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Fatty acid composition and fat-soluble vitamins content of sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) from Bulgarian Black Sea

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Abstract Sprat and goby are commercially important Bulgarian Black Sea fish species. The fatty acid (FA) composition was analyzed by Gas Chromatography with MS detector. Lipid extraction was done according to the Bligh and Dyer method. The monounsaturated FA accounted were 26.93 % for sprat and 30.38 % for goby and palmitoleic (C 16:1) and oleic (C 18:1) acids were dominants in this group. In comparison with other groups, the polyunsaturated FA showed the high level in goby – 37.60% including eicosapentaenoic (C 20:5 n3, EPA), docosahexaenoic (C 22:6 n3, DHA) acids, and lower level on sprat – 34.33%. The level of n 3 polyunsaturated fatty acid was higher than the total n 6 polyunsaturated fatty acid in the all analyzed Black Sea fish species. HPLC method was used for determination of Vitamin A (all-trans-retinol), Vitamin D_3 (cholecalciferol) and Vitamin E (α–Tocopherol) content. The results from fat-soluble vitamins show the differences between sprat and goby. The present studies suggest that both fish species are good sources of n 3 fatty acids and vitamins A, D_3 and E.

Keywords: Black Sea fish, fatty acids, PUFA, Vitamin A, Vitamin D_3, Vitamin E

1. Introduction

Fish is considered as a valuable source of essential fatty acids, vitamins and low levels on saturated fatty acids and cholesterol. The significance of long chain polyunsaturated fatty acids such as n-3 PUFA has gained attention because of their prevention of human cardiovascular diseases. The vitamins are organic compound that are necessary in very small amounts in the diet and fish is one of the main source of vitamins. Vitamins forms are heterogeneous group of substances and are vital nutrients and the absence of vitamins causes serious physiological problems. They regulate metabolic processes, control cellular functions and prevent different diseases.

Black Sea appears to be one of the important fish basins influencing greatly the economy of all countries around the basin. Bulgarian's fishery catch are mainly based on small pelagic fishes namely sprat (*Sprattus sprattus*), horse mackerel (*Trachurs trachurus*) and others. The fatty acids and vitamins data for different marine fish species especially originating from Canada, Norway, Japan are available in literature .However information about the fatty acids and vitamin contents of Bulgarian Black Sea fish species is lacking. One report was encounted in the literature, in witch was mentioned FA and vitamin E content in sprat and mackerel [16].

The objective of our study was to collect information on fatty acid composition fat-soluble vitamin content of two of commercially important Bulgarian fish species. Black sea sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) were selected. Their total lipids, fatty acid composition and vitamin A, D_3 and E contents were determined.

2. Experimental

2.1. Sampling of fish species

Samples of the commercially important Bulgarian fish species sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) from Kavarna (North Bulgarian Black seacoast) were purchased from Varna local fishmarket during non-spawning season (november 2008). Twenty-five specimens with

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middle body weight and length were selected for the analyzed two species. Biological characteristics were determined, and fishes were frozen, and stored at - 20°C until analysis.

2.2. Lipid extraction

Prior to analysis, the head, tail, fins, and viscera of the fish were removed. The fish were filleted and homogenized. The edible fish tissue was extracted by the method of Blight and Dyer. After phase separation, the chloroform extracts were evaporated until dryness and were quantified by weight. The total lipid content was determined gravimetrically.

