VERSITA doi: 10.2478/auoc-2014-0003
Ovidius University Annals of Chemistry Volume 25, Number 1, pp. 15-23, 2014

Fat soluble vitamins and fatty acid composition of wild Black sea mussel, rapana and shrimp

Albena MERDZHANOVA*, Diana A. DOBREVA, Mona STANCHEVA and Lubomir MAKEDONSKI

Department of Chemistry, Medical University of Varna, 55 Marin Drinov Str., Varna, Bulgaria

Abstract Many studies suggest that marine molluscs are one of the most important dietary sources of fat soluble vitamins (E, D_3 and A) and essential fatty acids (FA). The most commercially important species from the Bulgarian Black Sea are the Black mussel, rapana and shrimp. There is scarce information in the scientific literature about fat soluble vitamins and FA composition of these Black Sea molluscs. The aims of the present study are to determine and compare fat soluble vitamins content as well as relative daily intake, FA composition and atherogenic index (IA), thrombogenicity index (IT) and flesh-lipid quality index (FLQ) in wild Black Sea mussel (*Mytilus galloprovincialis*), rapana (*Rapana venosa*) and shrimp (*Crangon crangon*). Fat soluble vitamins were analysed simultaneously using RP-HPLC system. The FA profile was analysed by GC-MS. All of the analysed samples presented significant amounts of vitamin E, followed by vitamin A and D_3. Black Sea molluscs are excellent sources of fat soluble vitamins, especially for vitamin D_3 - one survey provides more than 100% of the RDI established in Bulgaria. The FA composition of total lipids showed significant differences and the present study revealed that SFA content was significantly higher than MUFA ($p<0.001$) and PUFA ($p<0.001$) (SFA>PUFA>MUFA) in shrimp and mussel whereas rapana showed opposite trends (PUFA>SFA>MUFA). The omega6/omega3 and PUFA/SFA ratios of the analysed species were greater than the FAO/WHO recommendations.

Keywords: Mytilus sp., Rapana sp., Crangon sp., fat soluble vitamins, fatty acids, Bulgarian Black Sea coast

1. Introduction

Mytilus galloprovincialis (Lamark, 1819) is a marine mollusk with the highest ecological and economic importance in the Black Sea ecosystem including in Bulgaria marine area, known as mass species and marine bio-resource potentially exploitable for human consumption [1]. Among the Black Sea mussels the Black mussel *(M. galloprovincialis)* is the most widespread. It can be found all over the Black Sea – in depth to 65 meters and in the Bay covering areas about 15–20 meters. *M. galloprovincialis* is a filter feeding animal, which depends on phytoplankton, organic detritus, bacteria and probably dissolved organic matter in the water as sources of food [2].

Rapana venosa is a marine snail with fast growth rate and tolerance to low salinity; high and low temperatures,water pollution and oxygen deficiency. This species have a documented impact on both natural and cultivated populations of mussels and other molluscs, and significant negative changes in the ecosystem. Rapa whelk (*R. venosa)* is a very voracious predator; introduced into the Black Sea in the early 1940s, it is blamed for the decline in the native, edible bivalve fauna. Since the 1980s, rapana has become a valuable commercial resource: its meat is exported to Japan for food and recently it has also been included in the diet of those native to the Black Sea area. According to some recent reports, annual Rapa whelk catches from Turkey and Bulgaria exceed 13,000 $t \cdot year^{-1}$ [3].

Crangon crangon (Linnaeus, 1758) is a marine coastal decapod species with a wide distribution range along the European coast including Black sea. Brown shrimp (*C. crangon)* inhabits mainly soft bottom (sandy, sandy-mud and muddy substrata), estuarine and marine shallow areas and may occur at

ISSN-1223-7221 © 2014 Ovidius University Press

depths of 15 to 90 m. *C. crangon* is omnivores but feeds predominantly on polychaetes, mollusks and small artropods. *C. crangon* has very high productivity and is an important food source for many birds, fish and crustaceans. It is commercially important species for human consumption in Black Sea countries [4].

Fat soluble vitamins are essential components of marine lipids and are exclusively provided by the diet. They control a variety of biologically important processes in the human body. All-trans retinol participates in photoreception, regulates gene expression, bone growth, reproduction etc. Cholecalciferol promotes and enhances the absorption and metabolism of calcium and phosphorus. The main role of alpha-tocopherol is as an antioxidant by protecting membrane structures, essential FAs and vitamins A from oxidation [5].

