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Fish Lipids as a Source of Healthy Components: Fatty Acids from Mediterranean Fish

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1. Introduction

From the time when the epidemiological work on Greenland Eskimos suggested a possible correlation between low incidence of heart disease and the consumption of seafood (Bang et al., 1971), a considerable number of studies have been done on the role of n-3 polyunsaturated fatty acids (n-3 PUFA) in human health and diseases. A substantial number of experiments have indicated that consumption of fish oils rich in n-3 PUFA has different health benefits including cardiovarscular health improving, proper fetal development, anti-inflammatory effects and chronic disease alleviation (Harris, 2010; Itua & Naderali, 2010; Lloret, 2010; Massaro et al., 2010; Roberts et al., 2010). The natural sources of n-3 PUFA are foremost fish lipids, especially those of marine origin. The two most important n-3 PUFA are eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA). EPA and DHA have been largely investigated and their positive biological effects have been demonstrated from feeding studies with fish or fish oil supplements (Smutna et al., 2009). Therefore, the nutritional importance of fish consumption is associated with their n-3 PUFA contents. These findings have created a new market for fish oil as a source of healthy components. Many products based on fish oil fatty acids such as dietary supplements and pharmaceuticals as well as other products with technical and cosmetic applications based on fish oil fatty acids have been developed and produced commercially (Driscoll et al., 2009; Martin et al., 2008; Raatz et al., 2009; Smutna et al., 2009). Knowledge about the presence of important constituents and the fatty acid composition of different lipid fractions is essential in the assessment of diet evaluation. Likewise, fatty acid compositional data are needed by food scientists and nutritionists for dietary formulation, processing and product development. In the last decade a significant number of fatty acid compositional data for a number of fish from different parts of the world have been published. However, the fatty acid composition of Adriatic Sea fish species lipids has not been investigated thoroughly. Therefore, the aim of our study was to determine the edible muscle tissue and/or liver fatty

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acid composition of the white sea bream, *Diplodus sargus,* L., the common two-banded sea bream, *Diplodus vulgaris*, L. and the sea eel, *Conger conger*, L. These are all appreciated fish species in the Mediterranean diet, which occupy an important place in the fishing activity of Croatia and other Mediterranean countries. This review summarizes some published data from our previous research (Baticic et al., 2009; Varljen et al., 2004; Varljen et al., 2003), and some new, unpublished data regarding the fatty acid characterization of fish liver and/or edible muscle tissue lipid fractions. The fatty acid compositions of neutral (triacylglycerols, TAG) and polar (phosphatidylinositol, PI; phosphatidylserine, PS; phosphatidylcholine, PC; and phosphatidylethanolamine, PE) lipid classes were described and their potential relevance as source of healthy components is proposed.

2. Materials and methods

The entire procedure of fish sample collection and preparation likewise the analytical procedures described below were previously published in details in the Journal of the American Oil Chemists' Society (Varljen et al., 2004).

2.1 Collection of fish species

Samples of two-banded sea bream (*Diplodus vulgaris,* L.), white sea bream (*Diplodus sargus,* L.) and sea eel (*Conger conger,* L.) were collected from the Kvarner Bay and the Šibenik basin, in the Adriatic Sea, Croatia by long-line at a depth of 10–15 m, overnight. Specimens of similar body weight and length were selected from all the captured specimens. Biological characteristics, namely body weight (g) and length (cm), were noted, and the fish were dissected immediately after catch. The head, tails, fins, viscera and skin were removed. Intact fish livers were set aside for the determination of moisture, lipid content and fatty acid composition. Samples of about 5 g of white fish muscle (edible muscle tissue) were taken from the left lateral region of the body prior to analysis, while about 1 g of muscle tissue samples were taken for moisture content analysis. Each sample was put into a plastic tube, sealed, and marked, then transported on ice to the laboratory of the Department of Chemistry and Biochemistry at the Faculty of Medicine, Rijeka. Samples for lipid extraction and determination of fatty acid composition in different fractions were preserved at –20°C for further analysis.

2.2 Water content analysis

The water content was determined in fish muscle tissue and liver samples having an average mass of 1 g. The samples, that were separated for water content analysis were preserved overnight at +4°C and analyzed immediately the following day, so as to obtain reliable results. The analyses were performed after drying the tissue at 105°C to a constant mass.

2.3 Extraction of total lipids

Total lipids were extracted from fish muscle tissue samples according to Folch et al. (1957). Briefly, a chloroform/methanol solvent mixture (2:1, vol/vol) was added to frozen samples in the ratio solvent/tissue of 20:1 (vol/wt). The samples were homogenized three times and each homogenization step was followed by cooling of the sample for 1 h at +4°C. The chloroform/methanol extracts were incubated overnight at +4°C to allow the organic (containing the extract of total lipids) and aqueous layers to separate completely. The upper (aqueous) layer was removed, and the lower (organic) layer was rinsed with

chloroform/methanol (2:1 vol/vol), then placed into a glass tube. The total lipid fraction was obtained by evaporating the lower phase. The solvent was removed in a rotary evaporator under vacuum at +40°C. These extracts, representing the total lipids, were weighed, and results were noted for each fish. Total lipid contents were determined gravimetrically. After that, each extract was dissolved once again in 2 mL of chloroform/methanol (2:1 vol/vol). The resulting extract of total lipids was stored at +4°C until further analysis.

2.4 Analysis of lipid classes

Polar and neutral lipid fractions were separated from the total lipid extract by thin layer chromatography. Chromatograms were developed on silica gel plates [Allurole Silica gel F254; Merck, Darmstadt, Germany; 20 × 20 cm, 0.2 mm, using petroleum ether/diethyl ether (80:20, vol/vol)] up to 18 cm, so as to allow the separation of polar and neutral lipids. A small quantity of the sample was applied separately at the edge of the plate. That part of the chromatogram was cut off after development, and the bands were visualized by spraying with 50% sulfuric acid in ethanol followed by heating for 1 h at 180°C. Polar lipids remained at the start line, whereas neutral lipids moved 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, visualized part of the plate. Neutral lipids (TAG) were scraped off the plate together with the silica gel into tubes for methylation and further analysis. The same plate was put into the polar-lipid reagent (chloroform/methanol/ammonium hydroxide 65:35:5, by vol), up to the part where neutral lipids were scraped off. Polar lipid fractions (PE, PC, PI, PS) were visualized by iodine staining and scraped off the plate together with silica gel into tubes for methylation. Samples of polar and neutral lipid fractions, obtained as described, were used for fatty acid analysis.

2.5 Fatty acid analysis

Fatty acid compositions of polar and neutral lipid fractions of fish muscle tissue and liver samples were determined by gas cromatography of the corresponding methyl esters. Fatty acid methyl esters were obtained by acid methanolysis of lipid fractions extracts. A capillary gas chromatograph equipped with a flame ionization detector was used. A nonpolar capillary column, HP Innowax cross-linked polyethyleneglycol (HP-5, 30 m × 0.32mm; Agilent, Zagreb, Croatia) containing 5% diphenyl and 95% dimethylpolysiloxane, was used for analysis, which were performed in duplicates. Fatty acid methyl esters were identified by comparing their retention times with those of commercial fatty acid methyl esters standards (GLC 68B; Nu-Chek-Prep, Inc., Elysian, MN). The relative share of each identified fatty acid for each polar and neutral lipid fraction was calculated automatically. The degree of unsaturation, expressed as the unsaturation index, according to Kates and Baxter (1962), was calculated as follows: Δ/mol = [% monoene + 2 (% diene) + 3 (% triene) + 4 (% tetraene) + 5 (% pentaene) + 6 (% hexaene)]/100.

2.6 Statistical analysis

The results of fatty acid composition were expressed as mean ± SD for each fatty acid, representing a percentage of their total. Differences between selected parameters were tested

by analysis of variance (ANOVA) followed by Scheffe post hoc test. The value of $P < 0.05$ was considered as statistically significant.