2.3. Fatty acids analysis

Fatty acid compositions of total lipids at edible fish tissue were determined by GC of the corresponding methyl esters. The residual lipid fraction was methylated by base-catalyzed transmethylation using 2M methanolic potassium hydroxide and n-hexane according to BDS EN 5509:2000 [18]. After 10 minutes centrifugation (3500 rps), the hexane layer was taken for GC analyses. Gas chromatography was performed by a model FOCUS Gas Chromatograph with autosampler A 2000, equipped with Polaris Q MS detector (Thermo Scientific, USA). The capillary column used was a TR-5 MS (Thermo Scientific, USA) universal column 30m length and 0.25mm i.d, with a wide range of applications from food analysis. Helium was used as a carrier gas at flow rate 1 ml/min. Chromatographic separation was achieved by temperature range: initial temperature – 40^{o}C for 4 min followed by 10^{o}C per minute until 235^{o}C and final temperature reach was 280^{o}C for 5 min. The sample volume was 1µl. The three parallel analyses were made from each methanolysed sample. The injector was a split/splitless injector operated in the split mode. Peaks were identified according to two parameters: Retention Time (RT) based on available FAME mix standard (SUPELCO F.A.M.E. Mix C4-C24) and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA). FAMEs was identified and quantified by comparison with the RT and peak areas of SUPELCO standards. Three replicate GC analyses were performed and the values of FA were expressed as percentage of total FA mass as a mean value and ± standard deviation (SD). All of the chemicals used in the experiments were analytical grade and GC grade (Sharlau).

2.4. Vitamin analysis

A reversed-phase high-performance liquid chromatographic (HPLC) system (Thermo Scientific Spectra SYSTEM, UV-Vis, FL, ODS2 Hypersil™ 250 x 4,6 mm, 5u) for the vitamin analysis of fishes was used. Vitamin A, D_3 and E were analyzed simultaneously. Analysis has followed the procedure of saponification and extraction. The sample preparation and analysis of fat-soluble vitamins was defined using the method of Sanchez-Machado et al. with small modifications [3].

An aliquot of the homogenized sample (1.000g ± 0.005g) was weighed into a 25 ml glass centrifuge tubes with screw cap and 2 % of methanolic L-ascorbic acid and 0.5 M methanolic potassium hydroxide were added. Ten parallel samples of fish edible tissue were shaked and saponified in 80°C for 20 min. The non-saponified components were extracted with n-hexane and the extract were evaporated under nitrogen. The dry residue was dissolved in MeOH. The samples (20µl) were injected into the liquid chromatograph system. Three fat-soluble vitamins were analyzed at the same time using HPLC technique with UV and FL (vitamin E) detectors. The mobile phase was 97:3 = MeOH:H_2O (v/v) with a flow rate of 1ml/min [10]. Their quantity was determined with a UV (at λ_{max} = 325nm – for vitamin A, λ_{max} = 265 nm for vitamin D_3) and fluorescence (at λ_{ex} = 288 nm and λ_{em} = 332 nm – for vitamin E), on the basis of comparing retention times and chromatographic peak areas of the respective standards (Retinol solution, Fluka; DL-alpha Tocopherol and cholecalciferol, Supelco).

2.5. Statistical analysis

Standard curves for all-trans-retinol, cholecalciferol and α-tocopherol were obtained using six different concentrations of standard solutions. The coefficients of correlation were 0.99877, 0.99896 and 0.98978 respectively. For the determination of the recoveries, samples of homogenized fish tissue were spiked with a

methanolic solution containing three fat-soluble vitamins. The recovery rates (of added vitamins) are calculated utilizing the external standard method. All samples were analyzed in triplicate. Results were expressed as average and standard deviation (mean ± SD) for fish samples.

Total lipid content of edible tissue was determined for each group (n=5) and the results were present as $g.100g^{-1}$ raw tissue ($g.100g^{-1}$r.t.). The significant differences of total lipids ($P<0.0001$) were observe. To assess the statistically significant differences of the results from vitamin and fatty acid analysis were used the t test (nonparametric tests) procedure of Graph Pad Prism 5 program, at a significance level of 5%. The significant variations of results for vitamin analyses were considered at $P<0.0001$.

3. Results and Discussions

3.1. Total lipids and Fatty acid composition

The fatty acids composition of Black sea fish species are given in **Table 1** as mean value (as percentage of total amount fatty acid) and ± standard deviation.