The nutritional benefits of sea food consumption are mainly attributed to the effects of omega-3 Polyunsaturated Fatty Acids (n-3 PUFAs), which have several potential cardio protective effects along with their antithrombotic action. Numerous studies have explored and supported the antiatherogenic, antithrombotic, and antiarrhythmic effects of n-3 PUFAs [6]. PUFAs can affect platelet function by interacting with membrane proteins and serving as precursors for secondary messengers [7]. Their effect depends on the fatty acid (FA) chain length and the degree of saturation. Individual saturated fatty acids (SFA) such as lauric (C12:0), myristic (C14:0) and palmitic (C16:0) increase LDL cholesterol and platelet aggregation [6, 8, 9].

To our knowledge, there are not recent studies on similar aspects regarding the fat soluble vitamins content, FA composition and IA, IT and FLQ in wild black mussels, rapana and brown shrimp from Bulgarian Black Sea coast. Generally, it is accepted that mollusks have some ability for PUFA biosynthesis but, such capability appears to vary among species depending on the enzymatic complement of desaturase and elongase enzymes involved in these metabolic reactions. Having in mind all these facts, the aim of this study was to determine and compare total lipids (TL), fat soluble vitamins content, FA composition, and lipid- quality indices as atherogenic (IA), thrombogenic (TI) and flesh-lipid quality (FLQ) index of these mollusks from Bulgarian Black Sea coast.

2. Experimental

2.1. Collection of mollusk samples

All mollusk samples were purchased from Varna local fish market during autumn 2011. They were collected in two ecologically non-polluted regions of the Black Sea Coast: Cape Galata and Kavarna, (Northwestern part). The samples were immediately frozen at -20^{o}C and stored in a fridge. Biometric characteristics as mean weight (g) and mean length (cm) were determined and presented in **Table 1.**

Table 1. Biometric characteristics (mean ± SD)

	Black mussel n=30	Rapa whelk n=15	Brown shrimp n=60
Mean weight	12.0±0.5	155.0±5.0	1.0±0.2
Mean length	5.5±0.5	10.0±0.5	4.5±2.0
Habitat	Demersal	Demersal	Demersal
Food habits	Herbivorous	Omnivores	Omnivores

n **-** number of specimens
SD - standard deviation

2.2. Sample preparation

Thirty specimens of mussels, sixty of shrimps and fifteen of rapana were used for fatty acid and vitamin analysis. All shucked mussels, rapana and shrimps were cut into small pieces and homogenized at 800 rpm for 5 minutes, using Moulinex blender.

2.3. Standards and reagents

All-trans-retinol was purchased from Fluka, cholecalciferol, alpha-tocopherol, and other HPLC-grade reagents - from Sigma-Aldrich^{TM}. Fatty Acid Methyl Esters (FAME.) mix standard (SUPELCO FAME. Mix C4-C24), nonadecanoic acid and methyl ester nonadecanoic acid standards were purchased from Sigma–Aldrich^{TM}. All used chemicals were of analytical, HPLC and GC grade (Sharlau, Spain).

2.5. Extraction of fat soluble vitamins and HPLC analysis

The sample preparation was performed using the method presented by Dobreva *et al.* [10]. An aliquot of the homogenized sample (1.000±0.005g) was weighed into a glass tube with a screw cap; L-ascorbic acid 1% in methanol and potassium hydroxide 1M in methanol were added. Six parallel samples of edible fish tissue were prepared and subjected to saponification at 80ºC for 20 min. The

components of interest were extracted with n-hexane and the extract was evaporated under nitrogen. The dry residue was dissolved in methanol and injected (20µL) into the liquid chromatography system.

Three fat soluble vitamins were analyzed simultaneously using HPLC system (Thermo Scientific Spectra SYSTEM) equipped with analytical column ODS2 Hypersil™ 250x4,6mm, 5um. All-trans retinol and cholecalciferol were detected by UV, alpha-TP by fluorescence detection. The mobile phase composition was MeOH:H_2O 97:3 v/v, 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-alpha Tocopherol, Supelco; Cholecalciferol, Supelco). The results are expressed as µg per 100 g wet weight (µg·$100g^{-1}$ww).

