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Potential Value of Hepatic Lipids from White Sea Bream (*Diplodus sargus*, L.) as a Good Source of Biomedical Components: Seasonal Variations

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Summary

The aim of this study is to evaluate the potential value of hepatic lipids of the white sea bream, *Diplodus sargus*, as a source of important biomedical components. Fatty acid compositions of hepatic neutral (triacylglycerols) and polar (phosphatidylinositol, phosphatidylserine, phosphatidylcholine, or phosphatidylethanolamine) lipid fractions were determined. In order to verify the influence of a season on the fatty acid compositions of liver lipid fractions, fish were captured and analyzed in winter, spring, summer and autumn. Eighteen different fatty acids were identified in the analyzed lipid fractions. 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), eicosapentaenoic (20:5 *n*-3) and docosahexaenoic acid (22:6 *n*-3) were the major constituents among polyunsaturated fatty acids. Their amounts and ratios differed significantly among seasons in different lipid fractions. Total unsaturated fatty acids in all analyzed lipid fractions were the highest in the winter period. Saturated fatty acids were the highest in the spring period in all lipid fractions. Eicosapentaenoic and docosahexaenoic acid achieved the highest values in triacylglycerols in the winter period. Unsaturation indices and *n*-3/*n*-6 values were also influenced by the season. This study revealed a seasonality pattern of *D. sargus* hepatic fatty acid composition.

Key words: Adriatic Sea, *Diplodus sargus*, fatty acid composition, fish lipids, seasonal variation

Introduction

The knowledge about positive health effects of fish consumption is based on a number of studies performed in the last three decades, showing that *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) have many benefits on human health (*1*). Eicosapentaenoic acid (20:5 *n*-3, EPA) and docosahexaenioc acid (22:6 *n*-3, DHA) are the most important *n*-3 PUFA. They have been largely investigated, showing their biological effects from feeding studies

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with fish or fish oil supplements (*2*). A wide spectrum of beneficial effects on cardiovascular and immune function, and on the development and function of the central nervous system have been proven. Clinically important anti-inflammatory effects of *n*-3 PUFA are suggested in inflammatory diseases, asthma, psoriasis and a range of other diseases (*3*).

Based on the importance of fish as part of a healthy diet, an outstanding promotion of fish and seafood consumption as a predominant source of *n*-3 PUFA has been published (*4*). Different products based on fish oil fatty acids such as dietary supplements and pharmaceuticals have been produced (*2*). Since *n*-3 PUFA occur in high amounts in seafood, knowledge about the fatty acid composition is essential. Thus, nutritionists and food scientists working on dietary formulations and product development find the data on the fish fatty acid composition of great benefit. Therefore, the objective of this study is to determine the potential value of fish hepatic lipids as a source of biomedical components.

Data on lipid and fatty acid composition of different marine, freshwater and captive fish from many countries in the world are available in the literature (*5*), but published fatty acid compositional data of fish from the Adriatic Sea is still scarce. Hence, we wanted to determine the liver polar and neutral lipid fatty acid composition of a commercially important fish species from the North Adriatic Sea, the white sea bream (*Diplodus sargus*, L.). It is known that the lipid content and fatty acid composition are influenced by different eco-physical parameters and environmental conditions, such as fish physiological properties, dietary habits, age, sex, water temperature and season of capture (*6–9*). No reports have yet been published about the effects of season on the fatty acid composition of this important fish species in the Adriatic Sea. For this reason, this study is dealing with the fatty acid profile of different lipid fractions of fish harvested during four seasons of the year. The total lipid and moisture contents were determined. Fatty acid compositions of neutral (triacylglycerols, TAG) and polar (phosphatidylinositol/phosphatidylserine, PI/PS; phosphatidylcholine, PC; phosphatidylethanolamine, PE) lipid classes were determined.