3. Results and discussion

Fish species described in this review were abundantly available throughout all seasons in the coastal region of the Kvarner Bay, North Adriatic Sea (*D. vulgaris* and *D. sargus*) and the Šibenik basin, middle Adriatic Sea, Croatia (*D. vulgaris* and *C. conger*). The two-banded sea bream, *D. vulgaris*, and the white sea bream, *D. sargus*, belonging to the same genus *Diplodus,* family *Sparidae*, are common marine teleosts in the Adriatic Sea, widely distributed along the Mediterranean and eastern Atlantic coasts. The sea eel, *C. conger* belongs to the family of *Congridae* and it is also relatively abundantly found in the Mediterranean, North and Irish Sea. The fatty acid compositions of the edible muscle tissue and/or liver were determined for different lipid fractions. Some analyses also included seasonal variations of fatty acid compositions.

3.1 Liver Fatty acid composition of fish originating from the North Adriatic Sea

3.1.1 *Diplodus sargus*, L.

Data regarding *D. sargus* length and mass, liver mass, total lipid content in liver, expressed as a fraction (%) on a wet mass basis, and moisture content in liver during four seasons are presented in Table 1.

	Winter	Spring	Summer	Autumn
Fish length (cm)	25.7 ± 2.0	29.4 ± 4.8	28.2 ± 1.3	20.4 ± 1.3
Fish body weight (g)	445.0 ± 97.1	435.0 ± 193.9	592.3 ± 32.2	354.1 ± 50.1
Liver weight (g)	3.79 ± 1.17	3.14 ± 0.33	3.38 ± 0.65	2.67 ± 0.36
Total lipids (%)	5.5 ± 1.5	6.5 ± 1.5	4.2 ± 1.0	4.6 ± 0.7
Moisture content (%)	71.5 ± 1.1	74.1 ± 0.6	76.9 ± 1.1	73.8 ± 2.4

Table 1. *Diplodus sargus,* L. biological characteristics in different seasons

Body mass and length of fish specimens analyzed in this study are within the limits reported in the literature (Jardas, 1996). The total lipid content in liver was the highest in spring ((6.5±1.5) %) and the lowest in summer ((4.2±1.0) %). The moisture content in the liver was the highest in summer ((76.9±1.1) %), while it was the lowest in winter ((71.5±1.1) %). The obtained results for *D. sargus* from the Adriatic Sea showed slightly lower values for total lipid content in the liver, while they are in agreement with the published results of moisture content for *D. sargus* from other parts of the Mediterranean Sea (Cejas et al., 2004; Perez et al., 2007).

The fatty acid compositions of neutral (TAG) and polar (PI/PS, PC, PE) lipid fractions of *D. sargus* liver, as well as other fatty acid parameters, have been determined during spring, summer, autumn and winter. Results are shown in Tables 2 to 5. The relative ratios of each fatty acid are expressed as mean values ± SD, representing the fraction (%) of total identified fatty acids. According to their characteristics and the nomenclature adopted in mariculture, the analyzed fatty acids were grouped as saturated (SFA), monounsaturated (MUFA), diunsaturated (DUFA), while tri-, tetra-, penta-, and hexaenoic fatty acids were grouped as polyunsaturated fatty acids (PUFA). The degree of unsaturation and the n-3/n-6 ratios were also determined.

	Percentage of total fatty acids in TAG[1]			
Fatty acid component	Winter	Spring	Summer	Autumn
14:0	3.4 ± 0.2	3.9 ± 0.9	5.7 ± 0.1	5.1 ± 1.1
14:1 n-5	0.8 ± 0.3	0.8 ± 0.5	0.7 ± 0.4	0.5 ± 0.2
16:0	16.8 ± 2.5	29.1 ± 2.1	25.4 ± 3.0	25.5 ± 2.0
16:1 n-7	10.0 ± 0.8	10.1 ± 1.2	8.4 ± 1.7	10.2 ± 1.3
18:0	4.4 ± 0.2	11.1 ± 4.2	9.1 ± 1.5	8.1 ± 0.8
18:1 n-9	19.3 ± 1.5	24.5 ± 7.2	11.4 ± 2.6	24.6 ± 3.5
18:2 n-6	3.6 ± 0.6	2.1 ± 1.5	0.8 ± 0.5	0.8 ± 0.3
20:0	0.1 ± 0.1	0.6 ± 0.5	0.6 ± 0.3	0.3 ± 0.2
18:3 n-3	1.0 ± 0.3	2.7 ± 2.5	0.1 ± 0.1	1.2 ± 1.5
20:1 n-9	2.3 ± 1.1	2.1 ± 1.7	2.7 ± 1.3	2.6 ± 1.7
22:0	0.3 ± 0.4	Trace	0.1 ± 0.1	1.7 ± 3.6
20:4 n-6	4.0 ± 1.3	2.7 ± 0.9	4.1 ± 2.7	4.4 ± 1.7
22:1 n-11	0.1 ± 0.2	0.8 ± 0.4	0.1 ± 0.1	0.1 ± 0.2
20:5 n-3	8.6± 0.8	3.0± 1.3	4.3± 2.6	4.8± 0.9
24:0	Trace[2]	0.3 ± 0.3	0.2 ± 0.0	0.1 ± 0.2
22:3 n-3	1.0 ± 1.0	1.7 ± 1.6	2.6 ± 1.5	1.9 ± 1.1
24:1 n-9	0.2 ± 0.2	0.4 ± 0.4	0.3 ± 0.2	1.7 ± 0.4
22:6 n-3	23.9 ± 5.2	4.3 ± 2.4	23.2 ± 5.9	6.6 ± 1.4
MUFA + DUFA	36.4 ± 4.1	40.7 ± 9.2	24.5 ± 2.8	40.4 ± 4.5
PUFA	38.6 ± 4.1	14.3 ± 4.9	34.4 ± 4.3	18.9 ± 2.8
Σ UFA	74.9 ± 3.1	55.0 ± 5.1	58.8 ± 4.7	59.3 ± 4.0
EPA + DHA	32.5 ± 5.6	7.3 ± 3.2	27.5 ± 5.7	11.4 ± 2.2
Σ SFA	25.1 ± 3.1	45.0 ± 5.1	41.2 ± 4.7	40.7 ± 4.0
Unsaturation index	2.49	1.07	2.11	1.32
n-3/n-6	4.53	2.43	6.07	2.79

Table 2. Fatty acid composition of triacylglycerols-TAG (neutral lipid fraction) of *Diplodus sargus,* L. liver with seasonal variation (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

	Percentage of total fatty acids in PI/PS[1]			
Fatty acid component	Winter	Spring	Summer	Autumn
14:0	1.6 ± 0.6	3.0 ± 0.8	0.9 ± 0.4	0.9 ± 0.2
14:1 n-5	0.8 ± 0.5	1.5 ± 0.8	0.4 ± 0.3	0.3 ± 0.1
16:0	26.0 ± 9.8	46.6 ± 5.3	23.2 ± 8.0	17.1 ± 3.0
16:1 n-7	3.9 ± 2.2	8.1 ± 2.1	1.8 ± 1.1	1.7 ± 0.9
18:0	30.0 ± 13.5	19.6 ± 8.6	40.7 ± 4.0	42.3 ± 6.7
18:1 n-9	10.0 ± 2.0	12.9 ± 1.5	9.4 ± 2.9	6.8 ± 0.7
18:2 n-6	0.6 ± 0.4	0.4 ± 0.2	1.1 ± 0.8	0.4 ± 0.2
20:0	0.2 ± 0.2	0.2 ± 0.3	0.5 ± 0.2	0.5 ± 0.2
18:3 n-3	Trace[2]	0.3 ± 0.0	Trace	Trace
20:1 n-9	0.7 ± 0.4	0.3 ± 0.2	0.8 ± 0.3	1.0 ± 0.5
22:0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.6 ± 1.3
20:4 n-6	12.0 ± 3.7	2.2 ± 1.9	10.2 ± 3.5	11.0 ± 4.0
22:1 n-11	Trace	0.1 ± 0.1	Trace	0.2 ± 0.3
20:5 n-3	4.5 ± 2.4	2.0 ± 2.3	1.6 ± 1.1	4.6 ± 2.3
24:0	Trace	Trace	0.3 ± 0.4	1.0 ± 1.2
22:3 n-3	1.2 ± 1.8	0.4 ± 0.4	4.0 ± 2.2	5.3 ± 2.2
24:1 n-9	0.1 ± 0.2	Trace	Trace	0.8 ± 0.6
22:6 n-3	8.1 ± 4.2	2.3 ± 2.6	4.8 ± 2.0	5.5 ± 2.7
MUFA + DUFA	16.1 ± 2.0	23.3 ± 2.7	13.6 ± 1.7	11.2 ± 1.9
PUFA	25.8 ± 3.8	7.2 ± 7.2	20.7 ± 4.4	26.4 ± 9.1
Σ UFA	41.9 ± 5.1	30.5 ± 7.9	34.3 ± 4.3	37.6 ± 9.5
EPA + DHA	12.1 ± 6.1	4.3 ± 4.8	6.5 ± 2.3	10.1 ± 4.9
Σ SFA	58.1 ± 5.1	69.5 ± 7.9	65.7 ± 4.3	62.4 ± 9.5
Unsaturation index	1.40	0.58	1.05	1.28
n-3/n-6	1.33	1.99	0.94	1.35