2.4. Lipid extraction and fatty acid analysis

Portions of freshly prepared homogenate (5.000±0.001g) were extracted in triplicate with chloroform: methanol (1:2 v/v) according to Bligh and Dyer procedure and 1% of methanolic BHT (2-terth-Butyl-4-hydroxyanisole) was added to all samples as antioxidant [11]. After phase separation the chloroform layers were evaporated on a rotary vacuum evaporator (Bushi 5200) until dryness and quantified gravimetrically. Total lipid content of edible tissue was determined for each group (n=6) and the results are presented as g per 100g wet weight (g·$100g^{-1}$ww). The chloroform fraction was methylated by base-catalyzed transmethylation using 2M KOH in methanol and n-hexane [12]

The hexane layer was separated and analyzed by GC-MS. Gas chromatography was performed by a model FOCUS Gas Chromatograph with autosampler A3000, equipped with Polaris Q MS detector (Thermo Scientific, USA). The capillary column used was a TR-5 MS, 30m length, film thickness 0.25 µm, 0.25mm i.d. The optimum temperature gradient was 40°C to 280°C (5°C/min) Injector temperature was 220° C and detector temperature was 250° C. Helium was used as a carrier gas at a flow rate 1 ml/min. Three parallel analyses were made from each methylated sample. Peaks were identified according to two parameters: Retention Time (RT) based on available FAME mix standard (SUPELCO 37 FAME Mix C4 - C24) and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA). The quantitation was done by the method of external calibration comparing the chromatographic peak areas of the corresponding standard (SUPELCO 37 FAME Mix C4-C24). Results were expressed as the percentage of each fatty acid with respect to the total fatty acids [13].

2.5. Nutrition quality indices (NQI)

Nutrition quality are estimated by several indices of fatty acid composition: the indices of atherogenicity (IA) and thrombogenicity (IT), flesh-lipid quality index (FLQ), according to Hosseini *et al.* [14]; n-6/n-3 and PUFA/SFA ratios, according to Simopolous [15].

IA and IT

Ulbritcth and Southgate [16] suggest two indices – IA and IT which might better describe the atherogenic and thrombogenic potential of different unsaturated FA.

IA indicates the relationship between the sum of the main saturates and that of the main unsaturated, the former being considered pro-atherogenic (favoring the adhesion of lipids to cells of the immunological and circulatory systems), and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol and phospholipids, thereby preventing the appearance of micro- and macrocoronary diseases) [16, 17].

IT shows the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic FA (MUFA, n-6 PUFA and n-3PUFA) [16, 17]

FLQ index indicates the percentage relationship in which the main n-3 PUFA (EPA+DHA) appearing in muscle with respect to the totality of the lipids. The higher value of this index is an indicator of the higher quality of the dietary lipid source [17, 18].

2.6. Statistical analysis

All analytical determinations were performed in triplicate. The results were expressed as a mean and standard deviation (mean ± SD). The obtained data was analyzed using Graph Pad Prism 5 software. Unpaired t-test statistical analysis was applied to estimate the differences between analized species. Thus the comparison was made for total lipids, fat soluble vitamins and individual FA and FA groups. The differences were considered significant at $p<0.05$.

3. Results and Discussions

3.1. Total lipid content

In this study total lipid (TL) content was highest for black mussel (2.49±0.15 $g \cdot 100^{-1}$g ww), followed by brown shrimp (1.35±0.05 $g \cdot 100^{-1}$ g ww), whereas Rapa whelk presented significantly lower value (0.55±0.05 $g \cdot 100^{-1}$g ww, $p<0.001$). These finding are similar to data reported for TL in mussels collected in other places of the Romanian Black Sea. The authors found seasonal dynamics in TL content in wild deep and rocky black mussel in range: form 1.09 to 2.69 $g \cdot 100^{-1}$ g ww [19]. For black mussel species from different seas as Adriatic Sea, Mar Grande of Taranto, different TL contents were reported in 2010 and 2008 [20, 21]. Compared to our results in 2011 there were reported showed lower lipid content (0.95$g \cdot 100^{-1}$g ww) for shrimp from Sinop Region (Black Sea) [22]. These patterns of temporal variability of TL in bivalve mollusks, rapana and shrimp reported in previous studies are the result of several environmental factors acting simultaneously, such as temperature, food availability, plankton composition and physiological factors [4, 20, 21, 22]. No data available in literature for TL value of rapa whelk and brown shrimp from Bulgarian Black Sea coastal waters.

3.2. Vitamins content

In this study significant differences ($p<0.05$) in retinol, cholecalciferol and alpha-tocopherol contents between the analized species were established. The results were expressed as average and standard deviation (mean ± SD). The amounts of vitamins were presented in **Table 2** as microgram per 100 grams wet weight ($\mu g \cdot 100g^{-1}$ww). Vitamin E functions primarily as an antioxidant and is very important for human health, due to its protective role to cell membranes from oxidative stress and in relation to fertility health. In this aspect brown shrimp is excellent source of alpha-tocopherol because of the almost six fold higher content compared to mussel and rapana.