Materials and Methods

Collection of fish specimens

The common white sea bream, *Diplodus sargus*, L. (Pisces, Perciformes, Sparidae) was analyzed in this study. Adult fish samples were collected from the Kvarner Bay, North Adriatic Sea, Croatia, during the four seasons of 2005. Fish were captured overnight by a longline at a depth of 10–15 m. *D. sargus* specimens of similar body mass and length were selected within all the captured specimens. The winter study group of fish comprised altogether 10 fish caught during two nights, the first night between January 20–21, 2005 and the second night between February 04–05, 2005. The spring study group comprised in total 8 fish caught at night between April 25–26, 2005. The summer study group comprised 9 fish captured at night between July 12–13, 2005. Finally, the autumn study group was composed of 10 fish in total, which were collected during two nights: the first night between September 24–25, 2005 and the second between October 08–09, 2005. Biological characteristics were determined after every catch, and body mass (g) and length (cm) were measured for each fish. Intact fish livers were set aside immediately after catch for determination of moisture, lipid content and the fatty acid composition of lipid fractions. Each liver sample was put into a plastic tube, sealed, marked, and then transported on ice to the laboratory of the Department of Chemistry and Biochemistry at the School of Medicine, Rijeka. Samples were stored at –80 °C until further analysis.

Moisture content analysis

The moisture content of fish liver samples was determined using a piece of each liver sample with average mass of 0.7 g. The samples that were separated for moisture content analysis were stored overnight at 4 °C and analyzed immediately the following day. The analyses were performed after drying the liver samples at 105 °C to a constant mass (*10*).

Total lipid extraction

Total lipids were extracted from fish liver samples according to Folch *et al.* (*11*). A chloroform/methanol solvent mixture (2:1) was added to the samples at the ratio of solvent/tissue=20:1. Each sample was homogenized three times for 1 min at 3000–4000 rpm and after every homogenization step cooled for 1 h at 4 °C. A volume of 4 mL of 0.034 % $MgCl_2$ was added to the extract for each gram of tissue to promote the separation of the organic (containing the extract of total lipids) and aqueous layers. The chloroform/methanol layers were removed and stored at 4 °C overnight. The following day, the upper, aqueous layer was removed, and the lower, organic layer was rinsed with chloroform/methanol=2:1. The solvent was removed from the organic layer in a rotary evaporator under vacuum at 40 °C, which furnished the total lipid fraction. The extracts of total lipids were determined gravimetrically, and the results were recorded for each fish. Each extract was dissolved once again in 2 mL of chloroform/methanol=2:1 solvent mixture and stored in dark vials at –80 °C until further analysis.

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 F_{254}, 20×20 cm, 0.2 mm thick, Merck, Darmstadt, Germany) using petroleum ether/diethyl ether=80:20 up to 18 cm, to allow the separation of polar and neutral lipids. A small amount of the sample was applied separately at the edge of the plate and cut off after development. The band containing the small sample at the edge of the plate was visualized by spraying with 50 % sulphuric 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. TAGs were

scraped off the plate into tubes for methylation and further analysis. The same plate was put into the polar reagent containing chloroform/methanol/ammonium hydroxide=65:35:5, up to the part where neutral lipids were scraped off the plate. Polar lipid fractions (PI/PS, PC, PE) were visualized by iodine staining and then also scrapped off the plate into tubes for methylation (*12*).

Methylation and gas chromatographic separation of fatty acids

Fatty acid methyl esters were prepared by acid methanolysis of lipid extracts using BF_3 in methanol for both TAG and phospholipid fractions, according to Metcalfe and Schmitz (*13*). Fatty acid compositions of polar and neutral lipid fractions of fish liver tissue samples were determined by gas chromatography of the corresponding methyl esters. Gas chromatographic analyses were carried out using an Autosystem XL (Perkin-Elmer, Norwalk, CT, USA) equipped with flame ionization detector (FID). An SP-2330 capillary column (Supelco, Bellefonte, PA, USA), 30 m×0.32 mm i.d., 0.20 μm film thickness, was used for the analysis. The column temperature was programmed for a linear increase of 4 °C/min from 120 to 220 °C. The injector temperature was 300 °C, and detector temperature was 250 °C. Helium was used as the carrier gas with split injection (100:1). Each analysis was performed twice. Fatty acid methyl ester was identified by comparing its retention times with those of commercial fatty acid methyl ester standards (GLC 68B; Nu-Check Prep, Inc., Elysian, MN, USA). The relative fraction of each identified fatty acid for each polar and neutral lipid fraction was calculated automatically. Chromatography software from Perkin-Elmer Nelson (Turbochrom 4, rev. 4.1.) was used for data acquisition from the FID.