Table 3. Fatty acid composition of phosphatidylinositol-PI/phosphatidylserine-PS (polar lipid fractions) of *Diplodus sargus,* L. liver with seasonal variation (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

	Percentage of total fatty acids in PC[1]			
Fatty acid component	Winter	Spring	Summer	Autumn
14:0	2.3 ± 0.8	2.4 ± 0.9	2.0 ± 1.2	1.8 ± 0.6
14:1 n-5	1.2 ± 0.6	0.5 ± 0.2	1.1 ± 0.1	0.8 ± 0.7
16:0	34.7 ± 5.9	24.1 ± 1.4	40.4 ± 4.4	37.0 ± 9.1
16:1 n-7	8.0 ± 2.2	2.9 ± 1.8	4.7 ± 2.5	4.8 ± 2.1
18:0	5.6 ± 0.7	35.6 ± 2.5	8.0 ± 0.6	6.5 ± 1.2
18:1 n-9	9.8 ± 1.5	11.8 ± 4.5	6.1 ± 3.6	8.4 ± 2.4
18:2 n-6	2.4 ± 2.9	0.8 ± 0.4	0.3 ± 0.0	0.8 ± 1.1
20:0	0.1 ± 0.0	Trace	0.4 ± 0.2	0.1 ± 0.2
18:3 n-3	0.2 ± 0.0	0.7 ± 0.2	Trace	0.1 ± 0.2
20:1 n-9	0.3 ± 0.1	0.6 ± 0.5	0.4 ± 0.3	0.5 ± 0.4
22:0	0.1 ± 0.0	0.1 ± 0.1	Trace	0.2 ± 0.1
20:4 n-6	4.6 ± 1.3	2.3 ± 0.8	6.3 ± 2.1	8.7 ± 1.7
22:1 n-11	Trace[2]	0.1 ± 0.1	Trace	Trace
20:5 n-3	7.6 ± 2.6	2.2 ± 1.3	6.6 ± 0.7	8.5 ± 2.5
24:0	0.1 ± 0.1	Trace	Trace	Trace
22:3 n-3	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.3	0.9 ± 0.4
24:1 n-9	0.3 ± 0.5	Trace	0.7 ± 0.5	1.3 ± 0.7
22:6 n-3	22.3 ± 4.8	15.5 ± 5.4	22.7 ± 5.7	19.5 ± 6.5
MUFA + DUFA	22.1 ± 2.1	16.7 ± 5.2	13.3 ± 3.7	16.6 ± 3.6
PUFA	35.1 ± 6.5	21.1 ± 5.9	36.0 ± 6.8	37.7 ± 9.9
Σ UFA	57.2 ± 6.7	37.8 ± 1.0	49.3 ± 4.6	54.3 ± 9.8
EPA + DHA	29.9 ± 6.3	17.7 ± 5.9	29.3 ± 5.8	28.0 ± 8.6
Σ SFA	42.2 ± 6.7	62.2 ± 1.0	50.7 ± 4.6	45.7 ± 9.8
Unsaturation index	2.16	2.16	2.09	2.15
n-3/n-6	1.82	6.22	4.52	3.04

Table 4. Fatty acid composition of phosphatidylcholine-PC (polar lipid fraction) of *Diplodus sargus,* L. liver with seasonal variation (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

	Percentage of total fatty acids in PE[1]			
Fatty acid component	Winter	Spring	Summer	Autumn
14:0	1.0 ± 0.5	2.8 ± 0.4	1.6 ± 0.4	1.3 ± 0.5
14:1 n-5	0.5 ± 0.2	1.6 ± 0.2	0.6 ± 0.3	0.4 ± 0.3
16:0	21.0 ± 3.7	38.0 ± 2.8	22.2 ± 3.5	23.4 ± 8.2
16:1 n-7	5.4 ± 1.9	8.2 ± 1.0	6.3 ± 1.2	4.9 ± 1.9
18:0	11.1 ± 1.0	13.9 ± 3.0	11.8 ± 0.4	13.4 ± 4.4
18:1 n-9	17.3 ± 3.9	12.5 ± 2.4	13.6 ± 2.7	12.3 ± 3.5
18:2 n-6	4.5 ± 4.7	0.4 ± 0.3	0.5 ± 0.3	0.9 ± 0.3
20:0	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2
18:3 n-3	1.5 ± 1.0	0.3 ± 0.2	0.4 ± 0.7	0.7 ± 0.7
20:1 n-9	0.4 ± 0.3	0.3 ± 0.1	0.8 ± 0.8	0.7 ± 0.8
22:0	0.2 ± 0.2	0.1 ± 0.0	Trace	0.7 ± 0.9
20:4 n-6	9.4 ± 2.7	6.2 ± 0.8	10.8 ± 1.4	8.2 ± 2.5
22:1 n-11	0.2 ± 0.3	Trace	0.3 ± 0.6	0.1 ± 0.3
20:5 n-3	7.7 ± 2.8	5.6 ± 2.2	7.4 ± 0.7	5.5 ± 3.6
24:0	Trace[2]	0.1 ± 0.3	0.3 ± 0.6	0.3 ± 0.9
22:3 n-3	1.1 ± 0.6	0.9 ± 0.3	0.8 ± 0.7	2.7 ± 3.6
24:1 n-9	0.3 ± 0.2	0.1 ± 0.1	0.4 ± 0.5	1.8 ± 1.4
22:6 n-3	18.5 ± 1.1	9.0 ± 4.7	22.0 ± 1.6	22.4 ± 9.9
MUFA + DUFA	28.4 ± 6.7	23.0 ± 2.3	22.6 ± 2.6	21.2 ± 4.6
PUFA	38.2 ± 5.5	22.0 ± 7.3	41.4 ± 1.2	39.5 ± 12.6
Σ UFA	66.6 ± 4.8	45.1 ± 5.3	64.0 ± 3.4	60.7 ± 10.4
EPA + DHA	26.2 ± 3.1	14.7 ± 6.4	29.4 ± 1.3	27.9 ± 11.3
Σ SFA	33.4 ± 4.8	54.9 ± 5.3	36.0 ± 3.4	39.3 ± 10.4
Unsaturation index	2.30	1.34	2.39	2.27
n-3/n-6	2.08	2.40	2.72	3.45

Table 5. Fatty acid composition of phosphatidylethanolamine-PE (polar lipid fraction) of *Diplodus sargus,* L. liver with seasonal variation (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

3.1.2 *Diplodus vulgaris*, L.

