

THE COMPARISON OF PROXIMATE COMPOSITION, FATTY ACIDS AND FAT-SOLUBLE VITAMINS CONTENT OF THE BLACK SEA SPRAT (*SPRATTUS SPRATTUS* L.) DURING CATCHING SEASONS

Albena Merdzhanova^{1*}, Diana Atanasova Dobрева¹, Veselina Panayotova¹
¹Department of Chemistry, Faculty of Pharmacy, Medical University of Varna,
84B "Tsar Osvoboditel" Blvd., Varna, Bulgaria

* Corresponding author's E-mail: a.merdzhanova@gmail.com

Abstract

The aim of the present study was to determine and compare the seasonal changes in proximate composition, fatty acid profile and fat soluble vitamins content in spring and autumn sprat (*Sprattus sprattus*) from the Bulgarian Black Sea waters. Crude protein was in the range 16.10 – 17.15%, fat content was from 4.20 to 6.65g/100g wet weight (w.w.). The fatty acid (FA) and vitamin's contents showed significant seasonal changes. The spring sprat was showed lower saturated fatty acid (SFA, 31.7%), higher mono unsaturated fatty acids (MUFA, 34.7%) and insignificantly lower polyunsaturated fatty acids (PUFA, 33.6%) compared to the autumn samples. In both seasons omega-3 (n-3) PUFA levels were higher than omega-6 (n-6) PUFA and presented over than 50% of total PUFAs. Different amounts of alpha-tocopherol were found in both seasons – 701.2 µg/100g ww (spring). The higher amounts of all-trans retinol (142.3 µg/100 g ww) and cholecalciferol (11.9 µg/100 g ww) were found in spring samples. Regardless of the observed seasonal changes in proximate and FA composition, fat soluble vitamins and n-3 PUFA contents sprat species caught from the Bulgarian part of Black Sea are excellent sources of the analysed components and can be recommended for healthy human diet.

Keywords: proximate composition, fat soluble vitamins, human health, *Sprattus sprattus*

Received: 21.01.2018

Reviewed: 20.02.2018

Accepted: 27.02.2018

1. INTRODUCTION

The quality of fish tissue is function of their body compositions and energy values, which vary among different seasons and species. Determination of proximate composition as protein, carbohydrates, lipids, and moisture contents is often necessary to ensure that fish tissues have a good nutrition quality and that they meet the requirements of food regulations and commercial specifications (FAO, 2010). In recent years a number of investigators have assessed proximate composition of fish species as shad, horse mackerel, garfish and goby from Southeast and Northwest part of the Black Sea (Guner et al., 1998; Boran and Karacam, 2011). However studies of seasonal changes in fat soluble vitamins contents and fatty acid profile of one of the most commercially important species as sprat from the Bulgarian part of Black Sea are lacking. During the last 20 years, the sprat species (*Sprattus sprattus* L.) has been

most abundant and main subject of perennial fish caught in the Western Black Sea and for Bulgarian local fish markets. Sprat is domestic (non-migrated) species. It forms big schools and undertakes seasonal movements between foraging (inshore) and spawning (open sea) areas (NAFA, 2007; Tserkova, 2013). It is well known that the seawater fish fatty acid (FA) composition is characterised by low levels of n-6 FA and high levels of n-3 PUFA and fat soluble vitamins as D₃ (Tocher, 2003). Many studies have been conducted on the effect of marine fish consumptions in the preventions of coronary heart disease, atherosclerosis, thrombosis and blood pressure (Santos-Silva *et al.*, 2002). Unfortunately, the fish consumption in Bulgaria is traditionally low (5.3 kg per capita per year) compared to the levels of consumption in the neighbouring countries such as Turkey and Greece, and the average European levels (23 kg annually per capita per year). Therefore, it would be beneficial to

increase the fish consumption and the contents of the beneficial fatty acids and fat soluble vitamins in locally produced fish like wild Black Sea sprat. Other sources of n-3 FA are scarce in the Bulgarian population diet but the fish demand has been increasing in recent years and consumers' interests turn off to the investigated species (HNR, 2009). Having in mind all these facts the aim of this work is a comparative evaluation of the seasonal changes of proximate composition, FA profile and fat soluble vitamins content of the edible tissue of the Black Sea sprat (*Sprattus sprattus*) caught from the Bulgarian Black Sea coast.

