

Lipid composition of raw and cooked *Rapana venosa* from the Black Sea

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Abstract. *Rapana venosa* is an edible mollusc with nutritional and economic importance. There is limited information about its lipid composition. The aim of the present study is to provide information about lipid composition, fatty acid profiles, fat soluble vitamins and cholesterol content of raw and cooked *Rapana venosa*. Cooking did not affect the ratio of lipid classes, but fatty acids composition varied significantly. Considerable variations were observed in fatty acid distribution of total lipids and neutral lipids. Fatty acid groups of phospholipids remained unaffected by temperature treatment. The most abundant fatty acids in all lipid classes of raw and cooked specimens were palmitic acid (C16:0) and eicosapentaenoic acid (C20:5n-3). The sum of omega-3 polyunsaturated fatty acids (PUFA) was higher than omega-6 PUFA in all lipid fractions. The results of the present study showed that cooking process affected cholesterol, fat soluble vitamins and carotenoids content differently. Larger variations were observed for vitamin A, β -carotene and astaxanthin and to lesser for vitamin E. Cholesterol and vitamin D₃ were also affected by the thermal stress. The present study revealed that *Rapana venosa* meat could be a good source of high quality nutritional lipids, which are well preserved even after culinary treatment.

Keywords: *Rapana venosa*, cooking, lipid composition, cholesterol, vitamins, carotenoids.

1. Introduction

The molecular diversity of chemical compounds found in marine animals is the result of the evolution of the organisms and their unique physiological and biochemical adaptations. To survive in a competitive environment and stress caused by various factors such as bacteria, temperature fluctuation, ultraviolet radiation, mechanical strength of the waves, etc., marine organisms synthesize a wide variety of biologically active metabolites with a variety of unique structures and biological effects [1].

Health benefits resulting from the consumption of seafood are the subject of intensive research over the past three decades. Seafood is rich source of polyunsaturated fatty acids (PUFA), phospholipids (choline), sterols, carotenoids, soluble vitamins (vitamin D), vitamin B₁₂ and various micronutrients and essential amino acids. In many researches it is emphasized that seafood provides the intake of long-chain omega-3 (LCn-3) PUFA, which main role is associated with the prevention of cardiovascular diseases. In terms of biological activity, omega-3 PUFAs of marine origin (fish, molluscs) are considered more effective than those of landcorp origin [2].

Veined rapa whelk or *Rapana venosa* is a predatory marine snail, once an invasive species in the Black Sea, now has become a valuable seafood with nutritional and economic importance. The black mussel is the main food source of rapana. *Rapana venosa* is not active during the winter months when is usually buried in the bottom sediments [3]. Studies show that *Rapana venosa* consumption improves the lipid profiles and antioxidant capacities in serum of rats fed an atherogenic diet [4], however authors conclude that more detailed

investigations are needed to identify and to determine the effects of separate bioactive ingredients of the species in prevention of atherosclerosis. The most abundant fatty acids in *Rapana venosa* lipids are palmitic (C16:0), eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) [5-7]. The high content of the latter two in the lipid extracts of the marine snail is associated with the accelerated healing of induced cutaneous burns in rats [8]. Although there are studies devoted to the characterization of *Rapana venosa* lipids, there is scarce literature on the changes occurring due to cooking processes. Very long-chain PUFAs (VLC-PUFA) are highly susceptible to oxidation. Thermal stress can cause losses in meat lipid quality by destruction of vitamins and pigments, and reduced nutrients resulting from leaching. The aim of the present study is to provide new information on the lipid content, lipid classes, fatty acid profiles, fat-soluble vitamins and cholesterol content of raw and cooked *Rapana venosa* meat.

2. Experimental

2.1. Sample preparation

Rapana venosa specimens were purchased in September 2017 from a local enterprise for fish and seafood processing, located near Varna, Bulgaria. Samples were harvested from the Northern part of the Black Sea coast. Live specimens were transported to the laboratory in ice boxes. They were washed and processed immediately. One hundred animals were chosen randomly to determine the sample mean. Specimens were divided into two groups for further processing. Cooking method applied was boiling. Rapa whelks were placed in an autoclave at 98 °C and ambient pressure. The temperature

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decrease to 82 °C for 15 min and then rises again to 90 °C. The moisture content was determined by drying in an oven for 3 h at 105 °C until constant weight [9].