Table 2 Fat soluble vitamins content in edible molluscs tissue, $\mu g \cdot 100g^{-1}$ (mean ± SD)

Vitamin	Black mussel	Rapa whelk	Brown shrimp
A	99.7±7.3	0.60±0.04	537.0±0.6
D_3	14.8±1.0	3.90±0.40	12.99±1.2
E	1688.9±40.3	925.4±37.4	7730.9±177.4

This difference correlates with the high PUFAs content in shrimp tissue. On the contrary, rapa whelk showed lowest amount of all analyzed vitamins, especially alpha-tocopherol, which correlated with lowest TL value. Although black mussel presented highest TL value, it contained significantly lower E and A vitamins amounts, and similar amount for vit.D_3 compared to brown shrimp. These probably species-specific differences are due to its metabolism and environmental factors.

Presented results are in good agreement with those published by other authors. In 2010 MacDonald found lower amounts – 38.7 $\mu g \cdot 100g^{-1}$ww for retinol and 740 $\mu g \cdot 100g^{-1}$ww for alpha-tocopherol in raw edible green shell mussel tissue compared to our results [23]. Similar results for vitamin A (34 $\mu g \cdot 100g^{-1}$ww) and for vitamin E (1100 $\mu g.100g^{-1}$ww) in mussels are published in 2011 in Whole food catalog database [24].

Danish food composition databank [25] and Öhrvik *et al*. [26] presented similar amounts for these vitamins in mussel – 84 $\mu g \cdot 100g^{-1}$ww and 66.5 $\mu g \cdot 100g^{-1}$ww for retinol, and two times higher (3500 $\mu g \cdot 100g^{-1}$ww and 3070 $\mu g \cdot 100g^{-1}$ww) for alpha-tocopherol.

The quantities of fat soluble vitamins provided by 100 g raw tissue calculated as a percentage of the average daily allowance (ADA) are presented in **Table 3**.

Dietary standards for fat soluble vitamins intake in Bulgaria are the same with those approved by the European Union [27, 28]. An exception is the

recommended daily intake (RDI) of cholecalciferol [29]. The rate for the RDI of vitamin D in European Union was updated in 2011, which is not yet been performed in Bulgaria.

Table 3 Percentage of the daily recommended intake (RDI) of fat soluble vitamins

Vitamin	Black mussel	Rapa whelk	Brown shrimp
A	13.3%	0.1%	71.6%
D_3	296.0%	79.0%	260.0%
E	11.3%	6.2%	51.5%

* Average value of the recommended daily intake for adults (male and female)

According to Bulgarian dietary standards for ADA of fat soluble vitamins, rapa whelk showed lowest percentage for RDI of the three vitamins, compared to the other two species [29]. Black mussel is a good source of the analyzed nutrients, especially vitamin D_3. A portion of 100 g edible tissue provides 296% of RDI for cholecalciferol. Similar RDI percentage value for vitamin D_3 was found for brown shrimp. These amounts are three times higher than the daily recommended intake. It should be noted that the portion of shrimp is a very good source of vitamin E – 51.5% of RDI.

3.3. Fatty Acid Composition

Twenty - nine fatty acids from C10:0 to C 22:6 n-3 were identified and compared among the different species. There were wide variations and significant differences ($p<0.05$) in the FA profiles of molluscs population in terms of total and individual saturated and unsaturated FAs. Traditionally bivalves are considered to be herbivores and it is assumed that phytoplankton form the main component of their diet and their FA profile, respectively. However, several studies have shown that bivalves can use other food sources such as detritus, bacteria, micro zooplankton and meso zooplankton, whereas gastropods (like rapana) and decapods (like shrimp) are omnivorous species and this affects their FA composition [22, 30].

Orban *et al.* [31] and Zlatanos [32] presented a relative pattern PUFA>SFA>MUFA in black mussel from the Adriatic coast and local Mediterranean mussel farm. A deflection of this pattern was observed for mussel samples in our investigation, in which SFA content was significantly higher than PUFA ($p<0.001$) and MUFA ($p<0.001$) (SFA> PUFA >MUFA). Similar FA distribution for black mussel and rapa whelk from Baia Mamaia zone-Park, Constanza (Black Sea) was presented by Badiu *et al.* [33]. Deflection of our results for FA composition of brown shrimp was reported by Turan *et al.* [22] for same species from Turkish part of Black Sea (Sinop) (SFA>PUFA>MUFA).

It is known that temperature and food availability are two of the most important factors regulating the growth of marine invertebrates, including bivalve mollusks [30]. During the study period analyzed species were exposed to essentially the same temperature regime but due to different position in the water column and area, their food sources may vary. Although their potentially accessible food (phytoplankton, heterotrophic flagellates, ciliates, zooplankton, detritus), have many common FAs, some differences in specific FAs and groups of acids respectively were found between wild mussel, shrimp and rapana. In this study the FA profile of all mollusks showed a considerable contribution of SFAs (mussel and shrimp) and PUFA (rapa whelk) in tissues, while MUFAs were less-abundant. **Table 4** presents FA profile of analyzed molluscs as percentage of total FA (mean ± standard deviation).