Statistical analyses

Results are presented as mean values±SD, calculated for each of the three polar and one neutral lipid fractions of each liver sample for 8–10 fish in each study group, using the program Microsoft Excel®. Values for different fatty acids are expressed as a fraction (%) of total identified fatty acids. Statistical testing in order to verify the differences between four different seasons of the year was performed using analysis of variance (ANOVA) followed by Scheffe post hoc test. The value of $p<0.05$ was considered as statistically significant. All statistical operations were carried out using Statistica v. 6.1. (StatSoft, Inc., Tulsa, OK, USA).

Results and Discussion

D. sargus were abundantly available throughout all seasons in the coastal region of the Kvarner Bay, North Adriatic Sea. Data regarding fish 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.

A cumulative study from January to October 2005 showed that body mass and length of *D. sargus* specimens analyzed in this study are within the limits reported in the literature (*14*). 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 (*7,9*). Furthermore, the results agree with the published results for *D. vulgaris*, a fish belonging to the same genus, also caught in the Adriatic Sea (*6,15,16*).

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 four different seasons and 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, expressed as unsaturation index, was calculated according to Kates and Baxter (*17*). The *n*-3/*n*-6 ratio was also determined.

Eighteen different fatty acids were identified in the analyzed *D. sargus* liver lipid fraction 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

Table 1. Fish characteristics in different seasons (fish length, body mass, liver mass, total lipids and moisture content)

	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 mass/g	445.0±97.1	435.0±193.9	592.3±32.2	354.1±50.1
Liver mass/g	3.79±1.17	3.14±0.33	3.38±0.65	2.67±0.36
w(total lipids)/%	5.5±1.5	6.5±1.5	4.2±1.0	4.6±0.7
w(moisture)/%	71.5±1.1	74.1±0.6	76.9±1.1	73.8±2.4

fatty acids. The same observation was made for this fish species captured along the eastern Mediterranean coast of Turkey (*8*,*18*). The seasonal changes in their levels were previously recorded for gilthead sea bream (*Sparus aurata*), also belonging to Sparidae as *D. sargus* (*19*), for Baltic herring (*Clupea harengus membras*) (*20*), and other fish species (*21*,*22*). Similar results were published for *D. vulgaris* liver, a fish belonging to the same genus as *D. sargus*, caught in other areas of the Mediterranean Sea (*23*).

The ANOVA test and post hoc analysis revealed statistically significant differences ($p<0.05$) within seasons in the content of 14:0, 16:0, 18:0, 18:1 *n*-9, 18:2 *n*-6, 22:1 *n*-11, 20:5 *n*-3, 24:1 *n*-9 and 22:6 *n*-3 in TAG (Table 2). Polar lipid fractions showed an even more extensive seasonal variation, with statistically significant ($p<0.05$) differences in the content of all detected fatty acids except for 18:2 *n*-6, 20:0, 22:0 and 22:1 *n*-11, 20:5 *n*-3 and 24:0 in PI/PS, as shown in Table 3. PC fatty acid composition was also related to the season (Table 4), with statistically significant ($p<0.05$) seasonal variations in all the detected fatty acids except for 14:0, 14:1 *n*-5, 18:2 *n*-6, 20:1 *n*-9, 22:1 *n*-11, 24:0 and 22:6 *n*-3. The seasonality of fatty acid composition in PE was also statistically significant ($p<0.05$) in the content of 14:0, 14:1 *n*-5, 16:0, 16:1 *n*-7, 18:1 *n*-9, 18:2 *n*-6, 18:3 *n*-3, 20:4 *n*-6, 24:1 *n*-9 and 22:6 *n*-3 (Table 5).