The fatty acid compositions of neutral (TAG) and polar (PI/PS, PC, PE) lipid fractions of *D. vulgaris* liver, as well as other fatty acid parameters, have been determined during four different seasons. Results are shown in Tables 6 to 9. The relative ratios of each fatty acid are expressed as mean values ± SD, representing the fraction (%) of total identified fatty acids. The degree of unsaturation, expressed as unsaturation index and the n-3/n-6 ratio were also determined.

	Percentage of total fatty acids in TAG[1]			
Fatty acid component	Winter	Spring	Summer	Autumn
14:0	5.5 ± 1.1	3.3 ± 1.7	5.2 ± 1.4	4.9 ± 2.1
14:1 n-5	0.9 ± 0.6	0.6 ± 0.1	0.7 ± 0.4	0.9 ± 0.7
16:0	21.8 ± 3.2	25.0 ± 4.5	24.4 ± 3.8	27.4 ± 4.5
16:1 n-7	9.1 ± 1.9	8.1 ± 4.0	9.0 ± 3.8	6.7 ± 2.9
18:0	6.8 ± 2.6	9.3 ± 4.1	11.6 ± 5.0	12.1 ± 4.0
18:1 n-9	20.6 ± 4.8	23.7 ± 5.3	22.4 ± 5.0	16.0 ± 5.3
18:2 n-6	1.1 ± 0.7	0.9 ± 0.3	0.8 ± 0.5	1.5 ± 1.1
20:0	0.4 ± 0.2	0.3 ± 0.3	0.2 ± 0.1	0.9 ± 0.8
18:3 n-3	0.8 ± 1.1	1.4 ± 1.5	0.1 ± 0.2	2.2 ± 1.0
20:1 n-9	0.9 ± 0.7	0.6 ± 0.7	1.3 ± 1.6	0.3 ± 0.2
22:0	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.4	1.0 ± 0.7
20:4 n-6	4.5 ± 1.3	5.5 ± 2.0	4.7 ± 1.5	4.7 ± 1.0
22:1 n-11	0.2 ± 0.7	0.2 ± 0.7	Trace	Trace
20:5 n-3	5.6 ± 1.5	5.7 ± 3.4	7.4 ± 3.3	6.4 ± 3.3
24:0	0.3 ± 0.7	Trace[2]	0.2 ± 0.4	0.4 ± 0.8
22:3 n-3	2.6 ± 1.9	2.7 ± 1.2	2.2 ± 1.8	4.2 ± 1.8
24:1 n-9	0.2 ± 0.3	0.1 ± 0.1	Trace	Trace
22:6 n-3	18.3 ± 4.3	12.4 ± 4.7	9.1 ± 2.6	10.4 ± 4.2
MUFA + DUFA	33.0 ± 7.0	34.2 ± 6.0	34.3 ± 7.8	25.5 ± 6.0
PUFA	31.9 ± 7.2	27.7 ± 9.1	23.6 ± 3.7	27.9 ± 6.3
Σ UFA	64.9 ± 6.2	61.9 ± 8.1	57.9 ± 8.5	51.7 ± 7.5
EPA + DHA	23.9 ± 4.7	18.1 ± 6.5	16.5 ± 4.0	16.7 ± 5.8
Σ SFA	35.0 ± 5.8	38.2 ± 8.0	42.1 ± 8.5	46.6 ± 5.1
Unsaturation index	2.01	1.72	1.53	1.59
n-3/n-6	4.92	3.50	3.49	3.77

Table 6. Fatty acid composition of triacylglycerols-TAG (neutral lipid fraction) of *Diplodus vulgaris,* L. liver with seasonal variation (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

	Percentage of total fatty acids in PI/PS[1]			
Fatty acid component	Winter	Spring	Summer	Autumn
14:0	1.4 ± 0.7	3.0 ± 1.5	2.0 ± 0.9	1.4 ± 0.6
14:1 n-5	0.1 ± 0.1	0.7 ± 0.3	0.8 ± 0.5	0.1 ± 0.1
16:0	14.4 ± 1.6	38.9 ± 7.0	43.0 ± 9.4	14.4 ± 1.4
16:1 n-7	1.3 ± 0.7	2.6 ± 1.2	2.7 ± 0.6	1.3 ± 0.6
18:0	38.6 ± 5.0	33.4 ± 4.7	30.8 ± 8.2	39.7 ± 5.2
18:1 n-9	13.9 ± 5.2	9.9 ± 4.9	8.2 ± 3.0	13.6 ± 4.6
18:2 n-6	0.7 ± 0.7	0.8 ± 0.5	0.6 ± 0.7	0.7 ± 0.6
20:0	0.6 ± 0.2	0.4 ± 0.1	0.5 ± 0.6	0.7 ± 0.3
18:3 n-3	0.2 ± 0.5	0.2 ± 0.2	0.1 ± 0.2	0.2 ± 0.4
20:1 n-9	0.8 ± 0.6	1.4 ± 0.8	0.4 ± 0.4	0.9 ± 0.6
22:0	0.7 ± 0.4	1.4 ± 1.4	0.6 ± 0.5	0.8 ± 0.4
20:4 n-6	8.8 ± 5.4	1.5 ± 1.9	3.9 ± 4.1	8.1 ± 5.0
22:1 n-11	0.1 ± 0.2	0.1 ± 0.1	Trace	0.4 ± 0.7
20:5 n-3	3.1 ± 1.7	1.1 ± 0.9	1.8 ± 1.7	2.6 ± 1.8
24:0	0.8 ± 1.1	0.2 ± 0.3	0.2 ± 0.6	0.7 ± 1.0
22:3 n-3	7.8 ± 3.5	2.6 ± 1.3	1.5 ± 1.4	8.5 ± 3.5
24:1 n-9	0.2	0.3 ± 0.3	Trace	0.1 ± 0.2
22:6 n-3	6.5 ± 2.7	1.5 ± 1.1	2.8 ± 2.5	5.7 ± 3.0
MUFA + DUFA	17.1 ± 5.1	15.8 ± 4.4	12.9 ± 2.8	17.2 ± 4.5
PUFA	26.4 ± 7.9	6.8 ± 3.1	10.1 ± 6.3	25.0 ± 7.6
Σ UFA	43.5 ± 5.6	22.7 ± 7.2	23.0 ± 8.4	42.2 ± 5.7
EPA + DHA	9.6 ± 3.5	2.6 ± 1.8	4.6 ± 3.1	8.3 ± 4.1
Σ SFA	56.5 ± 5.6	77.4 ± 7.2	77,0 ± 8.4	57.8 ± 5.7
Unsaturation index	1.32	0.45	0.60	1.23
n-3/n-6	2.54	1.70	2.19	2.54

Table 7. Fatty acid composition of phosphatidylinositol-PI/phosphatidylserine-PS (polar lipid fractions) of *Diplodus vulgaris,* L. liver with seasonal variation (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