Fish are usually classified into groups according to their overall lipid content including lean (<2%); low-fat (2–4%); medium-fat (4–8%) and high-fat (>8%). The present study indicated that the goby (2.62 $g.100g^{-1}$r.t.) and sprat (6.51 g/100g) are classified amongst those fish with medium-fat to highly fat fish . The variation of the fish lipid content is related with season, water temperature, feeds. The TL composition of sprat varies in the range of 8.54 to 15.69 $g.100g^{-1}$r.t. in the literature [14].

The FA compositions of fish species were found for goby 43.96% and 47.45% total sum saturated (SFA) for sprat. The total sum monounsaturated (MUFAs) for both species were found approximately 28.81%, whereas total sum of polyunsaturated (PUFAs) acids were 26.73 – 30.20%.

The FA composition of tissue lipids in fish strongly influenced by the FA in their dietary lipids [4, 5, 8, 17]. The data shown that the amount of FA varied widely among the species but in most studies the palmitic (C 16:0) and stearic (C 18:0) acids are the predominant SFA [5, 17].

Table 1 Fatty acids profile in edible fish tissue (mean ± SD)

Fatty Acid	European Sprat *Sprattus sprattus*	Black sea goby *Neogobius rattan*
C 12:0	1.75±0.01	3.83±0.01
C 13:0	1.13±0.02	0.13±0.002
C 14:0	3.75±0.20	4.88±0.30
C 16:0	26.18^{c}±0.8	10.88^{c}±0.25
C 17:0	1.20±0.05	2.77±0.10
C 18:0	2.70^{a}±0.03	5.80^{a}±0.07
C 20:0	2.30±0.01	4.92±0.001
C 21:0	1.77±0.01	0.00
C 22:0	2.64±0.01	4.29±0.02
C 23:0	1.36±0.01	0.63±0.01
C 24:0	2.66^{a}±0.01	5.84^{a}±0.01
Σ SFA	**47.44**	**43.96**
C 14:1	1.25±0.02	2.65±0.01
C 16:1	11.37^{a}±0.12	3.40^{a}±0.10
C 17:1	1.15±0.01	2.77±0.02
C 18:1 n9 c	4.70^{b}±0.11	6.62^{b}±0.10
C 18:1 n9 tr	1.13±0.01	1.98±0.01
C 20:1	1.77±0.01	3.06±0.02
C 22:1 n9	3.09±0.10	2.65±0.03
C 24:1	1.35±0.01	2.70±0.02
Σ MUFA	**25.78**	**25.83**
C 18:3 n6	1.23±0.01	1.26±0.02
C 18:2 n6 t	1.82±0.01	0.76±0.01
C 18:2 n6 c	3.34±0.01	1.26±0.01
C 18:3 n3	2.22±0.01	4.16±0.05
C 20:5 n3	1.32±0.01	3.04±0.01
C 20:4 n6	2.45±0.02	4.68±0.05
C 20:2	1.80±0.01	1.77±0.01
C 20:3 n3	2.87±0.02	0.00
C 20:3 n6	1.32±0.01	2.90±0.01
C 22:6 n6	6.76^{a}±0.23	8.88^{a}±0.30
C 22:2	1.60±0.03	1.51±0.01
Σ PUFA	**34.33**	**30.20**

Value across rows not sharing a common superscript letter are significantly different from each other, $P<0.05$; for value with superscript ^{a} – P=0.01; ^{b} – P=0.04; ^{c} – P= 0.0007

Tanakol et all, described that several Black sea fish species have the high levels of Palmitic acid (19.5% to 29.0% of the total FA) and the stearic acid

(4.2% to 5.3% of the total FA) [9]. The analyzed Black sea fish species present high concentrations of three quantitatively dominating SFA: palmitic (sprat - 26.18%, goby - 10% lower value), myristic and stearic acids were observed in higher levels in goby while in sprat they were in lowest amounts especially comparison in palmitic acid. The high levels of this fatty acid supports the results published in many similar studies conducted on seawaters fish [4, 9].