Tables 2 to 5 also show different fatty acid parameters in the analyzed lipid fractions in different seasons. Statistically significant differences ($p<0.05$) were found in MUFA, DUFA, PUFA, total UFA and SFA, and EPA+DHA values in all lipid fractions among different seasons. The results of our study revealed that total unsaturated fatty acids (UFAs) in all analyzed lipid fractions were the highest in the winter period. Likewise, the EPA+DHA values were the highest for all lipid fractions

Table 2. Fatty acid composition (expressed as a fraction of total fatty acids in %) and fatty acid parameters of triacylglycerols, TAG (neutral lipid fraction) of *Diplodus sargus* liver with seasonal variations

Fatty acid component	*w*(total fatty acids)/%			
	Winter	Spring	Summer	Autumn
14:0	3.4±0.2^{c,d}	3.9±0.9^{c}	5.7±0.1^{a,b}	5.1±1.1^{a}
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^{b,c,d}	29.1±2.1^{a,d}	25.4±3.0^{a}	25.5±2.0^{a,b}
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^{b,c,d}	11.1±4.2^{a}	9.1±1.5^{a}	8.1±0.8^{a}
18:1 *n*-9	19.3±1.5	24.5±7.2^{c}	11.4±2.6^{b,d}	24.6±3.5^{c}
18:2 *n*-6	3.6±0.6^{b,c,d}	2.1±1.5^{a}	0.8±0.5^{a}	0.8±0.3^{a}
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	<0.1	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^{b}	0.8±0.4^{a,c,d}	0.1±0.1^{b}	0.1±0.2^{b}
20:5 *n*-3	8.6±0.8^{b,c,d}	3.0±1.3^{a}	4.3±2.6^{a}	4.8±0.9^{a}
24:0	<0.1	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^{d}	0.4±0.4^{d}	0.3±0.2^{d}	1.7±0.4^{a,b,c}
22:6 *n*-3	23.9±5.2^{b,d}	4.3±2.4^{a,c}	23.2±5.9^{b,d}	6.6±1.4^{a,c}
MUFA+DUFA	36.4±4.1^{c}	40.7±9.2^{c}	24.5±2.8^{a,b,d}	40.4±4.5^{c}
PUFA	38.6±4.1^{b,d}	14.3±4.9^{a,c}	34.4±4.3^{b,d}	18.9±2.8^{a,c}
Σ(UFA)	74.9±3.1^{b,c,d}	55.0±5.1^{a}	58.8±4.7^{a}	59.3±4.0^{a}
EPA+DHA	32.5±5.6^{b,d}	7.3±3.2^{a,c}	27.5±5.7^{b,d}	11.4±2.2^{a,c}
SFA	25.1±3.1^{b,c,d}	45.0±5.1^{a}	41.2±4.7^{a}	40.7±4.0^{a}
Unsaturation index*	2.49	1.07	2.11	1.32
n-3/*n*-6	4.53	2.43	6.07	2.79

All values are mean±SD
Statistically significantly different ($p<0.05$) from: ^{a}winter, ^{b}spring, ^{c}summer, ^{d}autumn
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)
*According to Kates and Baxter (*17*)

Table 3. Fatty acid composition (expressed as a fraction of total fatty acids in %) and fatty acid parameters of phosphatidylinositol/phosphatidylserine, PI/PS (polar lipid fractions) of *Diplodus sargus* liver with seasonal variations