2.2. Extraction of total lipids (TL)

Three replicate samples of raw and cooked meat homogenates (5.000±0.001 g) were extracted following the Bligh and Dyer procedure [10]. Lipid content was determined each sample gravimetrically and the results were expressed as g per 100 g wet weight (g·100 g⁻¹ ww).

2.3. Separation of lipid classes

Total lipids were separated into different classes by column chromatography. Neutral lipids (NL) and phospholipids (PL) were obtained using a glass column (10 mm dia × 20 cm) packed with a slurry of activated silicic acid (70 to 230 mesh; Merck, Darmstadt, Germany) in chloroform. The fraction containing NL was eluted with chloroform, while PL were eluted with methanol. The amount of lipid classes obtained was determined by gravimetry. The purity of each fraction was tested by thin-layer chromatography, using Silica gel F254 plates (thickness = 0.25 mm; Merck, Darmstadt, Germany).

2.4. Preparation of fatty acids methyl esters (FAME) and GS-MS analysis

The dry residues of each fraction were methylated using 2% H₂SO₄ in methanol and *n*-hexane [11]. After transmethylation, samples were centrifuged (3500 rps) and the layer containing FAME was extracted three times with hexane. Combined extracts were filtered through anhydrous Na₂SO₄ and evaporated under gentle steam of nitrogen. FAMES were separated by Thermo Scientific FOCUS Gas Chromatograph with TR-5 MS capillary column (30 m, 0.25 mm i.d.) and MS detector (Polaris Q). For peaks identification mass spectra (ratio m/z) of FAME mix standard (SUPELCO 37 F.A.M.E. Mix C4 - C24) and internal Data Base (Thermo Sciences Mass Library, USA) were used. Stearidonic acid (STA) and docosapentaenoic acid (DPA) were identified and quantified by PUFA №3 from Menhaden oil (Sigma-Aldrich, Merck). Results for total lipid fraction were calculated using conversion factor for molluscs [12] and for the individual lipid classes – as a percentage of each FA with respect to the total FAs [13].

2.5. Saponification and extraction of vitamins, pigments and cholesterol

Alkaline saponification was used to analyze β -carotene, astaxanthin, cholesterol, all-trans-retinol, α -tocopherol, ergocalciferol, and cholecalciferol content in raw and cooked rapana meat. Sample preparation procedure was performed following the method of Dobrova *et al.* [14]. Briefly, an aliquot of the homogenized tissue (1.000±0.005 g) was weighed into a glass tube with a screw cap and 1% of methanolic L-ascorbic acid and 0.5 M methanolic potassium hydroxide were added. Six replicates of each group were prepared and subjected to saponification at 50 °C for 30 min. After cooling the analytes were extracted twice with *n*-hexane:dichloromethane = 2:1 (v/v) solution. The combined extracts were evaporated under nitrogen and redissolved in methanol:dichloromethane solution,

filtered (0.45 μ m syringe filter) and injected (20 μ l) into the HPLC system.

2.6. HPLC analysis

All-trans-retinol, ergocalciferol, cholecalciferol, α -tocopherol, β -carotene, astaxanthin and cholesterol were determined simultaneously using high performance liquid chromatography (HPLC). The chromatographic separation was achieved by reversed-phase analytical column (Synergi 4 μ Hydro-RP 80A pore 250x4.6 mm). The mobile phase consisted of acetonitrile: methanol: 2-propanol = 75:20:5 with a flow rate of 1.1 ml/min [14]. Ultraviolet detection (UV2000) was used for cholesterol (208 nm), ergocalciferol (265 nm), cholecalciferol (265 nm), β -carotene (450 nm) and astaxanthin (474 nm). Concentrations of all-trans-retinol (at λ_{ex} = 334 nm and λ_{em} = 460 nm) and α -tocopherol (at λ_{ex} = 288 nm and λ_{em} =332 nm) were measured by fluorescence (FL3000) detector.

2.7. Nutrition quality indices (NQI)

Nutrition quality was evaluated based on several indices and ratios: the indices of atherogenicity (AI), thrombogenicity (TI), cholesterolemic index (h/H); n-6/n-3 and PUFA/SFA ratios, according to Simopolous [15]. Ulbricht and Southgate [16] suggest two indices – AI and TI that may well describe the atherogenic and thrombogenic potential of fatty acids. The index h/H presents the functional effects of individual PUFAs on cholesterol metabolism (hypo- and hypercholesterolemic effect), and was calculated according to Santos-Silva *et al.* [17]. The cholesterol/SFA index (CSI) was calculated according to Connor *et al.* [18] to assess the potential effect of dietary lipids on serum cholesterol.