Saturated fatty acid

The analyzed Black Sea molluscs presented high concentrations of three quantitatively dominating SFA: palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acids witch followed next distributions: C16:0>C18:0>C14:0. The dominance of SFAs was mainly due to the high level of C16:0 contributing approximately to 70% of the total SFA content in the examined species. Black mussel contained highest C16:0 amount (29.04%), while rapa whelk presented lowest levels (24.21%).

Several authors reported lower values for C16:0 in mussels from Adriatic and Mediterranean Sea [31, 32], whereas Prato *et al.* [20] presented similar results for cultured mussels from Southern Italy. Turan *et al.* [22] founded higher C16:0 levels for Black Sea shrimp, whereas Mika *et al.* [34] shows similar C16:0 amount for Baltic Sea shrimp compared to our data. Other predominant SFAs are C18:0 varying from 4.25% (shrimp) to 4.94%

(rapana) and C 14:0, which was found in range from 1.10% (shrimp) to 3.15% (mussel).

Table 4 FA profiles (% of total FAs) in edible tissue of black mussel, rapa whelk and brown shrimp (mean ± SD)

Fatty Acid	Black mussel	Rapa whelk	Brown shrimp
Saturated fatty acids			
C12:0	1.84±0.06	1.38±0.05	0.70±0.02
C14:0	3.15±0.20	2.32±0.15	1.10±0.10
C16:0	29.04±0.54	24.21±0.48	27.38±0.62
C17:0	0.42±0.04	0.30±0.01	0.30±0.02
C18:0	4.79±0.05	4.94±0.06	4.25±0.15
C20:0	0.39±0.01	1.84±0.04	0.75±0.04
C21:0	0.20±0.01	nd	0.20±0.01
C22:0	0.80±0.03	1.92±0.10	0.86±0.03
C23:0	nd	nd	0.15±0.01
C24:0	1.05±0.08	1.15±0.05	0.55±0.01
ΣSFA	**41.91**	**38.06**	**36.49**
Monounsaturated fatty acids			
C14:1 n5	0.25±0.01	0.65±0.05	0.45±0.02
C16:1 n7	18.25±1.30	2.77±0.20	16.10±1.05
C17:1 n8	0.20±0.01	0.23±0.01	0.30±0.01
C18:1 n9	4.88±0.30	7.21±0.55	8.00±0.55
C20:1 n9	1.43±0.06	1.70±0.10	0.70±0.02
C22:1 n9	0.25±0.01	1.20±0.09	0.40±0.01
C24:1 n9	0.23±0.01	0.80±0.04	0.35±0.01
ΣMUFA	**25.49**	**14.56**	**26.30**
Polyunsaturated fatty acids			
C18:3 n6	0.21±0.01	1.15±0.14	0.85±0.05
C18:2 n6	2.38±0.30	11.82±0.95	7.53±0.20
C18:3 n3	1.72±0.14	1.58±0.10	1.85±0.08
C20:5 n3	3.35±0.30	12.33±1.05	5.95±0.45
C20:4 n6	5.56±0.55	3.34±0.40	3.85±0.37
C20:2 n6	0.58±0.02	1.09±0.08	0.53±0.02
C20:3 n3	1.20±0.18	3.81±0.20	0.80±0.04
C20:3 n6	2.08±0.26	2.37±0.17	0.80±0.01
C22:6 n3	15.25±1.50	8.53±0.85	14.75±1.15
C 22:2	0.27±0.01	1.36±0.12	0.30±0.01
ΣPUFA	**32.60**	**47.38**	**37.21**

Monounsaturated fatty acid

Both mussel and shrimp samples contained high levels of palmitoleic acid (C16:1 n7) - 72.0% (black mussel) and 61.0% (brown shrimp) from total MUFA's. Turan *et al.* [22] and Mika *et al*. [34] presented higher values for C18:1 n9 and lower for C16:1 n7 for brown shrimp from Black and Baltic Seas. The diet of *C. crangon* consists of demersal, epifaunal and infaunal organisms, but feeding is controlled by environmental (abiotic, extrinsic, e.g. salinity, temperature, dissolved oxygen) and biological (e.g. habitat structure) factors [4, 22]. The second abundant MUFA for these species is oleic acid (C18:1 n9), which has 2 to 3 times lower level than C16:1 n7 respectively. Several authors reported similar levels for C18:1 n9 and C16:1 n7 for *M. galloprovincialis* captured from Adriatic, Tyrrhenian and Mediterranean Seas [31, 32, 35]. In contrast, higher level of C18:1 n9 than C16:1 n7 in cultured black mussels from Mar Grande of Taranto was presented by Prato *et al* [20], which explains this fact as effect of carnivorous dietary inputs. Analyzed rapa whelk samples presented higher C18:1 n9 than C16:1 n7 levels. Badiu *et al* [33] reported similar results for *Rapana venosa*.

