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Lipid Classes and Fatty Acid Composition of *Diplodus vulgaris* and *Conger conger* Originating from the Adriatic Sea

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Summary

Lipid classes and fatty acid composition of polar (phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylserine) and neutral (triglycerides) lipid fractions of common two-banded seabream (*Diplodus vulgaris*, L.) and sea eel (*Conger conger*, L.) originating from the Adriatic Sea (the Šibenik basin) were determined. Total lipid content in *C. conger* (3.7 ± 0.2 %) was almost three times higher than in *D. vulgaris* (1.3 ± 0.2 %). Polar lipids in *D. vulgaris* were almost twice as high (28.1 ± 4.2 %) as in *C. conger* (15.5 ± 0.2 %). Neutral lipids were present in higher proportions (71.9 ± 4.2 %) in *D. vulgaris* and (84.5 ± 0.2 %) in *C. conger*. The fatty acid composition of triglycerides was much more complex than those of polar lipid fractions. There were 25 identified fatty acids in *Diplodus vulgaris* and 23 identified fatty acids in *Conger conger* muscle tissue samples. Palmitic (16:0, 20.3–63.9 %), stearic (18:0, 5.5–58.7 %) and oleic (18:1 n-9c, 3.8–23.1 %) acid were the most abundant fatty acids in both analysed fish species, but their amounts differed significantly. Appreciable quantities of docosahexaenoic acid (DHA 22:6 n-3, 0.5–15.4 %), eicosapentaenoic acid (EPA 20:5 n-3, 1.2–5.3 %), arachidonic acid (20:4 n-6, 0.7–7.8 %) and tetracosaeonic acid (24:1 n-9, 0.7–4.8 %) were also found. EPA + DHA values were much higher in the *Conger conger* lipid fractions in comparison with *Diplodus vulgaris* lipid fractions, except for phosphatidylethanolamine. Our study points out that both fish species contain appreciable levels of n-3 polyunsaturated fatty acids and would therefore be suitable for highly unsaturated low-fat diets.

Key words: *Diplodus vulgaris*, *Conger conger*, fish, lipid classes, fatty acid composition

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Introduction

Since the epidemiological work of Dyerberg, Bang and Nielsen on Greenland Eskimos (*1*) suggested a possible link between low incidence of heart disease and the consumption of seafood, a large number of studies have been published on the role of n-3 polyunsaturated fatty acids (PUFA) in human health and diseases. Over the last decade, a considerable number of experiments have indicated that consumption of fish oils rich in n-3 polyunsaturated fatty acids (n-3 PUFA) has health benefits (*2–5*). Fish lipids, especially those of marine origin, are their natural source. The consumption of lipids rich in saturated fatty acids and cholesterol increases atherogenesis, while lipids rich in monounsaturated (MUFA) and PUFA reduce atherogenesis and thrombogenesis, and thus the risk of cardiovascular diseases (*6*). These fatty acids, particularly eicosapentaenoic acid (20:5 n-3 or EPA) and docosahexaenoic acid (22:6 n-3 or DHA) have been proved to have beneficial effects in cardiovascular diseases and control of blood lipid levels (*7,8*), diabetes mellitus (*9*), depression (*10*), autoimmune disorders, rheumatoid arthritis and other inflammatory disorders (*11*). EPA and DHA are also known to play a major role in modulating the biosynthesis of eicosanoids (*12*). Moreover, DHA is found in high concentrations in membranes of important organs, possibly influencing membrane-lipid dependent functions, especially in retina and brain (*13*). Therefore, the nutritional importance of fish consumption is associated largely with the n-3 PUFA content (*14*). These findings have created a new market for fish oil as a food and dietary supplement (*15*). Several other products with technical and cosmetic applications based on fish oil fatty acids have also been developed and produced commercially (*16*). Fatty acid composition data are needed by food scientists and nutritionists to aid them in dietary formulation, processing and product development (*14*).

Fish is a major source of food for many human populations, which has stimulated many studies to collect information about the quality of fish species all over the world. Fatty acid compositional data for different marine, freshwater and farmed fish species, especially originating from the United States, Canada and Japan are available in the literature (*17–19*). However, published information about the composition of fatty acids in Adriatic Sea fish species is meager.