2. MATERIALS AND METHODS

Table 1. Biological characteristics (mean \pm SD) of analysed sprat samples

Parameters	Weigh (g)	Length (cm)	Habitat	Food habits
Spring, (n=31)	10.0 \pm 0.3	11.0 \pm 0.2	Pelagic	Planktivorous
Autumn, (n=29)	13.0 \pm 0.5	12.0 \pm 0.2		

2.3. Fatty acid 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). For the determination of the analytical recoveries, the samples were spiked with a methanolic solution containing a C19:0 (1 mg/ml). Gas chromatography was performed by a FOCUS Gas Chromatograph, equipped by Polaris Q MS detector (Thermo Scientific, USA). The capillary column used was a TR-5 MS (Thermo Scientific, USA), 30m, 0.25mm i.d. Chromatographic separation was achieved by temperature range: initial temperature – 40°C for 4 min followed by 10°C per minute until 235°C and the final temperature reached was 280°C for 5 min. Peak identification was done by: retention time (RT) based on fatty acid methyl esters (FAME) mix standard (SUPELCO F.A.M.E. Mix C4-C24), and mass spectra (ratio m/z) – compared to the internal Data Base (Thermo Sciences Mass Library; Thermo Corporation, Waltham, USA). FAMES were quantified by the method of

2.1. Sample preparation:

Samples of sprat were purchased in March and September in 2015 from Varna local fish markets. The species were caught from Kavarna (the North part of the Bulgarian Black Sea coast). Specimens of similar body weight and length were selected from captured species in both seasons. Biological characteristics, as body weight (g), length (cm) and habitats were determined (see Table 1). A minimum of twenty-nine specimens were randomly selected from 2 kg batches and from each individual season. The fish was gutted, minced immediately and stored at –20°C prior to analysis.

external standard. The FA content was expressed as a percentage of total FAs content (BDS EN ISO 5508: 2004). All of the chemicals used in the experiments were analytical grade and GC grade (Sharlau, Sharlab Sourcing Group, Spain).

2.4. Nutrition quality indices:

Nutrition qualities are estimated by several indices and ratios of FA composition: the indices of atherogenicity (AI), thrombogenicity (TI), cholesterolemic index (h/H); n-6/n-3 and PUFA/SFA ratios, according to Simopolous (2013). Ulbricht and Southgate (1991) suggest AI and TI which might better describe the atherogenic and thrombogenic potential of different unsaturated FA. h/H presents the functional effects of different PUFAs of cholesterol metabolism (hypo- and hypercholesterolemic effect), and it is calculated according to Santos-Silva et al., (2002).

2.5. Extraction of fat soluble vitamins and HPLC analysis:

The sample preparation was performed using the method of Dobreva et al., (2011). Three fat soluble vitamins were analysed simultaneously using HPLC system (Thermo Scientific Spectra SYSTEM) equipped with analytical column

ODS2 Hypersil™ 250x 4,6mm, 5µm. all-trans retinol and cholecalciferol were detected by UV, α-TP by fluorescence detection. The mobile phase composition was 97:3 = MeOH: H₂O, and the flow rate was 1ml/min. The qualitative analysis was performed by comparing the retention times of pure substances: at λ_{max} = 325nm for retinol; λ_{max} = 265nm for cholecalciferol and alpha-TP fluorescence at λ_{ex} = 288nm and λ_{em} = 332nm. The quantitation was done by the method of external calibration comparing the chromatographic peak areas of the corresponding standards (Retinol solution, Fluka; DL-α Tocopherol, Supelco; Cholecalciferol, Supelco). The results are expressed as µg per 100 g wet weight (µg.100g⁻¹ww).

2.6. Statistical analysis:

The results of analysis are presented as mean values ± standard deviation (SD). Column statistics was used for calculation of the means and standard deviations. Student's t-test was used to evaluate the differences between the

means. Statistical significance was indicated at p<0.05. Statistical analysis was done using Graph Pad Prizm 6.0, USA software.

3. RESULTS AND DISCUSSION

3.1. Proximate composition and energy value:

In fish edible tissue water, proteins, and lipids make up about 98% of the total mass (FAO, 2010). Proximate data on different fish species are collected in different databases however, the chemical composition of fish generally varies due to geographical locations, stages of maturity, seasons and sizes. The proportions of the constituents are species-specific and the main variations in proximate composition between species and seasons occur in moisture and lipid content (FAO, 2010; Boran and Karaçam, 2011; Simat and Bogdanovich, 2012). The seasonal changes in proximate composition of the Black Sea sprat are shown in Table 2.