2.8. Statistical analysis

The results were analyzed by Graph Pad Prism 6.0 software and expressed as mean and standard deviation (mean \pm SD). Unpaired *t*-test was applied to estimate the differences between the analyzed samples. The differences were considered significant at *p* < 0.05.

3. Results and discussion

3.1. Total lipids, lipid classes and moisture content

The results for total lipids (TL), neutral lipids (NL), phospholipids (PL) and moisture content are presented in Table 1. *Rapana venosa* was characterized by low lipid content, which is consistent with data reported in literature [5-7, 19]

Table 1. Total lipids (TL), neutral lipids (NL), phospholipids (PL) and moisture content in raw and cooked *Rapana venosa* meat

	Raw	Cooked
Mean length, cm	6.27±0.9	
TL, g·100g ⁻¹ ww	1.22±0.15	1.63±0.07 ^a
NL, % of TL	14.4±0.4	25.2±0.4 ^a
PL, % of TL	84.4±0.5	74.1±0.1 ^a
Moisture, %	76.1±0.1	73.8±0.5 ^a

^a Statistical differences, *p* < 0.05

Significant differences were observed in moisture content of raw and cooked samples. Water content was

reduced by 3% after cooking process. The amounts of TLs and NL increased significantly after treatment, while the PL ratio decreased. However, the lipid class distributions (PL > NL) in both raw and cooked rapana remained unchanged.

3.2. Fatty acids composition of total lipids

Notably, fatty acids composition of mollusks is, to some extent, affected by external factors such as dietary inputs, water temperature, salinity, pollution and sun irradiation [20]. Fatty acids composition of total lipids of raw molluscs and molluscs after treatment are presented in Table 2.

Table 2. FA composition (as mg per 100 g ww) in raw and cooked *Rapana venosa* total lipids (mean \pm SD)

FAME mg·100 g ⁻¹	Raw	Cooked
8:0	0.70 \pm 0.06	1.33 \pm 0.08 ^a
10:0	3.64 \pm 0.21	3.81 \pm 0.36
12:0	nd	nd
14:0	30.65 \pm 3.9	31.62 \pm 4.3
15:0	5.08 \pm 1.6	4.52 \pm 1.14
16:0	202.9 \pm 2.3	220.8 \pm 2.1 ^a
17:0	1.43 \pm 0.2	1.75 \pm 0.29
18:0	79.3 \pm 0.9	84.8 \pm 0.7
20:0	2.41 \pm 0.45	nd
21:0	nd	nd
22:0	1.65 \pm 0.41	2.58 \pm 0.47 ^a
23:0	nd	nd
24:0	5.20 \pm 0.42	5.66 \pm 0.18
SFA	333.0	360.1
14:1	6.65 \pm 0.73	7.36 \pm 0.46
15:1	nd	nd
16:1	16.03 \pm 1.21	17.8 \pm 0.9
17:1	3.66 \pm 0.89	3.84 \pm 0.81
18:1	22.4 \pm 1.87	23.3 \pm 1.06
20:1	6.87 \pm 0.14	8.58 \pm 1.03
22:1	4.64 \pm 0.28	9.19 \pm 1.9 ^a
24:1	2.40 \pm 0.49	8.08 \pm 0.79 ^a
MUFA	62.7	78.2^a
18:4n3 (Strd)	8.02 \pm 1.65	8.56 \pm 1.44
18:3n6	nd	nd
18:3n3	4.12 \pm 0.12	4.74 \pm 0.62
18:2n6 cis	14.40 \pm 2.5	21.2 \pm 2.3 ^a
18:2n6 tr	nd	nd
20:5n3 (EPA)	146.8 \pm 4.3	142.04 \pm 5.44
20:4n6 (ARA)	38.42 \pm 1.26	41.24 \pm 2.71
20:3n6	25.6 \pm 2.9	28.35 \pm 2.38
20:3n3	7.13 \pm 0.15	21.28 \pm 2.13 ^a
20:2n6	22.6 \pm 2.3	20.36 \pm 2.9
22:2n6	39.17 \pm 1.187	42.01 \pm 1.57
22:6n3 (DHA)	nd	nd