The aim of the present study was to collect information on fatty acid composition of two commercially important fish species in the Adriatic Sea. They occupy an important place in the fishing activity of Croatia because they represent an important food source. Two-banded seabream (*Diplodus vulgaris*, L.) and sea eel (*Conger conger*, L.) fish species, common in the Adriatic Sea, were selected and the amount of total, neutral and polar lipids as well as fatty acid composition of neutral (triglycerides–TG) and polar (phosphatidylethanolamine-PE, phosphatidylcholine-PC, phosphatidylinositol-PI/phosphatidylserine-PS) lipid classes were determined.

Materials and Methods

Collection of fish species

Two-banded seabream (*Diplodus vulgaris*, L.) and the sea eel (*Conger conger*, L.) were analysed. Fish samples were collected from the Šibenik basin, in the Adriatic Sea, Croatia, during the month of August 1996. Fish were captured by long-line at depth of 10–15 m, overnight. *D. vulgaris* and *C. conger* specimens were selected among the captured species. Ten specimens of similar body weight and lenght for both analysed fish species were collected. Biological characteristics of whole fresh fish were determined after every collection. Species, body weight (g) and length (cm) were noted and fish were dissected immediately after catch. Average body weight of *D. vulgaris* specimens amounted to 350 g with an average lenght of 18 cm. *C. conger* specimens had an average body weight of about 1000 g and an average lenght of 68 cm.

Prior to analysis, about 5 g of fish muscle tissue (head, tail, fins, viscera and skin were removed) was separated for the determination of fatty acid composition, and about 1 g of fish muscle tissue was separated for moisture content. Each sample was put into a plastic tube, well sealed, and marked for transport on ice. The samples were brought to the Department of Chemistry and Biochemistry laboratory at the Faculty of Medicine, Rijeka, frozen at –20 °C within the first 24 h and after that tissue samples were preserved at –70 °C for further analysis.

Moisture content

The samples, which were separated for moisture content analysis, were preserved overnight at +4 °C, and analysed immediately the following day. The moisture content was determined in fish muscle tissue samples with an average mass 1 g, using the standard drying method at 105 °C until constant mass was attained (*20*).

Extraction of total lipids

Total lipids were extracted from muscle tissue samples according to Folch *et al.* (*21*). A chloroform/methanol solvent mixture (2:1) was added to frozen fish muscle tissue samples in the ratio solvent : tissue = 20:1. The samples were homogenised 3 times for 10 min with 3000–4000 rpm.

Each homogenisation step was followed by cooling of the sample for 1 h at +4 °C. Four mL of 0.034 % $MgCl_2$ was added to the extract for each 1 g of tissue. The chloroform/methanol extracts were incubated overnight at +4 °C, allowing the separation of the organic (containing the extract of total lipids) and aqueous layers completely. Upper (aqueous) layer was removed, and lower (organic) layer was rinsed with chloroform/methanol (2:1), and placed into glass tubes. Evaporation of the lower phase furnished the total lipid fraction. The solvent was removed in a rotary evaporator under vacuum at +40 °C. These extracts, representing the total lipids, were weighted and dissolved once again in a small volume (1–2 mL) of chloroform/methanol (2:1). The resulting extract of total lipids was stored at +4 °C for further analysis.

Analysis of lipid classes

Polar and neutral lipid fractions were separated from the total lipid extract by thin-layer chromatography (TLC) (*22*). Chromatograms were developed on silica gel plates (»Merck», Darmstadt, Germany – Allurole Silica gel F_{254}) 20 x 20 cm, 0.2 mm, using petroliumether/diethylether (80:20) up to 18 cm, to allow the separation of polar and neutral lipids. At the edge of the plate, a small quantity of the sample was applied separately. That part of the chromatogram was cut off after development, and the bands were visualised by spraying with 50 % sulphuric acid in ethanol followed by heating for 1 h at 180 °C (*23*). Polar lipids remained at the start line, while neutral lipids were taken along the plate.

The position of the bands on the preparative part of the plate was determined by comparison with their position on the small, visualised part of the plate. Neutral lipids (triglycerides – TG) were scraped off the plate together with silica gel into tubes for methylation, for further analysis. The same plate was put into the polar-lipid reagent (chloroform/methanol/ammonium hydroxide 65:35:5), up to the part where neutral lipids were scraped off. Polar lipid fractions (phosphatidylethanolamine-PE, phosphatidylcholine-PC, phosphatidylinositol-PI/phosphatidylserine-PS) were visualised by iodine staining and scraped off the plate together with silica gel into tubes for methylation. Total lipid, neutral lipid and polar lipid contents were determined gravimetrically. Phospholipids were mineralised with a mixture of H_2SO_4 and $HClO_4$ and estimated spectrophotometrically (*24*).