Table 2 Seasonal changes in proximate composition (g/100g w.w.; means ± SD) and energy values (kJ/100g w. w., means± SD) of the Black Sea sprat

Components	Moisture	Lipid	Protein	Ash	Energy
Spring	76.90±1.05	4.30±0.10	17.50±0.50	1.30±0.03	427.3±0.20
Autumn	73.60±0.95**	6.65±0.15***	18.35±0.45**	1.40±0.02	539.2±0.35***

SD - standard deviation, *** - p < 0.001, Wet weight – w.w.

There exists an inverse relationship between the water and lipid content of fish and the summation of both frequently spans a range of 78 to 88 %. As shown in Table 3, the moisture content in the spring sprat was higher than in the autumn samples which correlated with TL content. Similar results were obtained by other authors for the Black Sea shad, red mullet, garfish and Adriatic anchovy (Boran and Karaçam, 2011; Simat and Bogdanovich, 2012).

The protein content tends to vary much less widely from seasons and from one species to another. Protein content of fish is considered low if it is below 15% (FAO, 2010). The analysed Black Sea sprat in both seasons showed protein contents in range 17.50–18.35 g/100g w.w. and in accordance with

supplement of Commission Regulation (EC) No 116/2010 can be classified as a high in protein source. Guner et al., (1998) present lower protein values for the sprat (15.40 g/100g w.w.) from the South Eastern part of Black Sea. Simat and Bogdanovich (2012) and Usyduş et al., (2011) reported small annual variation in the protein content of the Adriatic anchovy and Baltic sprat, which confirm our results. Pelagic fish species (e.g. Clupeidea, Osmeridae) are usually high in lipid content and energy levels. According to FAO, (2010) analysed Black Sea sprat could be classified as a medium-fat species (<8 g/100g w. w, see Table 2). It is known, that the temperature increase strongly affects the fat content of cold-tolerated sprat and due to, the TL amount was used as an indicator of fish food supply. In this

research TL content showed significant seasonal differences ($p < 0.001$) which correlated with temperature variation: low temperature-lower TL content. The presented results are in good agreement with species biology – mass spawning (during spring – March) when was determined lowest TL and intensive growth and fattening (during autumn – September) – higher TL content (Tserkova, 2013). Seafood shows variable composition of proteins and fat, and energy content is dependent on this distribution. The lipid level in particular has high significance for the calorie content of fish, with implications for calculations in dietary studies and databases. In this study calculated energy values ranged from 427.3 kJ/100g w.w. (spring) to 539.2 kJ/100g w.w. for autumn sprat samples ($p < 0.05$). An earlier investigation (Guner et al., 1998) showed significantly higher energy content for Black Sea sprat (889 kJ/100g w.w.) due to higher lipids content determined for this species. Current study presented energy values in four Black Sea fish species from Sinop Region (Boran and Karacam, 2011) in wide range: from 360 (garfish) to 1250 (shad) kJ/100g w.w. According to Danish Food

Database, Baltic sprat energy values (640 kJ) are higher compared to our results.

3.2. Fatty acid composition:

In the present work most of the individual FAs changed their levels during the seasons. In spring samples were observed the following FA distributions: MUFA>PUFA>SFA, whereas autumn sprat presented different allocation: SFA>PUFA>MUFA respectively. The SFA group increased significantly in autumn seasonal (8.1%, $p < 0.001$) on account of decreasing of MUFAs (8.9%, $p < 0.001$). In both seasons PUFA showed levels over than 33.5% of total FAs. Such significantly higher quantities in MUFA compared to PUFA ($p < 0.001$) and SFA ($p < 0.01$) were found for the Black Sea red mullet (Merdzhanova et al., 2013). A deflection of this pattern was observed for the Black Sea anchovy from the Southeast part of the Black Sea, with significantly higher SFA than MUFA and PUFA levels (Oksuz and Ozyilmaz, 2010) and for the spring sprat (PUFA>MUFA>SFA) from Bulgaria. (Stancheva et al., 2012). The seasonal changes in FAs composition of the Black Sea sprat are given in Table 3 as mean value \pm standard deviation (as percentage of total amount fatty acid).