Fatty acid component	*w*(total fatty acids)/%			
	Winter	Spring	Summer	Autumn
14:0	1.6±0.6[b]	3.0±0.8[a,c,d]	0.9±0.4[b]	0.9±0.2[b]
14:1 *n*-5	0.8±0.5	1.5±0.8[c,d]	0.4±0.3[b]	0.3±0.1[b]
16:0	26.0±9.8[b]	46.6±5.3[a,c,d]	23.2±8.0[b]	17.1±3.0[b]
16:1 *n*-7	3.9±2.2[b]	8.1±2.1[a,c,d]	1.8±1.1[b]	1.7±0.9[b]
18:0	30.0±13.5	19.6±8.6[c,d]	40.7±4.0[b]	42.3±6.7[b]
18:1 *n*-9	10.0±2.0[d]	12.9±1.5[c,d]	9.4±2.9[b]	6.8±0.7[a,b]
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	<0.1[b]	0.3±0.0[a,c,d]	<0.1[b]	<0.1[b]
20:1 *n*-9	0.7±0.4	0.3±0.2[d]	0.8±0.3	1.0±0.5[b]
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[b]	2.2±1.9[a,c,d]	10.2±3.5[b]	11.0±4.0[b]
22:1 *n*-11	<0.1	0.1±0.1	<0.1	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	<0.1	<0.1	0.3±0.4	1.0±1.2
22:3 *n*-3	1.2±1.8[d]	0.4±0.4[c,d]	4.0±2.2[b]	5.3±2.2[a,b]
24:1 *n*-9	0.1±0.2[d]	<0.1[d]	<0.1[d]	0.8±0.6[a,b,c]
22:6 *n*-3	8.1±4.2[b]	2.3±2.6[a]	4.8±2.0	5.5±2.7
MUFA+DUFA	16.1±2.0[b,d]	23.3±2.7[a,c,d]	13.6±1.7[b]	11.2±1.9[a,b]
PUFA	25.8±3.8[b]	7.2±7.2[a,c,d]	20.7±4.4[b]	26.4±9.1[b]
Σ(UFA)	41.9±5.1[b]	30.5±7.9[a]	34.3±4.3	37.6±9.5
EPA+DHA	12.1±6.1[b]	4.3±4.8[a]	6.5±2.3	10.1±4.9
SFA	58.1±5.1[b]	69.5±7.9[a]	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

All values are mean±SD
Statistically significantly different ($p<0.05$) from: [a]winter, [b]spring, [c]summer, [d]autumn
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)
*According to Kates and Baxter (*17*)

in the winter, except for PE, where EPA+DHA values were slightly higher in the summer period. In contrast, saturated fatty acids (SFA) were the highest in the spring period in all analyzed lipid fractions. Neutral lipid fraction contained more UFAs in comparison with polar lipid fractions during the year, except for PE in summer and autumn. The decrease in the amount of UFA 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 UFA were significantly lower ($p<0.001$) in all seasons in comparison with their highest values achieved in winter. In PE (Table 5), the content of UFA was statistically significantly higher ($p<0.05$) in all seasons compared to the lowest values in the spring. Similarly, PUFA content also showed seasonal variations, with two significant ($p<0.05$) reductions: from winter to spring in all lipid fractions and from summer to autumn in TAG, having an even more accentuated pattern of seasonality. Similar findings were reported by Donato *et al.* (*24*) for *D. sargus* originating from the Mediterranean Sea. We noticed that PI/PS showed the highest content of SFA in all seasons (Table 3), with the highest values in the spring. The lowest total SFA in *D. sargus* was found in winter in all lipid fractions, which is in agreement with previously reported findings for this fish species from other catch areas (*8*). This decrease in winter is most probably due to the catabolization of SFA in order to ensure the additional metabolic energy required in that period and also 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 thorough the year. It was the highest for TAG in winter and the lowest for PI/PS in spring, which reflects the fatty acid compositions in those seasons. It

Table 4. Fatty acid composition (expressed as a fraction of total fatty acids in %) and fatty acid parameters of phosphatidylcholine, PC (polar lipid fraction) of *Diplodus sargus* liver with seasonal variations