Samples of polar and neutral lipid fractions, obtained as described, were used for fatty acid analysis.

Fatty acid analysis

Fatty acid compositions of polar and neutral lipid fractions were determined by gas chromatography of the corresponding methyl esters. Fatty acid methyl esters were obtained by acid methanolysis of lipid extracts by adding 0.86 mL of benzene and 1.00 mL of BF_3 in methanol to triglycerides and phospholipid fractions (*25*). Hewlett Packard HP 5890 A capillary gas chromatograph equipped with flame ionization detector (FID) was used. A non-polar capillary column HP Innowax cross-linked polyethyleneglycol (HP-5, 30 m x 0.32 mm), containing 5 % diphenyl and 95 % dimethylsyloxan, was used for analysis. The column temperature was programmed for a linear increase of 4 °C/min from 150 to 210 °C. The injector and detector temperatures were 250 °C. Nitrogen was used as carrier gas. The analyses were performed twice. Chromatograms were printed on HP 3396 A GC integrator. Fatty acid methyl esters were identified by comparing their retention times with those of the commercial fatty acid methyl ester standards (GLC 68B Nu-Check-prep, Inc., Elysian, Minnesota). Relative share of each identified fatty acid for each polar and neutral lipid fraction was calculated automatically.

The degree of unsaturation, expressed as unsaturation index according to Kates and Hagen (*26*), was calculated as follows: Δ/mol = [% monoene + 2 (% diene) + 3 (% triene) + 4 (% quadriene) + 5 (% pentaene) + 6 (% hexaene)]/100.

Mann-Whitney U test was used as a nonparametric test for comparing the differences in fatty acid composition for 2 independent groups of fish. We used this test since it is the most appropriate one for biological samples with high variance.

Results

Fish muscle lipid composition

Data on moisture content, total lipids, polar and neutral lipid contents, expressed as a percentage (%) in analysed fish muscle tissue samples, are shown in Table 1.

It was found that the total lipids (TL, percentage of wet weight of muscle tissues) in *C. conger* (3.7 ± 0.2 %) were almost three times higher than in *D. vulgaris* (1.3 ± 0.2 %). Moisture content was also higher in *C. conger* (77.5 ± 2.1 %) in comparison with *D. vulgaris* (76.7 ± 1.3 %). Polar lipids (PL, % of total lipids) were almost twice higher in *D. vulgaris* (28.1 ± 4.2) than in *C. conger* (15.5 ± 0.2 %). Neutral lipids (NL, % of total lipids) were present in higher proportions, (71.9 ± 4.2 %) in *D. vulgaris* and (84.5 ± 0.2 %) in *C. conger*. But it must be emphasized that the total phospholipid content is significantly higher in young fish, when compared to adult fish (*27*).

Fatty acid composition

Neutral (TG) and polar (PI/PS, PC, PE) lipid fractions in fish muscle samples from *D. vulgaris* and *C. conger* were separated by one-dimensional TLC as described in Material and Methods and fatty acid composition was determined. Relative concentrations of each fatty acid for each fish species are expressed as percentages of their total. Results are presented in Tables 2 and 3, respectively. According to their basic characteristics and to the nomenclature adopted in mariculture, the analysed fatty acids were grouped as saturated (SFA), monoenoic and dienoic fatty acids as mono and diunsaturated (MUFA and DUFA) while tri-, tetra-, penta- and hexaenoic fatty acids were clustered as polyunsaturated (PUFA).

In the analysed muscle tissue samples, we identified 25 fatty acids in *D. vulgaris* and 23 fatty acids in *C. conger*. Thanks to the methodology used we were able to detect congeners of specific unsaturated fatty acids (18:1 n-9c/18:1 n-9t, 18:3 n-6/18:3 n-3, 20:3 n-3/20:3 n-6). TG from the analysed fish species in this study contained a great variety of different fatty acids. On the other hand, polar lipid fractions (PI/PS, PC, PE) were poorer in the diversity of fatty acids. All of the identified fatty acids were found in TG, except 22:2, which was found only in *D. vulgaris* PC. Polar lipid fractions contained a minor number of different fatty acids: 11 PI/PS in *D. vulgaris* and 7 in *C. conger*, 10 PC in *D. vulgaris* and 11 in *C. conger*, 8 PE in *C. conger* and twice as many *i.e.* 17 in *D. vulgaris*. The fatty acid composition of PL was much less complex than that of TG.