The Black Sea is the most isolated European semi-enclosed and costal sea 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. Zaitsev [36] supposed that the decline in populations of zooplankton and increasing of phytoplankton mass as a result from the Black Sea eutrophication, leads to the reductions of some MUFA levels as C 20:1 and increase of n-3 PUFAs in molluscs and fishes from this region. The results obtained in this study showed that all analyzed species have low levels of C20:1n9 ranged from 0.70% (shrimp) to 1.70% (rapana).

Polyunsaturated fatty acid

Significant variations in PUFAs groups between species were observed. Rapa whelk showed highest PUFA value (47.38%), followed by brown shrimp (37.21%) and Black mussel from Cape Galata (32.60%). PUFA contents of analyzed molluscs are in agreement with previously literature data. Some authors determined PUFA levels range from 24.63% to 68.00% for black mussels and rapana form Mediterranean, Black and Adriatic Sea, and our results are within these levels [30, 32, 33]. Only Prato *et al* [20] showed a significantly lower PUFA levels for mussels from Mar Grande of Taranto (7.55-11.16%) compared to our amounts.

In present investigation the major long–chain PUFAs were eicosapentaenoic acid (EPA, C20:5n-3), docosahexaenoic acid (DHA, 22:6 n-3), linoleic acid (LA, C18:2n-6) and arahidonic acid (ARA, C20:4n-6). Phytoplankton, algae and other plants are the base of the marine food chain and they are able to synthesize these high unsaturated PUFAs. The DHA was found to be the most dominant PUFA for mussel and shrimp. The highest obtained values for DHA are in black mussel (15.25%, which is 47% of total PUFA), followed by shrimp (14.75%, which is 40% of total PUFA). These results were similar in comparison to Zlatanos [32] and Saglik and Imre [37] investigations for Mediterranean and Black Sea mussels. In contrast, Orban *et al.* [31] and Badiu *et al.* [33] found higher EPA than DHA levels in black mussels from Adriatic, Tyrrhenian and Romanian Black Sea coasts. In this study rapa whelk showed highest EPA value (12.33%, which is 26% of total PUFA), whereas black mussel and shrimp contains significantly lower amounts (3.35-5.95%). Sriket *et al.* [38] reported close to presenting results (DHA> EPA) for black tiger and white shrimps from Thailand. Turan *et al.* [22] found significantly higher EPA levels for Black Sea shrimp compared to our results. The observed differences for EPA levels between analyzed mussels and shrimps, and literature data may be related to the type of food ingested by the molluscs. The conversion of EPA to DHA might also explain the differences observed between the two FA levels. Vernocchi *et al.* [35] supposed that proportion of EPA and DHA were strongly influenced by water temperature and DHA was the most abundant FA in warmest autumn months (such as analyzed samples). In present study, only black mussel EPA levels were found lower compared to ARA levels ($P<0.001$). Both analyzed Black Sea mollusks (shrimp and rapana) showed opposite trend and EPA values (5.95-12.33%) were significantly higher than ARA amounts (3.34-3.85%).

Despite the differences in the FA profile it can be concluded that all Black Sea mollusks are important sources of essential n-3 PUFA. N-3 and n-6 PUFA levels, n-6/n-3 and PUFA/SFA ratios, IA, IT, FLQ indices in studied Black Sea mollusks are presented in **Table 5**. Higher levels of n-3 PUFAs (49 - 66% of total PUFA) characterized edible tissue of all Black Sea molluscs, compared to n-6 PUFA levels. These results were similar in comparison to data presented by Badiu *et al.* [33] and Turan *et al.* [22] for Black Sea mussel, rapana and brown shrimp.