Fatty acid component	*w*(total fatty acids)/%			
	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	$24.1\pm1.4^{c,d}$	40.4 ± 4.4^{b}	37.0 ± 9.1^{b}
16:1 *n*-7	$8.0\pm2.2^{b,d}$	2.9 ± 1.8^{a}	4.7±2.5	4.8 ± 2.1^{a}
18:0	$5.6\pm0.7^{b,c}$	$35.6\pm2.5^{a,c,d}$	$8.0\pm0.6^{a,b}$	6.5 ± 1.2^{b}
18:1 *n*-9	9.8±1.5	11.8 ± 4.5^{c}	6.1 ± 3.6^{b}	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^{c}	$<0.1^{c}$	$0.4\pm0.2^{a,b,d}$	0.1 ± 0.2^{c}
18:3 *n*-3	0.2 ± 0.0^{b}	$0.7\pm0.2^{a,c,d}$	$<0.1^{b}$	0.1 ± 0.2^{b}
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	$<0.1^{d}$	0.2 ± 0.1^{c}
20:4 *n*-6	4.6 ± 1.3^{d}	$2.3\pm0.8^{c,d}$	6.3 ± 2.1^{b}	$8.7\pm1.7^{a,b}$
22:1 *n*-11	<0.1	0.1±0.1	<0.1	<0.1
20:5 *n*-3	7.6 ± 2.6^{b}	$2.2\pm1.3^{a,c,d}$	6.6 ± 0.7^{b}	8.5 ± 2.5^{b}
24:0	0.1±0.1	<0.1	<0.1	<0.1
22:3 *n*-3	0.4 ± 0.1^{d}	0.5±0.2	0.4±0.3	0.9 ± 0.4^{a}
24:1 *n*-9	0.3 ± 0.5^{d}	$<0.1^{d}$	0.7±0.5	$1.3\pm0.7^{a,b}$
22:6 *n*-3	22.3±4.8	15.5±5.4	22.7±5.7	19.5±6.5
MUFA+DUFA	$22.1\pm2.1^{c,d}$	16.7±5.2	13.3 ± 3.7^{a}	16.6 ± 3.6^{a}
PUFA	35.1 ± 6.5^{b}	$21.1\pm5.9^{a,d}$	36.0±6.8	37.7 ± 9.9^{b}
Σ(UFA)	57.2 ± 6.7^{b}	$37.8\pm1.0^{a,d}$	49.3±4.6	54.3 ± 9.8^{b}
EPA+DHA	29.9 ± 6.3^{b}	17.7 ± 5.9^{a}	29.3±5.8	28.0±8.6
SFA	42.2 ± 6.7^{b}	$62.2\pm1.0^{a,d}$	50.7±4.6	45.7 ± 9.8^{b}
Unsaturation index*	2.16	2.16	2.09	2.15
n-3/*n*-6	1.82	6.22	4.52	3.04

All values are mean±SD
Statistically significantly different ($p<0.05$) from: [a]winter, [b]spring, [c]summer, [d]autumn
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)
*According to Kates and Baxter (*17*)

was observed that unsaturation indices achieved their highest values in winter. This is in agreement with the previously published observation that a decrease in water temperature results in an increase in the degree of unsaturation (*25*). 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 (*26*). In comparison with the results of previous studies (*6,13,14*), *D. sargus* liver lipid fractions showed generally higher unsaturation indices than *D. vulgaris* muscle tissue lipid fractions, while they are in agreement with the unsaturation indices for liver lipid fractions.

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 (Table 2), following a sharp decline in the spring spawning period ($p<0.001$), and another from summer to autumn ($p<0.05$). This is in agreement with the study of Donato *et al.* (*24*). The lowest EPA+DHA values were always detected in PI/PS, but also showed seasonal variations (Table 3). Appreciable amounts of EPA+DHA in *D. sargus* liver make it possibly important for exploitation in pharmaceutical industry as a potential raw material for dietary omega-3 supplements.

Scientific evidence shows that *n*-3 fatty acids are important in the amelioration of cardiovascular disorders. Growing 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 fish liver samples. Fatty acids of *D. sargus* liver lipids have an *n*-3/*n*-6 ratio between 1 and 6, which is mostly in agreement with previously reported findings for this fish (*24*). The *n*-3/*n*-6 ratio is also a good marker for comparing nutritional value of fish oils, considered to be the most important indicator of fish lipid quality, which best reflects the quality of fish as food (*27*).

Table 5. Fatty acid composition (expressed as a fraction of total fatty acids in %) and fatty acid parameters of phosphatidylethanolamine, PE (polar lipid fraction) of *Diplodus sargus* liver with seasonal variations