The major constituents of total fatty acids in *D. vulgaris* and *C. conger* were saturates: palmitic (16:0) and stearic acid (18:0), monoenes: oleic (18:1) and palmitoleic acid (16:1), polyunsaturated: arachidonic (20:4 n-6), eicosapentaenoic (20:5 n-3) and docosahexaenoic acid (22:6 n-3), but their amounts differed significantly. Palmitic

Table 1. Moisture content, total lipids, polar lipids and neutral lipids in *Diplodus vulgaris* and *Conger conger*

Fish species	*w*(moisture)	*w*(total lipids)[1]	*w*(polar lipids)	*w*(neutral lipids)
	%	%	%	%
Diplodus vulgaris	76.7 ± 1.3	1.3 ± 0.2	28.1 ± 4.2	71.9 ± 4.2
Conger conger	77.5 ± 2.1	3.7 ± 0.2	15.5 ± 0.2	84.5 ± 0.2

[1] Expressed on wet weight basis

Table 2. Fatty acid composition of neutral (triglycerides – TG) and polar (phosphatidylethanolamine – PE, phosphatidylcholine – PC, phosphatidylinositol – PI/phosphatidylserine – PS) lipid mass fractions (*w*) of *Diplodus vulgaris* (expressed as percentage of total fatty acids)

Fatty acids (FA)	*w* (total fatty acids)[1]/ %			
	TG	**PI/PS**	**PC**	**PE**
14:0	7.0 ± 1.5	0.8 ± 0.9	2.3 ± 0.2	4.4 ± 1.5
14:1	trace[2]	n.d.[3]	n.d.	n.d.
15:0	0.7 ± 0.6	0.4 ± 0.8	1.9 ± 0.5	0.8 ± 1.1
16:0	25.4 ± 4.0	41.0 ± 22.2	63.9 ± 17.7	38.8 ± 13.1
16:1	12.5 ± 2.3	1.1 ± 1.5	3.7 ± 0.8	4.6 ± 3.3
17:0	1.4 ± 0.9	0.7 ± 1.0	2.3 ± 0.7	0.9 ± 1.3
17:1	0.3 ± 0.4	n.d.	n.d.	n.d.
18:0	10.5 ± 3.1	43.4 ± 30.0	14.9 ± 17.4	19.6 ± 7.7
18:1 n-9t	0.2 ± 0.5	n.d.	n.d.	n.d.
18:1 n-9c	20.4 ± 3.0	5.5 ± 1.8	8.8 ± 1.7	10.5 ± 1.4
18:2 n-6c	1.3 ± 0.8	n.d.	n.d	0.2 ± 0.5
18:3 n-6	0.1 ± 0.1	n.d.	n.d	n.d.
20:0	0.5 ± 0.5	n.d.	n.d	0.1 ± 0.3
18:3 n-3	0.1 ± 0.3	n.d.	n.d.	0.2 ± 0.4
20:1 n-9	1.3 ± 1.0	n.d.	n.d	0.1 ± 0.3
21:0	trace	n.d.	n.d.	n.d.
20:2	0.4 ± 0.5	n.d.	n.d.	2.0 ± 4.1
20:3 n-3	0.1 ± 0.2	n.d.	n.d.	n.d.
20:3 n-6	4.8 ± 1.6	1.2 ± 2.0	n.d	1.4 ± 1.8
22:1 n-9	0.1 ± 0.1	n.d.	n.d.	n.d.
20:4 n-6	7.8 ± 3.5	0.7 ± 1.5	n.d.	1.8 ± 2.6
22:2	n.d.	n.d.	1.0 ± 2.0	n.d.
20:5 n-3	1.4 ± 1.6	n.d.	n.d.	3.8 ± 5.7
24:1 n-9	0.7 ± 1.1	3.2 ± 5.0	0.8 ± 1.5	4.0 ± 4.0
22:6 n-3	3.0 ± 4.1	2.2 ± 3.1	0.5 ± 1.0	6.9 ± 12.5
MUFA+DUFA	37.2 ± 1.4	9.7 ± 5.1	14.2 ± 3.4	21.5 ± 3.0
PUFA	17.2 ± 5.5	4.1 ± 5.8	0.5 ± 1.0	14.0 ± 22.9
Σ UFA	54.4 ± 5.9	13.8 ± 7.0	14.7 ± 3.8	35.5 ± 21.2
SFA	45.6 ± 5.9	86.1 ± 7.0	85.3 ± 3.9	64.5 ± 21.4
EPA + DHA	4.3 ± 3.0	2.2 ± 3.1	0.5 ± 1.0	10.7 ± 18.1
Unsaturation index[4]	1.10	0.29	0.18	0.96
n-3/n-6	0.34	1.21	–	3.25

