of PUFA/SFA in food should be above 0.4, and according to that, all the fish species examined revealed a favourable PUFA/SFA ratio from 1.4 to 3.5. The DHA/EPA ratio for freshwater fish and most seawater fish analyzed in this study are in agreement with the literature data [27, 28, 30–33, 35]. Only in carp, the pattern n-3/n-6 ratio was less than 1 in present study, while in all other fish samples, the content of n-3 fatty acids was several times higher than content of n-6 fatty acids. According to the research conducted by Buchtova et al. [38] and Ćirković et al. [39], the carp grown on natural food had a high content of both n-6 and n-3 fatty acids. Ljubojević et al. [27] also reported n-3/n-6 ratio lower than 1 (0.48 ± 0.18) for common carp. Sardine had the highest ratio of these fatty acids at 10.2. The n-6/n-3 ratio should not exceed 4 for the prevention of cardiovascular, heart, and certain chronic diseases [40]. All studied species meet this suggestion.

As it was expected, the content of n-3 fatty acids, EPA and DHA, was significantly higher in seawater fish compared to freshwater fish group. The content of n-3 fatty acids should always be observed in relation to the lipid content of fish and in relation to the origin of fish (river, marine, cultivated). This fact justifies the dietary recommendation of a desirable intake of fatty fish in the diet, as only the fatty fish can provide a significant amount of EPA and DHA. For individuals who do not prefer eating fish, weekly intake of properly chosen species even in a small amount (about 10 g *per* day) may be sufficient to reach the mean value of the EFSA recommendation (400 mg EPA + DHA/day). Taking into account the limitation of the study, related to the small sample size and lack of evidence for seasonal variation of fat and fatty acid composition of fish species, we can only hypothetically suggest portions for achieving dietary recommendations. Having in mind insufficient data of fish consumption in Serbian population, further study should take into account more specific needs among different groups in relation to health benefits. The results of a 2015 cross-sectional study obtained from the Food frequency questionnaire (FFQ) among Serbian women in reproductive age indicated that freshwater fish (trout, catfish and carp) and salmon were consumed by 71% and 41% of examinees, respectively [41]. Increasing of California trout farming in Serbia, compared to previous years may be an indicator of new trends in fish consumption [15]. In the present study, the costs of EPA + DHA in the two fish categories were also calculated and this calculation proved there were few inexpensive sources of EPA + DHA on Serbian market. Sardine and mackerel were the least expensive and very affordable fish sources of EPA and DHA.

Index of atherogenicity indicates the relationship between the sum of the main saturated and the main unsaturated FA, the former being considered pro-atherogenic and the latter anti-atherogenic. Index of thrombogenicity shows the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic FA (MUFA, n-6 PUFA, and n-3 PUFA) [22, 26]. Flesh-lipid quality indicates the percentage correlation between the main n-3 LC-PUFA (EPA + DHA) and the total lipids. The higher value of this index is an indicator of the higher quality of the dietary lipid source [25, 26]. Ouraji et al. [42] and Stancheva et al. [30] reported that higher values of AI and TI (> 1.0) are detrimental to human health. The value of these parameters in the present study were lower than 1. The low values of AI and TI indicates that the tissue of all the studied fish is beneficial from a health point of view.

When considering the optimal fish intake there is one more aspect that should be taken into account. Due to the presence of various contaminants in the aquatic environment, fish may be contaminated with persistent chemicals resulting in potential risks to human health. Health benefit and health risk of fish consumption is generally known as the nutritional-toxicological conflict [43]. Contaminant concentrations in fish species depend strongly on the species itself, its metabolism and feeding habits, environmental conditions, chemical contaminations of the sediment and suspended particulate matter of the region where it was living before catch or during its production. In a recently published study [44], the content of metals (Hg, Cd, Pb, As) in three fish species from the Danube River in the Belgrade Region did not reach maximum levels established by The Commission of European Communities (EC 1881/2006, 2006) [45]. Also, a recent data on the mercury content in 9 fish species from the Adriatic sea have indicated that concentrations did not exceed the maximum level of 500 µg/kg [46]. In the study conducted by Janković et al. [47], total concentrations of Hg were measured in fish muscle and canned fish products available on Serbian market. Total of 651 samples were analyzed: 350 samples of marine fish, 34 samples of freshwater fish and 267 samples of canned fish products (tuna and sardines). Mercury concentrations in marine fish were in the range of 0.005-0.208 µg/g; in freshwater fish 0.005–0.099 µg/g and in canned products they were in the range of 0.005–0.642 µg/g. All analyzed samples contained mercury below the maximum level laid down by the European Union and Serbian regulation. Additionally, in a study on the Serbian population, the insignificant risk was demonstrated due to mercury, dichlorodiphenyltrichloroethane and polychlorinated biphenyls associated with fish consumption [48]. All mentioned data suggest that fish consumption in Serbia should not pose health risk derived from contaminants commonly found in fish. Beside the level of contaminants in fish, the frequency of fish consumption, type of fish consumed, as well as the size of the meal have to be taken into consideration for balancing the health benefits and risks of fish intake [49]. For example, hybrid of the Mediterranean-Dietary Approaches to Stop Hypertension (DASH) diets, called MIND (Mediterranean-DASH Intervention for Neurodegenerative Delay) emphasizes an optimal serving of just one meal of fish per week linked to neuroprotection and dementia prevention. This is opposed to 6 meals per week specified by the cardiovascular Mediterranean diet [50]. Our results on n-3 fatty acid content in freshwater and seawater fish indicated that weekly intake of some of the analyzed fish species that would enable achieving dietary recommendation is in accordance with MIND diet approach (sardine, mackerel, salmon, trout).

Conclusion

Among the results of analyses within species, significant variations in the content of certain classes of fatty acids were observed, which confirms that a large number of factors affect the lipid content. Although there were differences in lipid content and fatty acid composition, it was shown that both fish categories are good sources of PUFA. Among the investigated freshwater and seawater fish species, sardine and mackerel had the highest content of n-3 LC-PUFA and represented economically viable sources of EPA + DHA. Besides the fish, other dietary sources of n-3 fatty acids should be considered in further studies.

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