Table 5 Fatty acid groups, ratios and lipid quality indices (*mean ± SD)*

Fatty Acid	Black mussel	Rapa whelk	Brown shrimp
n-3	21.52±1.05	23.25±1.10	23.40±1.02
n-6	10.81±0.40	17.40±1.00	13.03±0.87
n-6/n-3	0.50±0.01	0.74±0.03	0.55±0.05
PUFA/SFA	0.78±0.03	1.24±0.17	1.02±0.15
IA	0.68±0.01	0.55±0.02	0.52±0.02
IT	0.49±0.01	0.32±0.01	0.36±0.01
FLQ	18.60±0.85	20.86±1.05	20.70±1.25

The n-6/n-3 ratio has been suggested to be a useful indicator when comparing relative nutritional values of sea foods. A decrease in the human dietary n-6/n-3 PUFA ratio is essential to help prevent coronary heart disease by reducing plasma lipids and to reduce the risk of cancer [8, 15]. In presented study this ratio ranged: from 0.50 to 0.74 and was found similar to earlier results presented in literature for Black Sea mollusks [22, 30, 31, 32, 33, 35, 36].

Another useful key factor for evaluation of sea food nutritional quality is PUFA/SFA ratio. Values of PUFA/SFA ratio greater than 0.45, are recommended by Department of Health (1994) [39]. In this study the PUFA/SFA ratio was found higher than cut-off value in all species (Table 4). Simopolous [15] reported that several studies have found inverse correlation between the PUFA/SFA ratios and cardiovascular diseases and suggested that replacement of SFA with PUFA in the human diet will decrease similar health problems.

The nutritional value of mollusk's meat is also determined by lipid quality indices, which depend on the relative proportions of some individual saturated and unsaturated fatty acids. These indices indicate the global dietetic quality of lipids and their potential effects on the development of coronary disease [17]. With regard to the quality indices considered (Table 5), FLQ showed insignificant statistical differences ($p=0.05$) between analyzed samples, as FLQ presented lower values in black mussel, coinciding with the lower EPA and DHA levels. IA and IT showed close values for analyzed samples. Turan *et al.* [22] reported significantly

higher IA (1.34) and similar IT (0.31) values for Black Sea brown shrimp. These values were lower than those found in lamb, beef and rabbit meats [40]. Higher values of IA and IT (> 1.0) are detrimental to human health [41] and presented values are beneficial for human nutrition and clearly showed the differences in FA patterns in edible tissue in analyzed species. To our knowledge, no data is available in literature for fat soluble vitamins content and lipid quality indices for mollusks from Bulgarian Black Sea coast.

4. Conclusions

Wild mollusks from Bulgarian Black Sea coast were analyzed with the aim to evaluate vitamin A, D_3 and E contents, FA composition and IA, IT and FLQ indices in relation to their nutritional quality. This preliminary study demonstrated that all analyzed species were low in total lipids. Both analyzed mussel and shrimp provide higher content of alpha-tocopherol and retinol and considerable amounts of cholecalciferol, and supply almost three times higher amount than the RDI for vitamin D_3.

Black Sea mollusks contained higher n-3 PUFA level and lower n-6 FAs. Regarding the n-6/n-3, and PUFA/SFA ratios all species are good sources of the identified biologically active substances. In this study all Black Sea mollusks present low IA and IT and high FLQ levels and therefore their consumption could be beneficial to influence the incidence of coronary heart disease in humans.

5. References

*E-mail address: a.merdzhanova@gmail.com

[1] E. Petrova and St. Stoykov, Agricultural Science and Technology **3** (4), 368 - 373 (2011).
[2] E. Petrova and St. Stoykov, Macedonian Journal of Animal Science **1** (1), 223–226 (2011).
[3] S. Moncheva, J. Namiesnik, R Apak, P. Arancibia-Avila, F. Toledo, S. Kang, S. Jung and S. Gorinstein, Chemistry and Ecology **27**, 1), 31–41 (2011).
[4] J. Campos, and H.W Van der Veer, Oceanogr. Mar. Biol. Annu. Rev. **46**, 65-104 (2008)
[5] Ribarova F. *Food and vitamins* Sofia, Bulgaria. (2007).
[6] K. W. Lee, A. D.Blann, Y.Gregory and H. Lip, Thrombosis Research **118**, 305-312 (2006).
[7] A. K. Dutta-Roy, Platelets **13,** 67–75 (2002).
[8] P. Kris - Etherton, W. Harris., L. Appel, Arterioscler Thromb Vasc Biol **23**, 20-30 (2003), http://www.atvbaha.org.
[9] *** FAO, Food and Nutrition, *Fats and fatty acids in human nutrition Report of an expert consultation*, Paper 91, FAO, (Final report). (2010).
[10] D.A. Dobreva, B.Galunska and M.Stancheva, Scripta Scientifica Medica, Varna Medical University **43,** 276-279 (2011).
[11] E. Bligh, and W. Dyer, Canadian Journal of Biochemistry and Physiology **37**, 913–917 (1959).
[12] *** BDS EN ISO 5509 (2000)
[13] *** BDS EN ISO 5508 (2000)
[14] H. Hosseini, M. Mahmoudzadeh, M. Rezaei, L. Mahmoudzadeh, R. Khaksar, N. Khosroshahi and A. Babakhani, Food chemistry **148,** 86-91 (2014)
[15] A. Simopoulos and L. Cleland, World Rev Nutr Diet. Basel, Karger **92**, 1-22 (2003).
[16] T. Ulbricht and D.Southgate, Lancet **338,** 985-992 (1991).
[17] L. Senso, M.D.Suarrez, T.Ruiz-Cara and M.Garcıŕa-Gallego, Food Chemistry **101,** 298–307 (2007).
[18] G. Abrami, F. Natiello, P. Bronzi, D. McKenzie, L. Bolis and E. Aggrade, Comparative Biochemistry and Physiology **1/2,** 71–82 (1992).
[19] R. Sirbu, A.Bechir, T.Negreanu-Pîrjol, C.Sava, B. Negreanu-Pîrjol, T. Zaharia, C. Ursache and R. M. Stoicescu, Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry **12** (3), 221 – 228 (2011).
[20] E. Prato, A.Danieli, M. Maffia and F. Biandolino, Zoological Studies **49, (2)**, 211-219 (2010).
[21] V. Ventrella, M. Pirini, A. Palgliarani, F. Trombetti, M.P. Manuzzi and A.R. Borgatti, Comparative Biochemistry and Physiology, Part B **149,** 241–250 (2008).
[22] H. Turan, Y, Kaya and E. Erdem, Journal of Aquatic Food Product Technology **20**, 100-107 (2011).
[23] G. MacDonald, *Identification of Health and Nutritional Benefits of New Zealand Aquaculture Seafood's,* Report, MacDonald and Associates Ltd, Nelson (2010).