[1] values are mean ± SD
[2] trace, <0.1 %
[3] n.d., not detected (<0.01 %)
[4] The degree of unsaturation, expressed as unsaturation index according to Kates and Hagen (*26*) (see text for complete formula)
MUFA: monounsaturated; DUFA: diunsaturated; PUFA: polyunsaturated; UFA: unsaturated; SFA: saturated fatty acids; EPA: eicosapentaenoic acid (20:5 n-3); DHA: docosahexaenoic acid (22:6 n-3)

Table 3. Fatty acid composition of neutral (triglycerides – TG) and polar (phosphatidylethanolamine – PE, phosphatidylcholine – PC, phosphatidylinositol – PI/ phosphatidylserine – PS) lipid mass fractions (*w*) of *Conger conger* (expressed as percentage of total fatty acids)

Fatty acids (FA)	*w* (total fatty acids)[1]/ %			
	TG	**PI/PS**	**PC**	**PE**
10:0	trace[2]	n.d.[3]	n.d.	n.d.
12:0	trace	n.d.	n.d.	n.d.
14:0	5.6 ± 1.1	n.d.	3.4 ± 0.4	5.8 ± 0.6
14:1	0.1 ± 0.2	n.d.	n.d.	n.d.
15:0	0.9 ± 0.3	n.d.	1.2 ± 1.2	5.3 ± 0.8
16:0	20.3 ± 1.3	18.9 ± 5.7	62.8 ± 6.3	44.8 ± 2.5
16:1	10.3 ± 2.0	n.d.	4.3 ± 1.1	3.0 ± 4.2
17:0	0.8 ± 0.2	0.5 ± 1.2	0.5 ± 0.7	1.4 ± 2.0
17:1	0.6 ± 0.3	n.d.	n.d.	n.d.
18:0	5.5 ± 0.7	58.7 ± 3.3	6.8 ± 1.4	22.1 ± 7.0
18:1 n-9c	23.1 ± 6.0	3.8 ± 3.8	13.6 ± 2.6	15.8 ± 3.2
18:2 n-6c	2.6 ± 0.9	n.d.	n.d.	n.d.
20:0	0.4 ± 0.2	n.d.	n.d.	n.d.
18:3 n-3	1.1 ± 0.6	n.d.	n.d.	n.d.
20:1 n-9	0.7 ± 0.7	n.d.	n.d.	n.d.
20:2	0.6 ± 0.4	n.d.	n.d.	n.d.
20:3 n-3	0.2 ± 0.2	n.d.	n.d.	n.d.
20:3 n-6	2.7 ± 0.7	n.d.	1.5 ± 2.9	n.d.
22:1 n-9	trace	n.d.	n.d.	n.d.
20:4 n-6	6.8 ± 2.2	n.d.	1.9 ± 3.6	n.d.
20:5 n-3	1.6 ± 1.7	5.3 ± 6.3	1.2 ± 1.7	2.0 ± 2.8
24:1 n-9	0.9 ± 0.6	4.8 ± 7.4	n.d.	n.d.
22:6 n-3	15.4 ± 5.2	8.1 ± 10.7	2.9 ± 2.0	n.d.
MUFA+DUFA	38.8 ± 7.9	8.6 ± 4.8	17.8 ± 2.2	18.8 ± 1.1
PUFA	27.7 ± 7.0	13.3 ± 9.3	7.6 ± 8.2	2.0 ± 2.8
Σ UFA	66.6 ± 1.3	22.0 ± 6.9	25.4 ± 6.1	20.8 ± 3.8
SFA	33.7 ± 1.4	78.1 ± 7.0	74.6 ± 6.1	79.4 ± 3.9
EPA + DHA	17.0 ± 5.0	13.3 ± 9.3	4.2 ± 2.7	2.0 ± 2.8
Unsaturation index[4]	1.81	0.83	0.54	0.29
n-3/n-6	1.53	–	1.20	–