Fatty acid component	*w*(total fatty acids)/%			
	Winter	Spring	Summer	Autumn
14:0	1.0±0.5[b]	2.8±0.4[a,c,d]	1.6±0.4[b]	1.3±0.5[b]
14:1 *n*-5	0.5±0.2[b]	1.6±0.2[a,c,d]	0.6±0.3[b]	0.4±0.3[b]
16:0	21.0±3.7[b]	38.0±2.8[a,c,d]	22.2±3.5[b]	23.4±8.2[b]
16:1 *n*-7	5.4±1.9[b]	8.2±1.0[a,d]	6.3±1.2	4.9±1.9[b]
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[d]	12.5±2.4	13.6±2.7	12.3±3.5[a]
18:2 *n*-6	4.5±4.7[b,c,d]	0.4±0.3[a]	0.5±0.3[a]	0.9±0.3[a]
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[b]	0.3±0.2[a]	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	<0.1	0.7±0.9
20:4 *n*-6	9.4±2.7	6.2±0.8[c]	10.8±1.4[b]	8.2±2.5
22:1 *n*-11	0.2±0.3	<0.1	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	<0.1	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[d]	0.1±0.1[d]	0.4±0.5[d]	1.8±1.4[a,b,c]
22:6 *n*-3	18.5±1.1	9.0±4.7[c,d]	22.0±1.6[b]	22.4±9.9[b]
MUFA+DUFA	28.4±6.7[d]	23.0±2.3	22.6±2.6	21.2±4.6[a]
PUFA	38.2±5.5[b]	22.0±7.3[a,c,d]	41.4±1.2[b]	39.5±12.6[b]
Σ(UFA)	66.6±4.8[b]	45.1±5.3[a,c,d]	64.0±3.4[b]	60.7±10.4[b]
EPA+DHA	26.2±3.1	14.7±6.4[c,d]	29.4±1.3[b]	27.9±11.3[b]
SFA	33.4±4.8[b]	54.9±5.3[a,c,d]	36.0±3.4[b]	39.3±10.4[b]
Unsaturation index*	2.30	1.34	2.39	2.27
n-3/*n*-6	2.08	2.40	2.72	3.45

All values are mean±SD
Statistically significantly different ($p<0.05$) from: [a]winter, [b]spring, [c]summer, [d]autumn
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)
*According to Kates and Baxter (*17*)

The fatty acid composition of marine fish lipids is multifarious, depending on fish biological and physiological conditions, diet, water temperature, and season (*6–9*). 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 such as proteins are mobilized from muscle, liver and visceral organs to gonads (*9,28*). *D. sargus* spawning period is in March, the spring period, when significant changes in the fatty acid composition of liver lipid fractions were noticed. The results concerning seasonal variations in our study generally agree with the previously reported findings for *D. sargus* liver fatty acid compositions studied in relation to the reproductive cycles, especially reductions in PUFA, including EPA and DHA. The influence of gender and sexual maturation on hepatic fatty acid composition has been established for *D. sargus* originating from other parts of the Mediterranean Sea (*9,24*). Likewise, a strong influence of nutritional habits on the fatty acid composition of fish lipids has been shown. In gilthead seam bream, also from the genus *Diplodus*, the fatty acid composition of liver and muscle tissue generally reflects the fatty acid composition of the diet (*29*). Other studies confirmed that the fatty acid patterns in fish are influenced by the fatty acid content of the dietary lipids (*30*).

The white sea bream, *D. sargus*, is a common marine hermaphroditic Sparidae fish species in the Adriatic Sea, found along the Mediterranean and Atlantic coasts (*9*). It is an omnivorous species which dominates fish assemblages in rocky infralittoral habitats, together with other species of the genus *Diplodus*. *D. sargus* feed throughout the year on bivalves, algae, sea urchins and barnacles. Polychaetes, decapods and amphipods are also common in its diet (*31*). Taking into consideration all previously

mentioned factors that influence the fatty acid composition, it can vary from species to species but also from specimen to specimen, as a consequence of individual fish characteristics.

Conclusion

The results of our study broaden the knowledge about the fatty acid composition of *D. sargus*, a commercially important fish species from the Adriatic and Mediterranean Sea. Our study revealed a seasonality pattern of its hepatic fatty acid composition. Due to the relatively high content of UFAs, the liver of *D. sargus* could serve as a good source of biomedically significant components if used as a raw material for the products based on fish oil fatty acids such as dietary supplements and pharmaceuticals. But it must be emphasized that the influence of season should be taken into consideration in order to obtain the most appropriate fatty acid composition. It should also be pointed out that the potential use of this type of oil is subjected to other analyses, since hepatic lipids can have a high quantity of toxic products, such as dioxins.

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