Table 3. Seasonal variation of fatty acids profile (FA, % of total FA), FA groups, ratios, lipid quality indices (mean \pm SD), EPA and DHA contents (g/100g w. w.) in fish tissue

Fatty Acid	Spring	Autumn	Fatty Acid	Spring	Autumn
C 12:0	0.35 \pm 0.05	1.75 \pm 0.15***	C 18:2 n6	7.18 \pm 0.30	6.16 \pm 0.31
C 14:0	3.50 \pm 0.36	3.75 \pm 0.25	C 18:3 n3	1.40 \pm 0.50	2.82 \pm 0.23***
C 16:0	23.80 \pm 1.03	26.18 \pm 1.50***	C 20:5 n3	0.79 \pm 0.02	1.95 \pm 0.10***
C 17:0	0.36 \pm 0.04	0.70 \pm 0.09	C 20:4 n6	4.82 \pm 0.40	3.40 \pm 0.30
C 18:0	2.70 \pm 0.24	3.20 \pm 0.20**	C 20:2 n6	nd	1.80 \pm 0.25***
C 20:0	0.47 \pm 0.01	1.30 \pm 0.15***	C 20:3 n3	0.31 \pm 0.03	2.71 \pm 0.22
C 21:0	nd	0.15 \pm 0.01	C 20:3 n6	0.27 \pm 0.05	2.55 \pm 0.01
C 22:0	0.39 \pm 0.01	1.24 \pm 0.23	C 22:6 n3	17.31 \pm 1.15	9.46 \pm 0.31***
C 23:0	0.02 \pm 0.01	0.16 \pm 0.11	C 22:2 n9	1.27 \pm 0.20	1.60 \pm 0.12
C 24:0	0.19 \pm 0.01	1.46 \pm 0.20	PUFA	33.60	34.30*
SFA	31.76	39.89***	Σ n-3	19.77 \pm 1.10	16.78 \pm 0.80***
C 14:1n5	0.03 \pm 0.01	1.35 \pm 0.10***	Σ n-6	12.25 \pm 0.60	13.21 \pm 0.70**
C 16:1n7	14.30 \pm 1.05	11.37 \pm 0.12**	n-6/n-3	0.62 \pm 0.02	0.78 \pm 0.03
C 17:1n8	0.35 \pm 0.05	1.15 \pm 0.01	PUFA/SFA	1.06 \pm 0.10	0.86 \pm 0.05*
C 18:1n9	10.90 \pm 0.80	6.33 \pm 0.41**	AI	0.56 \pm 0.02	0.72 \pm 0.03**
C 20:1n9	1.93 \pm 0.10	1.06 \pm 0.15	TI	0.35 \pm 0.01	0.57 \pm 0.01***
C 22:1n9	4.01 \pm 0.40	3.00 \pm 0.30**	h/H	1.48 \pm 0.15	0.80 \pm 0.07***
C 24:1n9	3.10 \pm 0.26	1.55 \pm 0.10**	EPA	0.030 \pm 0.005	0.116 \pm 0.01***
MUFA	34.70	25.81***	DHA	0.645 \pm 0.035	0.563 \pm 0.020**
C 18:3 n6	0.25 \pm 0.04	1.85 \pm 0.11***	EPA+DHA	0.675 \pm 0.030	0.679 \pm 0.032

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; AI – index of atherogenicity; TI – index of thrombogenicity; h/H – cholesterolemic index

According to Tocher (2003), the higher SFA and MUFA levels in fish may be the result of a lipidic diet. Moreover in addition to the diet, the SFA may be generated through non-lipidic carbon sources and then transformed into monounsaturated fatty acids. In this study the analysed sprat presented high concentrations of three quantitatively dominating SFA: palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acids which followed next distributions C16:0>C14:0>C18:0 regardless of the seasons. The prevalence of SFA group in autumn sprat was mainly due to increase of C16:0, C18:0 and very long chain acids C20:0-C24:0 ($p<0.001$). The domination of C16:0 and C14:0 (biomarkers of diatoms) in SFA group in autumn samples was connected with their accumulation related to feeding of phyto- and zooplankton, the presence and availability of which differed in food web and food habits of sprat from different seasons. Oksuz and Ozyilmaz (2010) and Stancheva *et al.*, (2012) reported similar results for the Black Sea anchovy and sprat SFA distributions. The amounts of MUFA's vary significantly between the seasons ($p<0.001$). Spring sprat contained the highest MUFA amount due to the highest levels of palmitoleic acid (C16:1n7) which is a 42.2% from total MUFA's followed by oleic acid (C18:1n9). This MUFA allocation remains unchanged in autumn season, but both monounsaturated FA levels significantly decreased – with 3.5% (18:1n9, $p<0.001$) and 5% (16:1n7, $p<0.001$) respectively. In addition, in this study was observed and decrease of long-chain (LC) monoenoic C20-C22 FA in autumn samples. The possible reason is those autumn sprats were lacking their main food items (cold-water copepods) and were forced to consume zooplankton species. Moreover Tocher (2003) reported that C18:1n9 FAs are the most important lipids for cold-water organisms with respect to their adaptation to temperature and depth and this is another possible reason for the C18:1n9 low levels observed in sprat tissue in autumn season.