[1] values are mean ± SD
[2] trace, <0.1 %
[3] n.d., not detected (<0.01 %)
[4] The degree of unsaturation, expressed as unsaturation index according to Kates and Hagen (*26*) (see text for complete formula)
MUFA: monounsaturated; DUFA: diunsaturated; PUFA: polyunsaturated; UFA: unsaturated; SFA: saturated fatty acids; EPA: eicosapentaenoic acid (20:5 n-3); DHA: docosahexaenoic acid (22:6 n-3)

acid (16:0) was the dominant fatty acid present (20.3–63.9 %) in both species, in each of the lipid classes, followed by stearic acid (18:0), but the differences in its distribution and amounts were extremely high (5.5–58.7 %). The amounts of both acids were higher in polar lipid fractions. Arachidonic acid (20:4 n-6) was detected in significant amounts in TG of both species, while its amount detected in polar lipid fractions was lower. Tetracosaeonic acid (24:1 n-9) was found in greater quantities in polar fractions in *D. vulgaris*, while it was not found in the same fractions in *C. conger* except in PI/PS. The two fish species that were analysed varied significantly regarding the content of polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA). DHA was found in a very appreciable quantity in *C. conger* triglycerides (15.4 %). Higher quantities of DHA were also found in *C. conger* PI/PS (8.1 %) and PC (2.9 %), but it was not found in its PE, while the content of DHA in PE from *D. vulgaris* was 6.9 %. DHA values in *D. vulgaris* were lower and amounted to 3.0 % in TG, 2.2 % in PI/PS and 0.5 % in PC. The highest content of EPA was found in PI/PS from *C. conger* (5.3 %), while it was not detected in the same fraction in *D. vulgaris*, nor in its PC. EPA + DHA values were much more higher for *C. conger* in comparison with *D. vulgaris*, except for PE.

The degree of unsaturation, expressed as unsaturation index also differed greatly between neutral and polar lipid fractions. It was the highest for *C. conger* TG (1.81). *D. vulgaris* TG also had a high unsaturation index (1.10). Polar lipid fractions had smaller unsaturation indices: 0.96 for PE, 0.29 for PI/PS, 0.18 for PC, while for the same polar fractions *C. conger* had unsaturation indices of 0.29, 0.83 and 0.54, respectively. These results also imply a heterogeneous distribution of fatty acids between the lipid fractions of analysed fish species.

Discussion

Selected fish species are very common in the Adriatic Sea and are also commercially important because they are appreciated in the Mediterranean type of diet. Comparing average body weights and lengths of the whole fresh fish analysed in this study with literature references (*28*), it is clear that values are within the reported limits.

Fish species analysed in this study are low-fat type fish, according to the lipid content classification (*14*). Total lipid content as well as polar and neutral lipid contents in *D. vulgaris* and *C. conger* confirm the results for different Mediterranean marine fish species reported by Passi *et al.* (*29*).

Apart from total lipids, the lipid composition and fatty acid content have been investigated in many fish lipids (*30*). In our study, TG formed the dominant class in fish muscle lipids and contained an entire spectrum of detected fatty acids in both analysed fish species. On the contrary, the fatty acid composition of PL was much less complex. Our results are in agreement with those published by Corraze and Kaushik (*31*) that showed that TG are the main part of stored lipids. Hayashi *et al.* also reported that TG are the predominant group in fish meat lipids (*32*).

Major fatty acids in our study were palmitic (16:0), palmitoleic (16:1), stearic (18:0) and oleic (18:1 n-9c) acid in all lipid classes, but their amounts and ratios differed significantly. A similar observation was reported by Andrade *et al.*, where palmitic acid was the predominant saturated fatty acid, accounting for 7–76 % of total saturated fatty acids and where oleic acid was the most abundant MUFA in 10 marine fish from Brazil (*33*). Hazra *et al.* also reported that saturates and monoenes comprise 60–70 % of total fatty acids in puffer fish (*34*). Palmitic acid (16:0) and oleic acid (18:1n-9c) were the predominant saturate and monoene in our study, respectively. A similar observation was also reported by McGill and Moffat (*35*) in liver lipids of cod, saithe, monkfish and other fish oils marketed commercially. In striped sea bass (*Morone saxatilis*), palmitic acid and oleic acid constitute the majority (62 %) of total fatty acids in every season (*36*). PUFA values for both fish species were generally similar to other fishes originating from the Mediterranean and from other parts of the world (*14,34,37,38*).