The amounts of MUFA's vary significantly especially in wild fish. The total MUFA content of sprat was 25.81%, respectively for goby – 25.83%. Probably this was due to high concentration of Palmitoleic Acid (C 16:1), Oleic acid (C 18:1 n 9) and 13-docosenoic acid (Erucic acid - 22:1 n 9). The palmitoleic acid was the main MUFA acid in all two species analyzed. The higher value of palmitoleic acid (11.40%) was obtained for sprat while goby present a maximum value for oleic acid (6.60%). The Erucic acid present a significant levels in both species – 3.09% for sprat and 2.64 % for goby. Many studies reported that oleic acid (C18:1 n9) is the main MUFA in seawater fish species, but this fatty acid has exogenous origin and usually reflects the type of diet of the fish [9]. Ozogul et al (2005), shown that Black sea turbot has an of oleic acid level – up to 13%, while on the other hand palmitoleic acid concentration - 4.28%. In contrast, in some Mediterranean and Aegean fish species such as mullet and sardine have a palmitoleic acid level - 17% and 12%, respectively while oleic acid was only 4.5%.

The Black sea is the most isolated European semi-enclosed and costal seas from the deep ocean. The biological consequence of the excess nutrient runoff from incoming rivers is the most intense eutrophication (in the world), thus transforming the Black sea ecosystem [9]. Tanakol et all, supposed that the decline in populations of zooplankton, increasing of phytoplankton mass and other as a response to the eutrophication in the Black sea and the fishes from that region had low levels in some MUFAs as C 20:1 and high levels of n-3 PUFAs. The results obtained in our study shown differences - C20:1 levels were low – 1,5% for sprat, while goby shown significant levels – 3.06%. This result confirms the peculiarity of the Black Sea basin.

The major fatty acids in PUFA group were eicosapentaenoic acid (EPA, C20:5 n3) and docosahexaenoic acid (DHA, 22:6 n-3), linoleic acid (LA, C18:2 n-6) and arahidonic acid (ARA, C20:4 n-6). Important long–chain fatty acids such as DHA, LA, EPA and ARA were found in significant levels. The DHA was found to be the most dominant fatty acid in all PUFA's groups. The obtained value of DHA was for goby (8.87%) and Sprat (6.76%) presents significant differences (P=0.0001). The PUFAs level - DHA and LA acid were found to be significant in two Black Sea species. They were the similarly comparison with Saglik et all (2001) investigations [5].

Table 2 Total lipid, PUFA/SFA and n6/n3 ratios, total sum of n3 and n6, EPA and DHA content in sprat and goby

	European Sprat *Sprattus sprattus*	Black sea goby *Neogobius rattan*
Total lipids, $g.100g^{-1}$r.t.	6.50	2.61
PUFA/SFA	0.56	0.69
Σ n 3 , %	13.17	16.17
Σ n 6 , %	4.99	8.83
n 6/ n3	0.38	0.55
EPA mg/100 g	5.00	3.15
DHA mg/100 g	50.00	10.00

Significance level is P<0.0001

They are analyzed the n-3 FA in Turkish wild seawater fishes (Bluefish, European anchovy) and reported that EPA and DHA occur in high amounts mostly in this fish species. In present study in both species – Sprat (1.32%) and Goby (3.03%) EPA levels was found lowest compared to ARA levels (P=0.0001) which were observed in goby in second order in PUFAs group- 4.67% for this species. The results shown in Table 2 indicate that all fish species analyzed were characterized by high level of Omega-3 (n-3) fatty acids series and low level of Omega-6 (n-6) series.

Fish are unable to synthesize any fatty acids of the n- 6 and n- 3 series unless a precursor with this omega FA structure is presented in the diet .The ability to elongate and desaturate fatty acids is not the same in all species of fish. The turbot was able to desaturate and elongate only 3-15 percent of 18:1

n9, 18:2 n6, or 18:3 n3 therefore in the rainbow trout, 70 percent of the label from 18:3 n3 was found in 22:6 n3 (FAO) [19]. These results agree with those obtained in other studies [4, 5]. Goby fish was found to be the riches source of n-3 fatty acids and that is why this species can serve as a valuable source of essential n-3 fatty acids especially DHA. The total sum of Omega-6 (n-6) acids series of analyzed fish samples sowed apparent difference between sprat and goby (P=0.04).