Among other ecological factors (as water temperature, salinity) the qualitative content of

food sources has been emphasized as a principal factor that defines the PUFA level in the tissues of fishes. In the presented study important LCPUFA such as eicosapentaenoic acid (EPA, C20:5 n3) docosahexaenoic acid (DHA, C22:6 n3), linoleic acid (LA, C18:2 n6), arachidonic acid (ARA, C20:4 n6) and alpha-linolenic (ALA, C18:3n3) FA were found in significant levels. The prevalent PUFA in Black Sea sprat tissue was DHA regardless of the seasons. The highest obtained DHA values were in spring sprat (52% of total PUFA), whereas in autumn sprat its value significantly decreased (up to 28 % of total PUFA, $p<0.001$). These results were similar in comparison with Usydus *et al.*, (2011) investigations for the Baltic Sea sprat, Stancheva *et al.* (2012) for the Black Sea sprat and Oksuz and Ozyilmaz, (2010) for the Black Sea anchovy. Furthermore some authors suggest that higher temperature (in autumn season) leads to decreased proportion of unsaturated FA, such as DHA) (Tocher, 2003). EPA n3 PUFA showed significant increase in autumn seasons (up to 5.8% of total PUFA, $p<0.001$). In the present study in both seasons EPA levels were found lower compared to ARA levels ($P<0.001$). Despite the differences in the FA profile it can be concluded that the analysed Black Sea sprat is a good source of essential n3 and n6 PUFA. In both seasons the analysed Black Sea sprat was characterized by higher levels of n3 PUFAs (48% - 58.8% of total PUFA) compared to n6 PUFA levels (36%-38% of total PUFA). The results presented in Table 3 indicated that spring sprat was characterized by higher level of n3 FA series and h/H indices, lower levels of AI and TI and the most balanced PUFA/SFA ratio. This findings for pelagic fish species as the Black Sea sprat are in agreement with those obtained in similar studies (Oksuz and Ozyilmaz, 2010; Usydus *et al.*, 2011). It is well known that the n6/n3 ratio is very important for human intake as both of these contains the same enzyme to synthesize prostaglandins derived from both n3 and n6 families. Further, Simopoulous (2013) suggested that a decrease

in the human dietary n6/n3 PUFA ratio is essential to help prevent coronary heart disease by reducing the plasma lipids. Another important key indicator for evaluation of fish nutrition quality is PUFA/SFA ratio. Several studies have found inverse correlation between this ratio and cardiovascular diseases (Simopoulos, 2013). Department of Health (1994) recommend values of PUFA/SFA ratio greater than 0.45, because foods with ratio under this value have the potential to raise blood cholesterol levels. In this study the PUFA/SFA ratio was found higher than the cut-off value in both seasons (Table 3). This ratio has decreased in autumn, and this was explained by the significant increase of SFA levels as preparing for upcoming wintering period. Discrepancies of this finding were reported by Usydus *et al.*, (2011) for the Baltic Sea sprat, which showed unchanged PUFA/SFA ratio in different seasons. Oksuz and Ozyilmaz, (2010) found similar FA ratio results for the Black Sea anchovy. The nutritional value of the analysed sprat is also determined by lipid quality indices. With regard to the quality indices considered, all three indices (AI, TI and h/H) showed significant statistical differences ($p < 0.001$) between seasons. AI and TI levels increased in

autumn samples, whereas h/H index showed opposite trend, due to higher C14:0 and C16:0 values, which have the most proatherogenic potential. Higher values of AI and TI (> 1.0) are detrimental to human health, whereas higher h/H levels ($> 1.0 \pm 0.2$) are recommended. The calculated values (see Table 3) are beneficial for human nutrition. To our knowledge, no data is available in literature which describes the seasonal changes in lipid quality indices for the Black Sea sprat caught from the Bulgaria Black Sea waters. The European Food Safety Authority (EFSA, 2012) recommended daily intake of 0.250 to 0.500g EPA+DHA. The percentage values of these FA were recalculated to g/100g of sprat edible tissue according to FAO/INFOODS Guidelines (2012). A 100 g of edible portion of sprat contained 0.670 g of EPA+DHA n3 PUFA regardless the seasons and provides 134% of the recommended EPA and DHA n3 PUFA daily intake.