Fatty acid profile of the two analysed fish species generally fits into the typical pattern for freshwater fish, where oleic acid is usually the major constituent (*39*), but only for TG fatty acid composition. High concentrations of stearic acid (18:0) were found in PL for both fishes (*D. vulgaris:* 1.9–43.4 %, *C. conger:* 6.8–58.7 %), which are not usually found in marine vertebrates. Our results showed much higher content of SFAs in PL in comparison with other marine fishes from the Adriatic and the Mediterranean Sea (*29,40,41*), which departs from the observation that phospholipids are characteristically rich in long chain PUFA, 20:5 n-3 and 22:6 n-3 often being the major fatty acids (*31*). TG showed more favorable fatty acid composition when compared to PL for both analysed fishes, containing more UFAs.

However, the fatty acid composition of marine fish lipids is complex. Lipid content as well as fatty acid composition in marine fishes vary remarkably with season, fishing ground, age and gender (*34,42–45*). Saito *et al.* showed the influence of sea temperature (*46*). Maia *et al.* noticed that fish nutritional habits influence their fatty acid composition (*47*). Many studies confirmed that the assimilation patterns of dietary fatty acids reflect the content of the dietary lipids source. The accumulation of dietary fatty acids in several fish species was comprehensively studied by Owen *et al.* (*48*), Watanable (*49*), Roche and Peres (*50*), Zhukova *et al.* (*51*), Montero *et al.* (*52*). Krajnovic-Ozretic *et al.* showed in their study on fatty acids in liver and muscle of farmed and wild sea bass (*Dicentrarchus lambrax*) from the Adriatic Sea (*40*) that the accumulation of saturated fatty acids in white muscle in farmed specimens under highly saturated diet was nearly 73 % and significantly correlated with the general fatty acid patterns from the dietary source. The fatty acid composition in marine fish lipids is conditioned by fish nutritional habits, but also by the possibility of transformation in the nutritional chain between sea organisms (*53*). As a result, the composition and content of fatty acids may vary not only from species to species but also to an even greater extent from specimen to specimen of the same species. Consequently, the variations in fatty acid compositions could be enormous. Taking this in consideration, this could be a possible explanation of our results.

Life cycles and feeding characteristics of two analysed fishes are different. The sea bream, *Diplodus vulgaris* is a marine teleost widely distributed on the Adriatic, Mediterranean and eastern Atlantic coasts. Like other marine littoral fish species, it has a life cycle that consists of a pelagic dispersal larval phase followed by a sedentary benthic adult phase (*54,55*). At the end of the planktonic stage, surviving larvae colonise shallow waters (up to 2 m deep), along rocky shores where, after a short period spent in the water column, they settle on boulder substrates and infralittoral rocky habitats (*56*). This is an benthopelagic, euryhaline marine species inhabiting rocky and sometimes sandy bottoms to depths of 160 m, but more commonly in less than 50 m. The young are sometimes found in seagrass beds. The analysis of the reproductive cycle showed an extensive spawning period: from December to March with a peak in intensity in January/February (*57*). The diet of *D. vulgaris* consists essentially of ophiuroids, polychaetes, amphipods and echinoids. They feed on crustaceans, worms and molluscs (*58*). Transition from a carnivorous to an omnivorous diet has also been observed (*59*).

The sea eel, *Conger conger* is a common and widespread species all around the Adriatic, Mediterranean, North Atlantic and Northern Africa's coasts. *Conger* eels can be found from the shoreline to the end of continental shelf. They favour rough ground and inhabit deep-water wrecks, reefs, broken ground, and highly three-dimensional habitats. *Conger* are bottom feeders more than capable of catching live food. This species is a carnivore, their diet consists on benthic organisms, including fish, crabs, cuttlefish and squid (*60*). The sexual maturity starts at the age of 5, when they migrate to the edge of the Sargasso Sea in the subtropical Atlantic to spawn at

deeps of 3000–4000 m (*61*). After spawning, the adults die; the larvae drift as lepticephali for two years (*62*) before they reach the shoreline and metamorphose into juvenile body form (*63*).