	Percentage of total fatty acids in PC[1]			
Fatty acid component	Winter	Spring	Summer	Autumn
14:0	3.2 ± 1.1	2.4 ± 0.6	2.0 ± 0.5	0.8 ± 0.6
14:1 n-5	0.8 ± 0.5	0.7 ± 0.5	0.8 ± 0.3	0.2 ± 0.1
16:0	37.0 ± 5.3	35.5 ± 7.8	37.2 ± 6.4	12.7 ± 1.4
16:1 n-7	5.5 ± 1.8	5.5 ± 2.6	3.9 ± 2.9	1.8 ± 0.3
18:0	8.7 ± 4.8	12.8 ± 9.2	13.7 ± 4.1	33.8 ± 7.4
18:1 n-9	13.8 ± 5.4	15.1 ± 3.7	11.0 ± 1.3	8.2 ± 1.2
18:2 n-6	0.7 ± 0.4	0.4 ± 0.4	0.7 ± 0.5	0.4 ± 0.2
20:0	0.3 ± 0.3	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.1
18:3 n-3	0.2 ± 0.4	0.1 ± 0.3	0.6 ± 0.5	0.7 ± 0.5
20:1 n-9	0.4 ± 0.5	0.5 ± 0.3	0.7 ± 0.5	0.3 ± 0.3
22:0	0.1 ± 0.1	0.4 ± 0.4	0.2 ± 0.2	0.8 ± 0.6
20:4 n-6	3.9 ± 1.4	7.1 ± 1.7	7.5 ± 3.3	12.3 ± 3.7
22:1 n-11	0.1 ± 0.2	0.3 ± 0.6	0.1 ± 0.1	0.8 ± 1.0
20:5 n-3	6.3 ± 1.6	6.2 ± 0.9	5.9 ± 1.7	4.5 ± 1.1
24:0	Trace[2]	0.1 ± 0.1	Trace	0.4 ± 0.5
22:3 n-3	1.1 ± 0.5	0.7 ± 0.5	1.2 ± 0.7	7.5 ± 4.9
24:1 n-9	0.2 ± 0.2	0.4 ± 0.3	0.4 ± 0.4	Trace
22:6 n-3	17.6 ± 6.9	11.5 ± 6.7	14.1 ± 5.1	14.7 ± 5.2
MUFA + DUFA	21.4 ± 6.4	23.0 ± 1.1	17.6 ± 2.7	11.7 ± 1.2
PUFA	29.2 ± 8.7	24.3 ± 8.8	29.2 ± 7.7	39.6 ± 7.2
Σ UFA	50.6 ± 5.1	47.3 ± 8.5	46.8 ± 5.5	51.4 ± 7.2
EPA + DHA	24.0 ± 8.0	16.2 ± 8.4	20.0 ± 5.7	19.1 ± 5.8
Σ SFA	49.4 ± 5.1	52.4 ± 8.1	53.2 ± 5.5	48.6 ± 7.2
Unsaturation index	1.79	1.54	1.67	1.96
n-3/n-6	5.67	2.68	2.37	2.43

Table 8. Fatty acid composition of phosphatidylcholine-PC (polar lipid fraction) of *Diplodus vulgaris,* L. liver with seasonal variation (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

	Percentage of total fatty acids in PE[1]			
Fatty acid component	Winter	Spring	Summer	Autumn
14:0	1.1 ± 0.3	2.2 ± 0.5	2.0 ± 0.5	2.0 ± 0.8
14:1 n-5	0.2 ± 0.1	0.6 ± 0.3	0.8 ± 0.3	0.5 ± 0.3
16:0	21.5 ± 2.8	37.0 ± 8.5	37.2 ± 6.4	26.0 ± 3.0
16:1 n-7	4.6 ± 0.9	7.2 ± 0.2	3.9 ± 2.9	6.9 ± 2.3
18:0	14.2 ± 4.1	11.8 ± 1.4	13.7 ± 4.1	14.9 ± 2.0
18:1 n-9	24.2 ± 4.5	17.8 ± 2.6	11.0 ± 1.3	11.4 ± 2.0
18:2 n-6	0.5 ± 0.3	0.6 ± 0.4	0.7 ± 0.5	0.7 ± 0.3
20:0	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1
18:3 n-3	1.0 ± 1.3	0.3 ± 0.4	0.6 ± 0.5	0.2 ± 0.1
20:1 n-9	0.5 ± 0.8	0.7 ± 0.2	0.7 ± 0.5	0.2 ± 0.2
22:0	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.3 ± 0.1
20:4 n-6	5.6 ± 3.7	4.9 ± 2.2	7.5 ± 3.3	6.7 ± 1.5
22:1 n-11	Trace[2]	Trace	0.1 ± 0.1	0.2 ± 0.2
20:5 n-3	5.2 ± 1.6	5.4 ± 1.9	3.8 ± 2.1	5.3 ± 1.1
24:0	Trace	0.1 ± 0.2	Trace	Trace
22:3 n-3	1.5 ± 0.8	1.1 ± 0.4	1.8 ± 1.4	1.7 ± 0.8
24:1 n-9	0.4 ± 0.5	0.3 ± 0.3	0.1 ± 0.2	Trace
22:6 n-3	19.0 ± 7.1	9.7 ± 4.7	8.5 ± 4.2	22.9 ± 2.7
MUFA + DUFA	30.4 ± 3.6	27.2 ± 2.5	26.4 ± 5.9	19.9 ± 3.9
PUFA	32.3 ± 9.2	21.3 ± 8.8	20.4 ± 6.5	36.8 ± 4.0
Σ UFA	62.7 ± 6.4	48.5 ± 9.4	46.8 ± 7.7	56.7 ± 2.6
EPA + DHA	24.1 ± 7.3	15.1 ± 6.4	12.3 ± 4.7	28.2 ± 3.1
Σ SFA	37.3 ± 6.4	51.5 ± 9.4	53.2 ± 5.5	43.3 ± 2.6
Unsaturation index	2.01	1.36	1.67	2.17
n-3/n-6	4.37	3.12	2.37	4.23

Table 9. Fatty acid composition of phosphatidylethanolamine-PE (polar lipid fraction) of *Diplodus vulgaris,* L. liver with seasonal variation (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

Eighteen different fatty acids were identified in analyzed *D. sargus* and *D. vulgaris* liver lipid fractions samples. The major constituents of total fatty acids were saturates: palmitic (16:0) and stearic acid (18:0); monounsaturated fatty acids: oleic (18:1 n-9) and palmitoleic acid (16:1 n-7), while arachidonic acid (20:4 n-6), EPA (20:5 n-3) and DHA (22:6 n-3) were the major constituents among polyunsaturated fatty acids. The fatty acid amounts and ratios differed significantly among seasons. Palmitic acid was the predominant saturated fatty acid. Oleic acid and DHA were the predominant unsaturated fatty acids. An accentuated seasonality pattern was found for these fatty acids. The same observation was made for *D. sargus* captured along the eastern Mediterranean coast of Turkey (Ozyurt et al., 2005; Imre & Saglik, 1998). The seasonal changes in the contents of these fatty acids were previously recorded for gilthead sea bream (*Sparus aurata*) (Grigorakis et al., 2002), for Baltic herring (*Clupea harengus membras*) (Aro et al., 2000), and some other fish species (Luzia et al., 2003; Tanakol et al., 1999). Furthermore, observations regarding the seasonality of fatty acid composition in *D. vulgaris* caught in other areas of the Mediterranean Sea that were previously published (Donato et al., 1984) are in agreement with the results of this study.

The results of our study revealed that total unsaturated fatty acids (UFAs) in all analyzed lipid fractions were the highest in the winter period in both *D. sargus* and *D. vulgaris,* except for PC in *D. vulgaris* where slightly higher total UFAs were found in the autumn perion. Likewise, the EPA+DHA values were the highest for all lipid fractions in both fish in the winter period, except for PE in *D. sargus*, where EPA+DHA values were slightly higher in the summer period while in *D. vulgaris* in the autumn period. In contrast, saturated fatty acids (SFA) were the highest in the spring and summer period in all analyzed lipid fractions. Neutral lipid fractions contained more UFAs in comparison with polar lipid fractions during the year, except for PE in summer and autumn (*D. sargus*) and autumn period (*D. vulgaris*). The decrease in the amount of UFAs in the analyzed fractions from winter to spring was noticed, followed by an increase in the UFA content in summer and autumn. In TAG, the UFAs were lower in all seasons in comparison with their highest values achieved in winter in both fish species. In PE, the content of UFAs was higher in all seasons compared to the lowest values in the spring also in both fish species. Similarly, PUFA content also showed seasonal variations, having an even more accentuated pattern of seasonality. Similar findings were reported by Donato et al. (1997) for *D. sargus* originating from the Mediterranean Sea. We noticed that PI/PS had the highest content of SFAs in all seasons with the highest values in the spring in both fish species. The lowest total SFA in *D. sargus* and *D. vulgaris* were found in winter in all lipid fractions, except for PC in *D. vulgaris*, where the lowest content of SFAs was determined in the autumn period. These results are in agreement with previously reported findings for this fish species from other catch areas among the Mediterranean coasts (Ozyurt et al., 2005). The observed decrease in total SFA in the winter period is most probably due to the catabolization of SFA in order to ensure the additional metabolic energy required in that period. Likewise, they could be necessary for the increase in PUFA required for spawning in spring and used in gonadal development.