The Omega-3/Omega-6 ratio has been suggested to be a useful indicator for comparing the relative nutritional value of fish. The ratio 0.2 – 1.0 would constitute a healthy human diet. That ration was in the recommended level for two fish species. The highest n-6/n-3 ratio was found to be 0.55 for goby followed by 0.38 for sprat. In modern nutrition studies, namely the values of EPA and DHA to be used as one of the key biochemical characteristics of products consumed by human population. A lot of studies recommended dietary intakes for Omega-6 and Omega-3 Fatty Acids and described the importance of reducing the n-6 and increase the n-3 PUFA in diet of both adults and newborns for optimal brain and cardiovascular health and function [6,7]. Dietary DHA can only be found in marine foods such as fish and other sea life. Intake of n-3 fatty acids in the United States is less than 1.6 g/d of which 1.4 g is a-linolenic acid (ALA; 18:3) and 0.1–0.2 g is EPA and DHA [15].

3.2. Vitamins content

For simultaneous determination of the fat-soluble vitamins was used multichanel detector. All-trans-retinol, cholecalciferol and α-tocopherol amount in present fishes were quantified by reversed-phase HPLC with UV detection (vitamin A and vitamin D_3) or fluorescence detection (vitamin E). The data were obtained after averaging of 10 parallel samples and an use of external calibration.

The quantities of the three vitamins for each fish species were calculated as $\mu g.100g^{-1}$ raw tissue and showed as mean ± SD. Reproducibility, estimated as the coefficients of variation for determinations of ten parallel samples, was in the range of 3.42% - 7.44%. Recoveries, evaluate on the determinations after spiking samples with known amounts of standards, were in ranges 72.8% - 92.7%. All calculated data were presented in table 3. The higher levels of all-trans-retinol and cholecalciferol were found in sprat 33.18 ± 1.55 and 10.51 ± 0.54 $\mu g.100g^{-1}$, respectively. Inversely α-tocopherol content is higher for goby fish - 614.90 ± 40.30 $\mu g.100g^{-1}$r.t.. The quantities of vitamins A and D_3 in the samples rise with the increase of the total lipid content but not in the case with vitamin E. Statistical results showed significant differences in analyzed fishes for all fat-soluble vitamins.

Table 3 Vitamins content ($\mu g.100g^{-1}$ r.t.) and statistical results of two fish species

	Black Sea Goby *neogobius rattan*	European Sprat *sprattus sprattus*
Vitamin A	14.83 ± 0.70	33.18 ± 1.55
Vitamin D_3	2.50 ± 0.20	10.51 ± 0.54
Vitamin E	614.90 ± 40.30	284.85 ± 44.50
% recovery		
Vitamin A	76.6	72.8
Vitamin D_3	81.5	92.7
Vitamin E	85.6	89.6
Coefficient of variation		
Vitamin A	4.73	4.97
Vitamin D_3	5.03	3.42
Vitamin E	7.44	6.38

Significance level is P<0.0001

4. Conclusions

Total lipids, fatty acids profile and vitamin content in the Black Sea sprat and goby were defined and compared. The following considerable differences between fish species were noticed: The sprat (autumn 2008) exhibited the highest level of total lipid content. SFA was the group with the highest level in two fish species, which correspond to their total lipid content. The differences in PUFA values that are associated to the high-level concentrations of DHA were determined for sprat ($50mg.100g^{-1}$). The n6/n3 ratio varied within 0.38 to 0.55.

The results for the fat-soluble vitamins content in the analyzed fishes are same order of magnitude with those reported by other groups [11, 12]. Regarding to the lipid contents, n6/n3 ratio and high level of all analyzed fat-soluble vitamins we may

conclude that these Black Sea fish species have good nutritional quality.

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6. References

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