Fatty acid content of two analysed fish species in this study shows a very heterogeneous distribution. When comparing the fatty acid composition data between *D. vulgaris* and *C. conger*, statistically significant differences ($p<0.05$) were found in neutral lipids. The contents of 16:0, 18:0, 18:2n-6c, 18:3n-3, 20:3n-3, 22:6n-3 were statistically significantly different ($p<0.05$) between the two fish species in their TG. When analysing PL fractions, statistically significant differences were found only in PC, in the amounts of 14:0, 18:1n-9c and 22:6n-3. Generally, *C. conger* showed a greater content of UFA, especially EPA and DHA, which makes its fatty acid profile more favourable. This could be due to different nutritional habits of the two fish species, but also because of a natural variation in the accumulation of fatty acids and the differences in environmental conditions. Nevertheless, the fatty acid composition of both fish species generally showed a similar distribution.

In recent years, n-3 PUFA have been acclaimed for greater potency in the amelioration of heart and cardiovascular disorders than n-6 PUFA. Therefore, the n-3/n-6 ratio is a marker of biomedical significance for fish oils and could be an index of biomedical application (*7*,*64*). N-3/n-6 ratios were calculated for fatty acids in analysed fish muscle samples. These ratios amounted between 0.34 and 3.25 and also showed different values between analysed lipid classes and between analysed fish species, too. All the n-3/n-6 ratios for different lipid fractions were higher than 1, except for *D. vulgaris* TG, which accords with the observation reported for different Mediterranean marine species of fish and shellfish (*29*), confirming the great importance of fish as a significant dietary source of n-3 PUFAs.

Conclusion

Our results showed a considerable amount of saturated fatty acids, especially in polar lipid fractions. Although not typical, the results of our study are in agreement with many other published results about the fatty acid content in different fish species originating from different locations in the world. The fatty acid distribution is very individual from species to species and it depends on many factors like season, temperature, fishing ground, fish species, age, gender or nutritional habits. Fatty acid composition clearly shows a heterogeneous distribution and varies not only between the analysed fish species but also between lipid fractions of analysed fish muscle tissue samples. Generally, the fatty acid compositions of *D. vulgaris* and *C. conger* show similar patterns, although *C. conger* showed more favourable fatty acid composition, containing more UFAs. Both fish species contain appreciable levels of n-3 polyunsaturated fatty acids and would be suitable for inclusion in the formulation of highly unsaturated low-fat diets.

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Lipidi i sastav masnih kiselina u *Diplodus vulgaris* i *Conger conger* iz Jadranskoga mora

Sažetak

Ispitivan je udjel lipida te sastav masnih kiselina polarnih (fosfatidiletanolamin, fosfatidilkolin, fosfatidilinozitol, fosfatidilserin) i nepolarnih (trigliceridi) lipidnih frakcija dviju vrsta ribe: fratra (*Diplodus vulgaris*, L.) i ugora (*Conger conger*, L.) iz Jadranskoga mora (iz Šibenskoga zaljeva). Količina je ukupnih lipida bila gotovo triput veća u *C. conger* (3,7 ± 0,2 %) u usporedbi s *D. vulgaris* (1,3 ± 0,2 %), a udjel polarnih lipida dvaput veći u *D. vulgaris* (28,1 ± 4,2 %) nego u *C. conger* (15,5 ± 0,2 %). Puno je veća bila količina neutralnih lipida, (71,9 ± 4,2 %) za *D. vulgaris* te (84,5 ± 0,2 %) za *C. conger.* Sastav masnih kiselina triglicerida bio je puno složeniji od sastava frakcija polarnih lipida. Identificirano je ukupno 25 masnih kiselina u analiziranim uzorcima tkiva mišića *Diplodus vulgaris*, odnosno 23 masne kiseline u uzorcima tkiva mišića *Conger conger.* Najzastupljenija količina masnih kiselina za obje vrste ispitivanih riba bila je: palmitinske (16:0, 20,3–63,9 %), stearinske (18:0, 5,5–58,7 %) te oleinske kiseline (18:1 n-9, 3,8–23,1 %), a njihovi su se udjeli znatno razlikovali. Vrlo je dobar udjel dokozaheksaenske (DHA 22:6 n-3, 0,5–15,4 %), eikozapentaenske (EPA 20:5 n-3, 1,2–5,3 %), arahidonske (20:4 n-6, 0,7–7,8 %) te nervonske kiseline (24:1 n-9, 0,7–4,8 %). Vrijednosti EPA + DHA bile su puno veće u frakcijama lipida *Conger conger* nego *Diplodus vulgaris*, osim za fosfatidiletanolamin. Obje vrste ribe sadržavaju značajnu količinu n-3 polinezasićenih masnih kiselina te su stoga pogodne za niskokaloričnu prehranu, bogatu nezasićenim masnim kiselinama.