[24] *** Whole Food Catalog (2011), http://wholefoodcatalog.info/food/mussel(raw)/
[25] *** Danish Food Composition Databank, Department of Nutrition, National Food Institute (2009).
[26] V. Öhrvik, A. Malmborg, I. Mattisson, S. Wretling and C. Åstrand, *Fish, shellfish and fish products - analysis of nutrients,* LIVSMEDELS VERKET, NATIONAL FOOD AGENCY, Sweden (2012).
[27] *** FNB (2011): *Dietary reference intakes for Calcium and Vitamin D,* http://books.nap.edu/ openbook.php?record_id=13050.
[28] *** EFSA, *Tolerable upper intake levels for vitamins and minerals, Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies,*(2006) http://www.efsa.europa.eu/en/ndatopics/docs/ndatolerableuil.pdf
[29] *** Ordinance № 23 / 19.07.2005 on the physiological feeding of population http://bg.wikipedia.org/ wiki /Физиологични _норми _за_хранене#cite_note-echo-9
[30] D. Ezgeta-Balić, M. Najdek, M. Peharda and M. Blažina, Aquaculture **334–337**, 89–100 (2012).
[31] E. Orban, G. Di Lena, T. Nevigato, I. Casini, A. Marzetti and R. Caproni, Food Chemistry **77**, 57–65 (2002).
[32] S. Zlatanos, EJEAFChe, 7(6), 3015-3019 (2008).
[33] D. Badiu, A. M.Balu, L. Barbes, R. Luque, R. Nita, M. Radu, E. Tanase and N. Rosoiu, Lipids **43**, 892-841 (2008).
[34] A. Mika, M. Golebiowski, E.Skorkovski and P. Stepnowski, International Journal of Oceanography and Hydrobiology **41** (2), 1-7 (2012).
[35] P. Vernocchia, M. MaVei, R. Lanciotti, G. Suzzi and F. Gardini, Food control **18,** 1575–1583 (2007).
[36] Y.P. Zaitsev, *Fisheries Oceanography* **1** (2), 180-189 (1992).
[37] S. Saglik and S. Imre, Turkish J Marine Sciences, **3** (3), 179-189 (1997).
[38] P. Sriket, S. Benjakul, W. Visessanguan and K. Kijroongrojana, Food Chemistry **103,** 1199–1207 (2007).
[39] *** Department of Health, *Nutritional aspects of cardiovascular disease:* Health and social subsects. HMSO, London (1994)
[40] L. Morbidini, Sarti D.M., Pollidori P. and Valigi A. Opinions Mediterraneenes. Serie A: Seminares Mediterraneens **46**, 29-33 (2001).
[41] H.Ouraji, B.Shabanpur, A.Abediankenari, A. Shabani, A. Nezami, M. Sudagar and S. Faghani, Journal of the Science of Food and Agriculture, **89,** 993-997 (2009).

Submitted: April 4th 2014
Accepted in revised form: May 7th 2014