FAME mg·100 g ⁻¹	Raw	Cooked
22:5n3 (DPA)	33.9 \pm 2.5	29.49 \pm 2.57
PUFA	340.16	359.3^a
n-3	199.9	206.1
n-6	140.2	153.2

^a $p < 0.05$; nd – not detected

SFA was the most abundant FA group in raw samples, followed by PUFA and MUFA. The same pattern was observed in the processed meat (SFA > MUFA > PUFA). In previous studies [5-7], autumn samples of *Rapana venosa* from the Black Sea showed different distribution: PUFA > SFA > MUFA. The amount of each FA group as milligram per 100 grams wet weight increased after boiling, which results in higher content of total fat. The possible reasons for observing differences are that the cooking technique used facilitated the extraction of total lipids or the increased levels are probably due to the loss of moisture. Palmitic acid (C16:0) was the most abundant FA in SFA group, oleic acid (C18:1n9) in MUFA, and eicosapentaenoic acid (C20:5 n3, EPA) in PUFA group. Marine mollusks are characterized by the predominance of essential n-3 PUFA, mainly EPA and docosahexaenoic acid (C22:6 n3, DHA), which constitute usually almost half of the total FAs [21]. In contrast, *Rapana venosa* did not show this property: EPA represented 20.6% of total FAs in raw, and decreased to 18.4% of total FAs in cooked tissue. DHA was a minor component and was not detected in the present study. Despite this fact, another LCn-3 PUFA – docosapentaenoic acid (C22:5n3, DPA) was found in significant amounts in rapa whelk TL. Presented results showed that cooking process insignificantly affect its quantity. Higher intake of DPA is associated with improved cardiovascular health [22]. Moreover, studies reveal that DPA was the most effective omega-3 FA in the prevention of raised insulin resistance in high-fat fed rats [23]. The observed differences in C₂₀-C₂₂ LCn-3 PUFAs amounts are mostly related to enzymes activities in mollusc's tissues. Various factors may influence the processes of elongation and desaturations of PUFAs, as the ambient temperature being an important one. In the present study autumn specimens (September) were analyzed, when the temperature of Black Sea water body is high (approx. 26°C). Consequently, one possible reason for lack of DHA in rapana TLs is significant decrease of n-3 LCPUFA metabolism under the influence of high seawater temperature [24]. SFA content in raw samples represented 27.3% of TL and decreased to 22.1% in cooked samples, MUFA was 5.1% in raw and 4.8% in cooked samples and PUFA – 26.0% in raw and 12.6% of cooked samples TL. The group of PUFA was mostly affected by the cooking method, which could be related to higher susceptibility to oxidation.

Despite the changes in fatty acids composition, cooking method seemed to not affect the nutritional quality indexes of *Rapana venosa* meat lipids (see Table 3). The Black Sea gastropod presented well-balanced and beneficial FA profile. The n-6/n-3 and PUFA/SFA ratios are traditionally used to indicate relative nutritional

values of food lipids. There were no significant differences between raw and cooked rapana.

Table 3. Fatty acid contents, FA ratios, nutrition quality indices in raw and cooked *Rapana venosa*

	Raw	Cooked
<i>n-6/n-3</i>	0.70	0.74
<i>PUFA/SFA</i>	0.95	0.93
<i>AI*</i>	0.81	0.79
<i>TI*</i>	0.44	0.46
<i>h/H*</i>	0.97	0.92
<i>CSI*</i>	3.05	3.29
<i>mg Vit. E/g PUFA</i>	17.8	18.7