3.3. Fat soluble vitamins content:

The seasonal changes of vitamin contents and percentage of the daily recommended intake (RDI) of fat soluble vitamins in analysed fish are presented in table 4 as micrograms per 100 grams wet weight ($\mu\text{g}/100\text{g w. w.}$).

Table 4 Total content, (mean \pm SD) and percentage of the daily recommended intake (RDI) of fat soluble vitamins

Analyte	Total content ($\mu\text{g}\cdot 100\text{g}^{-1}$ w.w.)		RDI (%)	
	Spring	Autumn	Spring	Autumn
all-trans-Retinol	143.2 \pm 13.2	33.2 \pm 1.6***	19.0%	4.4%***
Cholecalciferol	11.9 \pm 0.7	10.5 \pm 0.5	238.0%	210.0%
α -Tocopherol	701.2 \pm 56.7	284.9 \pm 44.5***	4.7%	1.9%***

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Significant differences in retinol and alpha-tocopherol contents ($p < 0.001$) between seasons were established in this study. Two and a half time reduction of vitamin A amounts in autumn samples were observed ($p < 0.001$). Even more significant reduction of vitamin E content was found in the autumn sprat tissue (almost four times), compared to spring season levels. Only vitamin D₃ levels showed minor variation during the seasons. The observed changes in fat-soluble vitamins content in this study

showed an inverse correlation with TL quantity. Alpha-Tocopherol is the vitamin E compound with the highest biological activity, which is fat soluble antioxidant, protecting the fish against in vivo lipid oxidation, membrane structures and essential PUFAs. The observed autumn decreasing of vitamin E content also correlated with preserved of PUFA levels in the same season in Black Sea sprat. Stancheva *et al.*, (2010) showed a similar result for vitamin E content in autumn sprat (300 $\mu\text{g}/100\text{g w. w.}$)

from Bulgarian markets, whereas significantly higher values for this vitamin were presented from Danish Food Database (2500 µg/100g w. w.) for Baltic sprat and Ahmadnia et al., (2008) for Caspian sprat species (8100 µg/100g w. w.). Several studies reported that vitamin D₃ content was 4 - to 9-fold higher in wild than in cultured fish (Guner et al., 1998; Ohrvik et al., 2012). Moreover oily fish is rich in vitamin D, containing 3.6-16.1 mg/100g w. w. (Ruxton and Derbyshire, 2009). The presented results of this study are in citing range in both seasons and we can classify Black Sea sprat as a very good source of vitamin D₃. Ahmadnia et al., (2008) reported significantly higher amounts for vitamin D₃ in Caspian Sea sprat compared to our results. Earlier investigations of Black Sea sprat and goby (Stancheva et al., 2010) showed species specific differences in vitamin D₃ content. Possible reason for observing differences is that the governing factor for vitamin D content is most likely diet (Ohrvik et al., 2012).

It is known that phytoplankton is a good source of vitamin A. Due to the fact that sprat is planktivorous species was observed higher value in spring season, whereas autumn samples showed significant decrease of vitamin A content. The possible reason is that the sprat diet composition changes because of decrease of lipid-rich cold-water zooplankton among sprat food items in autumn season. Current investigation of composition and dynamics of the Black Sea benthopelagic plankton reported that its share decreased in the progression: winter-spring-summer-autumn due to increasing growth and reproduction of the pelagic species (Vereshchaka and Anokhina, 2014). Danish Food Date Base presented for Baltic sprat similar results for vitamin A content (140.0µg/100g w. w.) compared to our results for spring samples. Earlier investigations of spring Black Sea sprat showed similar values for vitamin A content (Stancheva et al., 2010).