The degree of fatty acid unsaturation, expressed as unsaturation index, differed among the analyzed lipid fractions in both fish species thorough the year. It was the highest for TAG in winter and the lowest for PI/PS in spring both in *D. sargus* and *D. vulgaris*, which reflects

the fatty acid compositions in those seasons. It was observed that unsaturation indices in different lipid fractions achieved their highest values mostly in the winter period. This is in agreement with the previously published observation that a decrease in water temperature results in an increase in the degree of unsaturation (Henderson & Tocher, 1987). This could be explained by the fact that a higher degree of fatty acid unsaturation is essential to maintain the flexibility of membrane phospholipids at lower temperatures (Lovell, 1991).

The content of n-3 PUFA, EPA and DHA is especially important for their beneficial effects. The highest EPA+DHA values were noticed in TAG in the winter period in both fish species, except for PE in *D. vulgaris*, where the highest EPA+DHA values were determined in the autumn period. On the other hand, the lowest but still appreciable EPA+DHA values were always detected in PI/PS, and also showed seasonal variations. Considerable amounts of EPA+DHA in *D. sargus* and *D. vulgaris* liver make them potentially important for exploitation in pharmaceutical and other industries as a potential raw material for dietary omega-3 supplements and other fish-based oil products.

Growing scientific evidence shows that n-3 fatty acids are important in the prevention and amelioration of different chronic disorders (Lloret, 2010). Increasing knowledge suggests that the n-3/n-6 ratio could be used as a biomedical index. The n-3/n-6 ratios were calculated for all lipid fractions in both fish liver samples. Fatty acids of *D. sargus* and *D. vulgaris* liver lipids have an n-3/n-6 ratio between 1 and 6, which is mostly in agreement with previously reported findings for these fish genus (Donato et al. 1997). The n-3/n-6 ratio is also a good marker for comparing nutritional value of fish oils. It is considered to be the most important indicator of fish lipid quality, which best reflects the quality of fish as food (Hu et al., 2002).

3.2 Edible muscle tissue fatty acid composition of fish originating from north Adriatic Sea

3.2.1 *Diplodus vulgaris*, L.

D. vulgaris edible muscle tissue was analyzed and fatty acid compositions of neutral and polar lipid fractions in winter and summer were determined. Body weights of analyzed *D. vulgaris* specimens ranged from 200 to 400 g, with average lengths from 16 to 20 cm. Those values are within the limits reported in the literature (Jardas, 1996). The total lipid content, expressed on a wet weight basis (%, w/w), amounted to 1.0 ± 0.4% in the winter period and 0.9 ± 0.3% in the summer period. According to the lipid content classification, this fish species belongs to low-fat fish (Ackman, 1989). The water content in fish tissue samples amounted to 77.8 ± 2.7% in the winter period and 76.6 ± 1.7% in the summer period.

The fatty acid compositions of neutral (TAG) and polar (PI/PS, PC, PE) lipid fractions of *D. vulgaris* edible muscle tissue, as well as other fatty acid parameters, have been determined during summer and winter periods. Results are presented in Table 10 and 11. The relative ratios of each fatty acid are expressed as mean values ± SD, representing the fraction (%) of total identified fatty acids. The analyzed fatty acids were also grouped as saturated (SFA), monounsaturated (MUFA), diunsaturated (DUFA), while tri-, tetra-, penta-, and hexaenoic fatty acids were grouped as polyunsaturated fatty acids (PUFA). The degree of unsaturation, expressed as unsaturation index, and the n-3/n-6 ratio were also determined.

	Percentage of total fatty acids in winter period[1]			
Fatty acid component	TAG	PI/PS	PC	PE
14:0	5.9 ± 1.0	2.2 ± 1.9	1.4 ± 0.3	4.6 ± 4.3
16:0	21.9 ± 3.6	24.0 ± 8.5	44.7 ± 7.6	25.2 ± 7.0
16:1 n-7	10.7 ± 1.7	1.8 ± 2.0	5.3 ± 0.5	4.3 ± 1.9
18:0	6.6 ± 0.9	17.2 ± 6.1	9.5 ± 4.0	20.7 ± 10.5
18:1 n-9	32.8 ± 3.9	24.4 ± 15.0	19.9 ± 6.8	19.9 ± 5.8
18:2 n-6	1.9 ± 0.7	1.8 ± 1.8	1.6 ± 0.8	3.9 ± 4.0
20:0	0.6 ± 0.4	0.2 ± 0.5	0.2 ± 0.3	0.8 ± 0.9
18:3 n-3	2.6 ± 2.3	Trace[2]	0.8 ± 0.9	1.2 ± 1.5
20:1 n-9	2.5 ± 1.8	4.2 ± 3.9	0.9 ± 0.4	2.2 ± 1.4
22:0	0.3 ± 0.5	1.5 ± 2.0	1.5 ± 2.3	1.0 ± 2.5
20:4 n-6	4.3 ± 1.8	7.4 ± 6.3	4.2 ± 4.8	6.7 ± 2.0
22:1 n-11	1.0 ± 1.6	2.1 ± 2.6	1.3 ± 2.5	0.3 ± 0.7
20:5 n-3	4.1 ± 0.9	0.9 ± 1.6	3.7 ± 2.7	2.7 ± 1.3
24:0	0.1 ± 0.2	1.0 ± 1.6	0.4 ± 0.4	0.4 ± 0.8
22:3 n-3	2.2 ± 1.2	9.9 ± 9.1	1.1 ± 1.0	0.9 ± 0.8
22:6 n-3	2.6 ± 1.8	1.3 ± 2.3	3.6 ± 2.7	5.3 ± 2.4
MUFA + DUFA	48.8 ± 4.9	34.3 ± 14.8	29.0 ± 7.0	30.6 ± 7.9
PUFA	15.7 ± 4.0	19.6 ± 12.3	13.3 ± 10.0	16.8 ± 7.9
Σ UFA	64.5 ± 3.3	53.8 ± 6.3	42.3 ± 9.1	47.4 ± 15.8
EPA + DHA	6.7 ± 2.6	2.2 ± 3.8	7.3 ± 4.5	8.0 ± 3.7
Σ SFA	35.4 ± 3.3	42.6 ± 6.3	57.7 ± 9.1	52.7 ± 26.0
Unsaturation index	1.18	1.08	0.93	1.13
n-3/n-6	1.85	1.32	1.59	0.95

Table 10. Fatty acid composition of neutral (triacylglycerols, TAG) and polar (phosphatidylinositol, PI; phosphatidylserine, PS; phosphatidylcholine, PC; and phosphatidylethanolamine, PE) lipid fractions of *Diplodus vulgaris,* L. edible muscle tissue in the winter period (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