**AI* = [(C12:0 + (4 × C14:0) + C16:0)] / (n6PUFA + n3PUFA + MUFA)

**TI* = (C14:0 + C16:0 + C18:0) / [(0.5MUFA) + (0.5n6PUFA) + (3n3PUFA) + (n3PUFA/n6PUFA)]

**h/H* = (C18:1n9 + C18:2n6 + C18:3n3 + C20:4n6 + C20:5n3 + C22:6n3) / (C14:0 + C16:0)

**CSI* = (1.01 × SFAg · 100⁻¹ g ww) + (0.05 × cholesterol mg · 100⁻¹ g ww)

The *n-6/n-3* ratios were below 1.0, which indicates the possible beneficial properties of mollusks lipids to human health [15]. A decrease in the dietary *n-6/n-3* ratio is essential for reducing plasma lipids and thus preventing coronary heart disease and the risk of metabolic diseases. *PUFA/SFA* values for raw and cooked meat were 0.95 and 0.93, respectively. This ratio is recommended to be higher than 0.45 (> 0.45) [25]. Popova et al. [6] reported lower values for *n-6/n-3* and higher for *PUFA/SFA* ratios for autumn samples of *Rapana venosa* from the region of Varna Bay. Same discrepancy was observed in our previous study [5]. The differences are mainly due to the content of DHA, which was not detected in the present study. Koral and Kiran [7] reported higher *PUFA/SFA* ratio but higher *n-6/n-3* ratio for autumn samples of *Rapana venosa* from the Eastern Black Sea. The latter was higher because of the lower EPA content.

Rapana venosa lipids could be characterized with good anti-atherogenic, anti-thrombogenic and hypocholesterolaemic properties (*AI*, *TI* < 1 and *h/H* > 1). Anti-atherogenic and anti-thrombogenic indexes of cooked samples were found higher than those presented by Popova et al. [6]. All nutritional indexes values remained unaffected by the cooking process. Ghribi et al. [26] also reported stable values for *AI* and *TI* of boiled and steamed shellfish from Tunisian coast. The low levels of *CSI* are associated with the low *SFA* content in the rapana lipids. This fact is observed for shellfish species, despite of their higher cholesterol content [27].

Raederstorff et al. [28] supposed that 0.5 mg vitamin E per g *PUFA* is the minimum content of this antioxidant necessary to protect and metabolize dietary unsaturated FA. Results obtained in the present study confirmed higher lipid quality of the Black Sea mollusks meat.

3.3. Fatty acids composition of lipid classes

The differences detected for fatty acids between raw and cooked rapana meat were related to the changes in the amounts of phospholipids (PL) and neutral lipids (NL). Higher amounts of NL compared to PL provide higher levels of *SFA* and lower *PUFA* [29]. Neutral lipid

fractions represented 14.4% of raw *Rapana venosa* and increased to 25.2%, while the amount of PL decreased from 84.4% to 74.1% of total lipids. PL were characterized by higher *PUFA* content, compared to NL fraction (Table 4). The distribution of FA groups showed the following patterns: *PUFA* > *SFA* > *MUFA* for NL (raw), *SFA* > *PUFA* > *MUFA* for NL (cooked) and *PUFA* > *SFA* > *MUFA* for PL (raw and cooked). In both fractions the sum of *SFA* increased after cooking, NL increased by 7% and PL – by 8.3%.

MUFA increased in NL fraction (9%) and decreased in PL (20%), while *PUFA* decreased in both lipid fractions after culinary treatment. The main saturates in both fractions were C16:0, C18:0 and C14:0 (C16:0 > C18:0 > C14:0). NL serve as energy sources in mollusk tissues, consequently they contained higher levels of long chain SFAs (such as C16:0, C18:0, C20:0 and C22:0) which catabolism is preferred. Polar lipids, being important membrane constituents have similar *SFA* profile, which is homeostatically regulated, independently of dietary intake. In addition, membrane physical properties (for example fluidity) are influenced by FA chain length, as short chain FAs compared to long chain SFAs reduced the possibility for membrane adaptations at lower temperature [30]. Observed results confirm these statements and similarly higher levels of C16:0 and C18:0 were found in PL fractions. Total *SFA* increased in both lipid fractions after treatment, due to increase of C16:0 and C18:0 (26%, NL) and C16:0 (44%, PL).

In this study the levels of hypercholesterolaemic SFAs (C12:0 and C14:0) detected in all lipid fractions were low. Differences between *MUFA* levels in NL and PL classes were found after culinary treatment. Among *MUFA* C16:1n7 and C18:1n9 prevailed in all lipid fractions. Neutral lipids *MUFA* increased (9%), due to increase of C18:1n9 (25%), C20:1 and C24:1, whereas opposite trend was found for PL *MUFAs*, which levels significantly decreased (20%, *p* < 0.001), because of decrease of C18:1n9 (10%) after treatment.