There are dietary standards in Bulgaria for relative daily intake (RDI) of fat soluble vitamins, which are in accordance with the

European Union standards with the exception of those for vitamin D₃ (5 µg for adults in Bulgaria against 10 µg per day in the European Union). The fat soluble vitamins provided by 100 g raw fish tissue as a percentage of the average daily allowance were compared with the RDI values for all-trans retinol, cholecalciferol and alpha-tocopherol amounts (see Table 4). According to the Bulgarian dietary standards for average daily intake of fat soluble vitamins (Ordinance 23, 2005), the Black Sea sprat showed low percentages for the daily recommended intake of retinol (4.4 – 19.0% RDI, Table 4) regardless of the seasons. One portion of fillets provides in both seasons even lower levels of alpha-tocopherol (1.9 – 4.7% RDI, Table 4) compared with vitamin A. In contrast, cholecalciferol content in studied fish samples surpasses the recommended daily needs in range 210.0% -239.0% RDI and sprat can be classified as an excellent source of vitamin D₃ in all year.

4. CONCLUSION

Presented data, for small indigenous fish as *S. sprattus* is essential to supply information on nutritious healthy foods from Western part of Black Sea Region. The observed results indicated that the sprat caught from the Bulgarian part of the Black Sea has moderate lipid and higher protein content; higher SFA and PUFA levels, lower MUFA amounts in the autumn season compare to spring period. During the year sprat species have relatively high proportion of n3 PUFA because DHA was found to be the most dominant PUFA. The values of AI, TI and h/H indices showed good anti-thrombogenic, anti-atherogenic and hypocholesterolemic properties of sprat lipids. An edible portion of sprat (100 g) provides average 0.670 g EPA + DHA n3 PUFA. The fat soluble vitamins showed significant seasonal changes: vitamin A and E contents decreased in the autumn season. Regardless of the seasons, the Black Sea sprat could be assigned as a valuable source of vitamin D₃ and 100g fillets per serving provide almost three times of the

established RDI in Bulgaria. Based on presented findings *S. sprattus* can be classified as nutrient-rich small fish regardless of the catching seasons. On the other hand the observed differences in proximate and lipid composition could also influence postharvest processing and storage techniques of this species. In addition, with the raising of fish consumption of Black Sea countries, this information could be useful in developing nutrient-balanced, cost-effective diets for human nutrition.