	Percentage of total fatty acids in summer period[1]			
Fatty acid component	TAG	PI/PS	PC	PE
14:0	4.9 ± 1.1	1.5 ± 0.9	2.2 ± 1.7	0.7 ± 0.1
16:0	23.1 ± 2.4	29.6 ± 5.0	22.5 ± 8.9	39.6 ± 10.3
16:1 n-7	7.3 ± 2.2	2.6 ± 2.9	3.6 ± 3.6	2.9 ± 0.9
18:0	11.4 ± 2.2	32.6 ± 16.3	36.5 ± 16.8	24.3 ± 20.4
18:1 n-9	21.7 ± 2.5	5.9 ± 6.4	11.7 ± 8.5	15.0 ± 1.1
18:2 n-6	2.8 ± 0.9	1.1 ± 1.0	1.5 ± 1.1	0.4 ± 0.3
20:0	0.3 ± 0.2	0.8 ± 0.1	0.5 ± 0.4	Trace
18:3 n-3	0.5 ± 0.6	0.8 ± 1.1	0.4 ± 0.5	0.1 ± 0.2
20:1 n-9	1.8 ± 1.7	0.8 ± 1.4	0.5 ± 0.4	0.2 ± 0.2
22:0	0.7 ± 0.3	1.8 ± 1.4	1.1 ± 0.5	0.5 ± 0.7
20:4 n-6	6.6 ± 2.7	7.7 ± 9.9	5.7 ± 4.2	4.9 ± 5.5
22:1 n-11	Trace[2]	1.0 ± 1.4	Trace	0.1 ± 0.1
20:5 n-3	6.0 ± 2.0	1.7 ± 2.2	1.4 ± 1.6	2.1 ± 2.8
24:0	0.1 ± 0.2	1.9 ± 4.0	0.3 ± 0.2	0.1 ± 0.1
22:3 n-3	5.1 ± 0.5	6.6 ± 3.3	7.5 ± 2.1	3.6 ± 2.3
22:6 n-3	7.9 ± 1.0	3.7 ± 5.3	4.7 ± 2.9	5.5 ± 5.7
MUFA + DUFA	33.6 ± 2.5	11.3 ± 6.9	17.4 ± 11.6	18.5 ± 1.3
PUFA	26.0 ± 4.8	20.5 ± 13.7	19.7 ± 5.9	15.9 ± 13.4
Σ UFA	59.6 ± 4.3	31.8 ± 14.1	37.1 ± 11.8	34.4 ± 14.3
EPA + DHA	13.8 ± 2.8	5.4 ± 6.4	6.1 ± 3.8	7.3 ± 8..6
Σ SFA	40.4 ± 4.3	68.2 ± 14.1	64.5 ± 10.8	65.3 ± 14.1
Unsaturation index	1.56	0.96	1.01	0.93
n-3/n-6	2.07	1.45	1.94	2.13

Table 11. Fatty acid composition of neutral (triacylglycerols, TAG) and polar (phosphatidylinositol, PI; phosphatidylserine, PS; phosphatidylcholine, PC; and phosphatidylethanolamine, PE) lipid fractions of *Diplodus vulgaris,* L. edible muscle tissue in the summer period (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

Sixteen different fatty acids were identified in *D. vulgaris* edible muscle tissue lipid fractions. The major constituents of total FA in winter and summer were saturates: palmitic (16:0) and stearic acids (18:0); monoenes: oleic (18:1n-9) and palmitoleic acids (16:1); and polyunsaturates: arachidonic acid (20:4n-6), EPA (20:5n-3), and DHA (22:6n-3). The amounts and ratios of major FA identified in our study (16:0, 18:0, and 18:1n-9) differed significantly between the two seasons and between lipid fractions. A similar observation for this fish species in other areas of catch in the Adriatic Sea is available in literature (Donato et al., 1984). A statistically significant difference ($P < 0.0001$) in oleic acid (18:1n-9) content was found between summer and winter. This FA showed the greatest seasonal variation in our study, followed by 18:0 and 16:0. Values for 18:0 in TAG and PC were found to be statistically different ($P < 0.0001$) during the two periods. The content of 18:0 was considerably higher in summer, when the relative ratio of 18:0 was almost two times higher for TAG and almost four times higher for PC than in the winter period. No statistically significant seasonal variation was detected in the relative ratio of 16:0 in TAG and PI/PS, but it was noticeable in PC and PE ($P < 0.05$). Values for 16:0 were twice as high in winter in PC. In contrast, for PE the relative ratio of 16:0 was much higher in the summer. The content of 18:1n-9 significantly decreased from winter to summer ($P < 0.05$). These results are also in agreement with the results of Donato et al. (1984) for *D. vulgaris* originating from the Adriatic Sea.

The concentrations of n-3 PUFA, EPA, and DHA are significant for their confirmed biomedical importance. Greater amounts in EPA and DHA were found in TAG in the summer period. No such enhanced difference was found in polar lipid fractions. EPA + DHA values were twice as high in the summer period in TAG and PI/PS. Appreciable quantities of 20:4n-6 and 22:3n-3 were also found in all the lipid fractions, with statistically significant seasonal differences ($P < 0.0001$) in TAG, PC, and PE for 22:3n-3. Seasonal variation in the content of 20:4n-6 was significant only in TAG ($P < 0.05$).

Generally, MUFA + DUFA values were significantly higher in winter. On the other hand, PUFA values were higher in summer, especially in TAG. SFA values were also higher in summer. The diminution of the MUFA content in the summer was clearly accompanied by an increase in PUFA content. This is in agreement with the observations of Donato et al. (1984).

The TAGs serve as a store for SFA for energy purposes, and they also may be a temporary PUFA reservoir (Napolitano et al., 1988). They could be forwarded to the synthesis of structural lipids or directed to specific metabolic pathways. Statistically significant seasonal differences ($P < 0.05$ and $P < 0.0001$) were most conspicuous in TAG for all detected FA except 16:0, 20:0, 18:3n-3, 20:1n-9, 22:1n-11, and 24:0. Pazos et al. (1996) reported a similar observation. On the other hand, statistically significant differences ($P < 0.05$ and $P < 0.0001$) in polar lipid fractions (PI/PS, PC, and PE) were found to be less noticeable, especially in PI/PS, where statistically significant seasonal variation was found only for 18:1n-9 ($P < 0.0001$).

The degree of unsaturation, expressed as the unsaturation index, also differed between neutral and polar lipid fractions. It was highest in TAG during the summer while the lowest index was determined in PC n the winter and PE in the summer period.

Emphases on n-3 PUFA over n-6 PUFA propose that the n-3/n-6 ratio could be applied as a biomedical index. Therefore, the n-3/n-6 ratio is a biomedical marker for fish lipids. N-3/n-6 ratios were calculated for all the lipid fractions in analyzed fish muscle tissue

samples. FA in *D. vulgaris* muscle tissue lipids have an n-3/n-6 ratio between 1 and 2, which is relatively good. But it must be emphasized that all the ratios were higher in the summer period.

Results of our study indicate that *D. vulgaris* is a good source of natural n-3 PUFA and would therefore be suitable for inclusion in highly unsaturated low-fat diets. Our results are in agreement with other published results for teleost fish species originating from the Mediterranean and Adriatic Sea (Donato et al., 1984; Passi et al., 2002).

Seasonal variations of FA composition have previously been studied for different fish species (Mayzaud et al., 1999; Pazos et al., 1996, Donato et al. 1984). An inverse relationship between water temperature and the amount of PUFA in tissue lipids of fish and invertebrates has been shown (Hazel, 1979). Seasonal variation of n-3 PUFA seems to be linked to the diet as well as the reproductive cycle (Donato et al., 1984).

In this study, the FA composition in edible muscle tissue of *D. vulgaris* showed a significant variation from winter to summer. The seasonal variations in *D. vulgaris* lipids reflected fluctuations mainly in TAG. But it must also be emphasized that the reproductive cycle of *D. vulgaris* correlates with those seasons, since previtellogenesis occurs in winter and vitellogenesis occurs in summer (Donato et al., 1984). It can be concluded that, although the FA composition of fish is complex and depends on many factors, it clearly shows a seasonal pattern of distribution.

3.3 Edible muscle tissue fatty acid composition of fish originating from middle Adriatic Sea

Diplodus vulgaris, L. and *Conger conger*, L. edible muscle tissue fatty acid compositions were also determined. Fish were caught in the Šibenik basin, Middle Adriatic Sea as previously described. 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 12. 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.*

Fish species	Moisture content (%)	Total lipids (%)	Polar lipids (%)	Neutral lipids (%)
Diplodus vulgaris. L.	76.7 ± 1.3	1.3 ± 0.2	28.1 ± 4.2	71.9 ± 4.2
Conger conger, L.	77.5 ± 2.1	3.7 ± 0.2	15.5 ± 0.2	84.5 ± 0.2

Table 12. Moisture content, total lipids, polar lipids and neutral lipids in *Diplodus vulgaris*, L. and *Conger conger*, L. edible muscle tissue.