Table 4. FA (as relative %) composition of NL and PL in raw and cooked *Rapana venosa*

FA	Raw		Cooked	
	Lipid classes			
	NL	PL	NL	PL
8:0	0.2±0.01	0.04±0.0	0.1±0.01	nd
10:0	0.1±0.00	0.3±0.02	0.2±0.00	0.02±0.0
12:0	0.2±0.01	nd	0.6±0.04	nd
14:0	2.9±0.14	4.56±0.2	3.62±0.2 ^a	0.4±0.02 ^a
15:0	0.5±0.04	0.3±0.02	0.8±0.07	nd
16:0	24.2±1.2	18.1±1	25.7±1.3	26.1±1.3 ^a
17:0	0.1±0.01	1.3±0.07	0.6±0.04	0.1±0.01 ^a
18:0	9.9±0.5	6.9±0.3	12.5±0.1 ^a	7.7±0.04
20:0	0.2±0.006	1.55±0.07	0.3±0.02	0.13±0.01
21:0	0.1±0.01	nd	nd	0.1±0.004
22:0	2.4±0.1	0.9±0.005	nd	3.32±0.15 ^a
23:0	nd	nd	nd	nd

FA	Raw		Cooked	
	Lipid classes			
	NL	PL	NL	PL
24:0	1.3±0.05	0.93±0.04	1.4±0.05	nd
SFA	42.1	34.9	45.7^a	37.8^a
14:1	0.6±0.04	0.39±0.02	0.4±0.02	0.19±0.01
16:1n7	4.8±0.2	1.4±0.07	3.0±0.15 ^a	2.8±0.13 ^a
17:1	nd	0.1±0.006	0.4±0.02	0.2±0.01
18:1n9	3.5±0.16	1.9±0.1	4.4±0.2 ^a	1.7±0.1
20:1	1.6±0.06	1.0±0.04	2.4±0.05 ^a	nd
22:1	nd	nd	0.5±0.03	nd
24:1	1.1±0.07	1.2±0.06	1.6±0.09	nd
MUFA	11.7	6.1	12.8	4.9^a
18:4n3	0.8±0.04	1.8±0.07	1.6±0.06 ^a	1.9±0.07
18:3n6	0.8±0.04	0.9±0.05	0.6±0.04	nd
18:3n3	0.7±0.04	0.14±0.006	0.7±0.04	0.4±0.02
c18:2n6	1.3±0.06	1.0±0.05	0.8±0.05	1.3±0.06
t18:2n6	nd	nd	nd	nd
20:5n3	18.5±1	25.9±1.2	17.9±0.9	21.4±1.1 ^a
20:4n6	8.5±0.4	6.5±0.3	9.8±0.5	10.7±0.5 ^a
20:3n6	4.9±0.2	6.7±0.3	nd	9.9±0.5 ^a
20:3n3	0.6±0.04	2.0±0.08	nd	2.7±0.14
20:2 n6	0.9±0.07	2.7±0.12	nd	2.2±0.12
22:2n6	3.8±0.2	nd	7.2±0.4 ^a	6.8±0.3 ^a
22:6n3	2.4±0.1	nd	nd	nd
22:5n3	2.9±0.1	11.5±0.4	2.8±0.1	nd
PUFA	46.2	59.04	41.4^a	57.3^a
n3	25.9	41.34	23.0	26.4 ^a
n6	20.3	17.80	18.4	30.9 ^a
n6/n3	0.78	0.43	0.80	1.17 ^a
P/S	1.10	1.70	0.91	1.52

^a $p < 0.05$ (raw vs cooked); nd – not detected

The PUFA levels show significant changes in both lipid classes after cooking processes. The major differences were found in NL PUFAs (11%, $p < 0.05$) due to decrease of long chain C₂₀ and C₂₂ as EPA (3.3%) and DHA (nd). Polar PUFAs showed insignificant increase of C₁₈ PUFA (C18:4n3, C18:3n3, C18:2n6), whereas C₂₀ and C₂₂ n3 PUFAs significantly decreased: EPA (17%, $p < 0.01$) and DHA (nd). Opposite trends were observed for omega 6 PUFAs, where C20:4 n6 increased in NL (15%) and PL (40%). The following tendency was observed: lower C₁₈ PUFAs – higher C₂₀₋₂₂ PUFAs in NL and PL after cooking.