5. REFERENCES

- [1]. FAO. (2010). Nutrition elements of fish <http://www.fao.org/fishery/topic/12319/en> on 26/8/2010
- [2]. Guner, S., B. Dincer, N. Alemdag, & Colak A. (1998). Proximate composition and selected mineral content of commercially important fish species from the Black Sea. *Journal of Scientific and Agriculture*, **78**: 337–342.
- [3]. Boran, G. & Karaçam H. (2011). Seasonal Changes in Proximate Composition of Some Fish Species from the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, **11**(2), 01-05.
- [4]. NAFA, National Strategic Plan for Fisheries and Aquaculture. 2007–2013. Ministry of Agriculture and Forestry; National Agency for Fisheries and Aquaculture.
- [5]. Tserkova, F. (2013). Growth parameters of the Black Sea sprat (*Sprattus sprattus* L.) during the period November 2010 – March 2012 along the Bulgarian Black Sea coast. *Bulgarian Journal of Agriculture Science*, **19** (1): 109–113.
- [6]. Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Review Fishery Sciences*, **11**: 107–184.
- [7]. Santos-Silva, J., R.J.B. Bessa & Santos-Silva F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livestock Production Science*, **77**:187-194.
- [8]. HNR, Health of the Nation Report, (2009). Available at: <http://ncpha.government.bg/files/Doklad%20Zdrave%20na%20naciata%202009.pdf>.
- [9]. AOAC (1991). Moisture in meat and meat products. Air Drying Method, Official methods of analysis: Association of Official Analytical Chemists 16th Edition USA: Washington D.C.
- [10]. BDS 9374: 1982. Meat and meat quality. Determination of crude protein in meat. 2nd ed. Bulgarian Institute of Standardization, Sofia, Bulgaria.
- [11]. Bligh, E. & Dyer W.J. (1959). A rapid method of total lipid extraction and purification. *Can. J. of Biochemistry and Physics*, **37**: 913–917.
- [12]. BDS EN ISO 5509:2000. Animal and vegetable fats and oils. Preparation of methyl esters of fatty acids. 3th ed. Bulgarian Institute of Standardization, Sofia, Bulgaria.
- [13]. BDS EN ISO 5508: 2004. Animal and vegetable fats and oils. Gas chromatographic analysis of methyl esters of fatty acids. 2nd ed. Bulgarian Institute of Standardization, Sofia, Bulgaria.
- [14]. Simopoulos, A. (2013). Dietary Omega-3 Fatty Acid Deficiency and High Fructose Intake in the Development of Metabolic Syndrome, Brain Metabolic Abnormalities, and Non-Alcoholic Fatty Liver Disease. *Nutrients*, **5**(8): 2901-2923.
- [15]. Ulbricht, T. & Southgate D. (1991). Coronary heart disease: seven dietary factors. *Lancet*, **338**:985-992.
- [16]. Dobрева, D.A., B. Galunska & Stancheva M. (2011). Liquid chromatography method for the simultaneous quantification of fat soluble vitamins in fish tissue. *Scripta Scientifica Medica*, **43**: 276–279.
- [17]. Simat, V. & Bogdanovich T. (2012). Seasonal changes in proximate composition of anchovy (*Engraulis encrasicolus*, L.) from the central Adriatic. *Acta Adriatica*, **53**(1): 125 – 132.
- [18]. Commission Regulation (EU) No 116/2010 of 9 February 2010 amending Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims.
- [19]. Usydus, Z, J. Szlinder-Richert, M. Adamczyk & Szatkowska U. (2011). Marine and farmed fish in the Polish market: Comparison of the nutritional value. *Food Chemistry*, **126**: 78–84.
- [20]. Danish Food Composition Databank. 2009. Department of Nutrition, National Food Institute. http://www.foodcomp.dk/v7/fcdb_details.asp?FoodId=0082.
- [21]. Merdzhanova, A., L. Makedonski & M. Stancheva. (2013). Black Sea shad and red mullet as sources of omega-3 fatty acids. *Scripta Scientifica Medica*, **45** (3): 18-23.
- [22]. Öksüz, A. & Ozyilmaz K. (2010). Changes in fatty acid compositions of Black Sea anchovy (*E. encrasicolus* L.1758) during catching season. *Turkish Journal of Fishery Aquatic Sciences*, **10**: 381 –385.
- [23]. Stancheva, M., Galunska, B., Dobрева, A. D., & Merdzhanova, A. (2012). Retinol, alpha-tocopherol and fatty acid content in Bulgarian Black Sea fish species. *Grasas y aceites*, **63**(2): 152-157.

- [24]. Department of Health (1994): Nutritional aspects of cardiovascular disease: Health and social subjects. HMSO, London.
- [25]. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2012): Scientific opinion on the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *EFSA Journal*, **10**: 2815–2863.
- [26]. FAO/INFOODS Guidelines. (2012). FAO/INFOODS Guidelines for Converting Units, Denominators and Expressions, version 1.0. FAO, Rome.
- [27]. Stancheva, M., A. Merdzhanova, D. Dobрева & Makedonski L. (2010). Fatty acid composition and fat-soluble vitamins content of sprat (*Sprattus sprattus*) and goby (*Neogobius rattan*) from Bulgarian Black Sea. *Ovidius University Annals of Chemistry*, **21(1)**: 23-28.
- [28]. Ahmadnia, A., M. A. Sahari, M. Barzegar, S.J. Seyfabady & M. Abdollahi M. (2008). Vitamins contents of some commercially important fish species from south Caspian Sea. *American-Eurasian Journal of Sustainable Agriculture*, **2**: 285–293.
- [29]. Öhrvik. V., A. Malmberg, I. Mattisson, S. Wretling & Astrand C. (2012). Fish, shellfish and fish products - analysis of nutrients, The National Food Agency Report Series no 1, p.145.
- [30]. Ruxton, C. & Derbyshire E. (2009). Health impact of vitamin D: are we getting enough? *British Nutrition Foundation. Nutrition Bulletin*, **34**: 185–197.
- [31]. Vereshchaka, A. L. & Anokhina L.L. (2014). Composition and Dynamics of the Black Sea Benthopelagic Plankton and Its Contribution to the Near-Shore Plankton Communities. *PLoS ONE*, **9(6)**: e99595. doi:10.1371/journal.pone.0099595.
- [32]. Ordinance No 23/19.07.2005 on the Physiological Feeding of Population. Available online: <http://www.lex.bg/laws/lddec/2135508428>.