	Percentage of total fatty acids[1]			
Fatty acid component	TAG	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]	Trace	Trace	Trace
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	Trace	Trace	Trace
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	Trace	Trace	Trace
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	Trace	Trace	0.2 ± 0.5
18:3 n-6	0.1 ± 0.1	Trace	Trace	Trace
20:0	0.5 ± 0.5	Trace	Trace	0.1 ± 0.3
18:3 n-3	0.1 ± 0.3	Trace	Trace	0.2 ± 0.4
20:1 n-9	1.3 ± 1.0	Trace	Trace	0.1 ± 0.3
21:0	Trace	Trace	Trace	Trace
20:2	0.4 ± 0.5	Trace	Trace	2.0 ± 4.1
20:3 n-3	0.1 ± 0.2	Trace	Trace	Trace
20:3 n-6	4.8 ± 1.6	1.2 ± 2.0	Trace	1.4 ± 1.8
22:1 n-9	0.1 ± 0.1	Trace	Trace	Trace
20:4 n-6	7.8 ± 3.5	0.7 ± 1.5	Trace	1.8 ± 2.6
22:2	Trace	Trace	1.0 ± 2.0	Trace
20:5 n-3	1.4 ± 1.6	Trace	Trace	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
EPA + DHA	4.3 ± 3.0	2.2 ± 3.1	0.5 ± 1.0	10.7 ± 18.1
Σ SFA	45.6 ± 5.9	86.1 ± 7.0	85.3 ± 3.9	64.5 ± 21.4
Unsaturation index	1.10	0.29	0.18	0.96
n-3/n-6	0.34	1.21	-	3.25

Table 13. Fatty acid composition of neutral (triacylglycerols, TAG) and polar (phosphatidylinositol, PI; phosphatidylserine, PS; phosphatidylcholine, PC; and phosphatidylethanolamine, PE) lipid fractions of *Diplodus vulgaris,* L. edible muscle tissue (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

	Percentage of total fatty acids[1]			
Fatty acid component	TAG	PI/PS	PC	PE
14:0	5.6 ± 1.1	Trace	3.4 ± 0.4	5.8 ± 0.6
14:1	0.1 ± 0.2	Trace	Trace	Trace
15:0	0.9 ± 0.3	Trace	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	Trace	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	Trace	Trace	Trace
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	Trace	Trace	Trace
20:0	0.4 ± 0.2	Trace	Trace	Trace
18:3 n-6	1.1 ± 0.6	Trace	Trace	Trace
20:1 n-9	0.7 ± 0.7	Trace	Trace	Trace
20:2	0.6 ± 0.4	Trace	Trace	Trace
20:3 n-3	0.2 ± 0.2	Trace	Trace	Trace
20:3 n-6	2.7 ± 0.7	Trace	1.5 ± 3.6	Trace
22:1 n-9	Trace[2]	Trace	Trace	Trace
20:4 n-6	6.8 ± 2.2	Trace	1.9 ± 3.6	Trace
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	Trace	Trace
22:6 n-3	15.4 ± 5.2	8.1 ± 10.7	2.9 ± 2.0	Trace
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
EPA + DHA	17.0 ± 5.0	13.3 ± 9.3	4.2 ± 2.7	2.0 ± 2.8
Σ SFA	33.7 ± 1.4	78.1 ± 7.0	74.6 ± 6.1	79.4 ± 3.9
Unsaturation index	1.81	0.83	0.54	0.29
n-3/n-6	1.53	-	1.20	-

Table 14. Fatty acid composition of neutral (triacylglycerols, TAG) and polar (phosphatidylinositol, PI; phosphatidylserine, PS; phosphatidylcholine, PC; and phosphatidylethanolamine, PE) lipid fractions of *Conger conger,* L. edible muscle tissue (expressed as percentage of total identified fatty acids). [1]Values are mean ± SD; [2]Trace, <0.1%.

D. vulgaris and *C. conger* belong to low-fat type fish, according to the lipid content classification (Ackman, 1989). Total lipid content as well as polar and neutral lipid contents in *D. vulgaris* and *C. conger* accord with the results for different Mediterranean marine fish species (Passi et al., 2002).

TAG formed the dominant lipid fraction 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 polar lipid classes was much less complex. Our results are in agreement with previously published results which showed that TAG are the main part of stored lipids (Corraze & Kaushik, 1999).

Major fatty acids detected in *D. vulgaris* and *C. conger* in this 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. Palmitic acid (16:0) and oleic acid (18:1n-9c) were the predominant saturate and monoene, respectively. PUFA values were higher in neutral lipid fractions, especially in *C. conger*. However, high concentrations of stearic acid (18:0) were found in polar lipid fractions 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 polar lipids fractions in comparison with other marine fish from the Adriatic and the Mediterranean Sea (Passi et al., 2002). This departs from the observation that phospholipids are characteristically rich in long chain PUFA, with EPA and DHA often being the major fatty acids. TAG showed more favourable fatty acid composition when compared to polar lipid fractions for both analysed fishes, containing more UFAs.

Fatty acid contents of *D. vulgaris* and *C. conger* from the Middle Adriatic Sea show a very heterogeneous distribution. When comparing the fatty acid composition data between these two fish species, statistically significant differences ($P < 0.05$) were found in neutral lipids, in the contents of 16:0, 18:0, 18:2n-6c, 18:3n-3, 20:3n-3, 22:6n-3 in TAG. When analysing polar lipid 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. The most accentuated changes in total lipid and fatty acid composition of fish were previously noticed by other researchers during the reproduction period, when the storage of lipids and other compounds are mobilized from muscle, liver and visceral organs to gonads (Guler et al., 2007; Perez et al., 2007).

N-3/n-6 ratios were calculated for fatty acids in analysed fish edible muscle tissue samples. These ratios amounted between 0.34 and 3.25, also showing different values between analysed lipid classes and between analysed fish species. All n-3/n-6 ratios for different lipid fractions were higher than 1, except for *D. vulgaris* TAG. This findings accord with the observation reported for different Mediterranean marine species of fish and shellfish (Passi et al., 2002), confirming the great importance of fish as a significant dietary source of n-3 PUFAs.

4. Conclusion

This review summarizes data about our research of fatty acid compositions in different lipid fractions of marine fish from the Adriatic Sea, Croatia. Due to the relatively high content of unsaturated fatty acids, Adriatic Sea fish edible muscle tissue could be recommended for

inclusion in the Mediterranean type of diet, as low-fat food with elevated content of highly unsaturated fatty acids. Furthermore, livers from those fish, which are even more rich in polyunsaturated fatty acids in all lipid fractions, could be a good source of biomedically significant components if used as a raw material for products based on fish oil fatty acids such as dietary supplements and pharmaceuticals. Obtained results indicate that fatty acid composition in Adriatic Sea marine fish edible muscle tissue and liver lipid fractions show an accentuated pattern of seasonality. The fatty acid composition of marine fish lipids is multifarious and changes are complex, depending on fish biological and physiological conditions, diet, water temperature, fishing ground and season. Therefore, the influence of season and other factors should be taken into consideration in order to obtain the most appropriate fatty acid composition for industrial and pharmaceutical needs.

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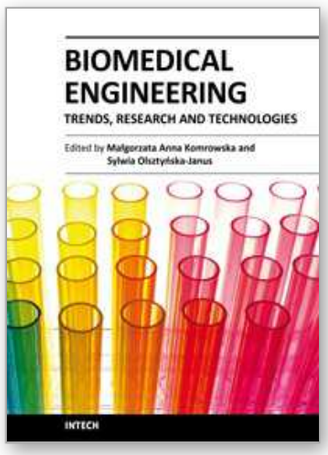

Biomedical Engineering, Trends, Research and Technologies

Edited by Dr. Sylwia Olsztynska

ISBN 978-953-307-514-3
Hard cover, 644 pages
Publisher InTech
Published online 08, January, 2011
Published in print edition January, 2011

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