Membrane permeability is positively correlated with highly unsaturated FAs (such as EPA, ARA and DHA). There were significant changes in total amount of n-3 ($p < 0.01$) and n-6 PUFAs. The levels of n-3PUFAs were higher compared to n-6 PUFAs in NL (raw and boiled) and raw PL lipid fractions. For both classes n-3 PUFAs decreased: with 12% in NL and 36% in PL fractions. In contrast, n-6 decreased only in neutral lipids (10%), and significantly increased in PL fractions (70%).

Meanwhile, significant difference ($p > 0.05$) was observed in the proportion of n6/n3 between raw and cooked rapana. Generally, after cooking process the ratio increased in both lipid fractions – 2.5% (NL) and three times in PL.

3.4. Fat soluble vitamins, carotenoids and cholesterol

The results for the fat soluble vitamins, cholesterol and carotenoids contents are presented in Table 5. Vitamin A, D₃, astaxanthin and beta-carotene are expressed as microgram per 100 grams wet weight ($\mu\text{g}\cdot 100^{-1}\text{ g ww}$), vitamin E and cholesterol are expressed as milligram per 100 grams wet weight ($\text{mg}\cdot 100\text{ g}^{-1}\text{ ww}$).

Table 5. Fat soluble vitamins, cholesterol, carotenoids in in raw and cooked *Rapana venosa* meat (mean \pm SD)

	Raw	Cooked
Cholesterol ($\text{mg}\cdot 100^{-1}\text{ g}$)	54.3±1.50	58.6±2.0 ^a
Astaxanthin ($\mu\text{g}\cdot 100^{-1}\text{ g}$)	62.1±2.45	19.5±1.85 ^a
β -carotene ($\mu\text{g}\cdot 100^{-1}\text{ g}$)	390.0±25.0	266±18.4 ^a
Vitamin D ₂ ($\mu\text{g}\cdot 100^{-1}\text{ g}$)	1.16±0.10	1.06±0.08
Vitamin D ₃ ($\mu\text{g}\cdot 100^{-1}\text{ g}$)	27.2±1.05	35.0±0.97 ^a
Vitamin A ($\mu\text{g}\cdot 100^{-1}\text{ g}$)	16.84±0.65	nd
Vitamin E ($\text{mg}\cdot 100^{-1}\text{ g}$)	5.65±0.15	6.30±0.22

^a $p < 0.05$; nd – not detected

Vitamin E, astaxanthin and beta-carotene are important components with high antioxidant activity. Carotenoids are vital components, which affect normal growth, metabolism and reproductivity of rapana. Some snail species can synthesize astaxanthin from beta-carotene by oxidative metabolic pathway and accumulate this component in their tissues [31]. Based on the results observed we can assume that high levels of beta-carotene are related to low metabolic activities, influenced by low water temperature and preparation and adaptation of marine organism for over-wintering (all samples were from September 2017).

Significant differences were observed for vitamin A, E and D₃ between row and cooked samples. Vitamin D₃ content was higher in cooked species and presented was two folds higher than the recommended daily intake [32]. Thus, analyzed *Rapana venosa* can be classified as excellent source of vitamin D₃, which increases its nutritional value.

Similar amounts of cholesterol were found in raw and cooked rapana TLs (approx. $56\text{ mg}\cdot 100^{-1}\text{ g ww}$). Badiu et al. [8] reported that cholesterol accounted 72% of total sterols in *Rapana venosa* from Romanian Black Sea coast. There was no comparable information for cholesterol content of rapa whelk from different parts of Black Sea. Based on presented result we can conclude that cooked rapana also contain high amounts of bioactive lipids with antioxidant properties as beta-carotene, astaxanthin, vitamin E, compared to raw samples.

4. Conclusions

Rapana venosa lipids may be regarded as a very interesting source of some biologically active compounds, which are well preserved even after culinary treatment. Presented cooking method is mild and

preferable for rapana treatment. The edible meat of this gastropod may be utilized for special dietary applications requiring high amounts of long chain n-3 PUFA as-EPA and DPA, high PL and vitamin D₃ contents, lower n6/n3 and PUFA/SFA ratios. The present study illustrated the high quality of rapana lipids in agreement with the recommendations of various health organizations. *Rapana venosa* lipids could be classified as appropriate for daily consumption being part of a healthy diet. In addition, the results for the lipid quality indices confirm the good anti-atherogenic, anti-thrombogenic, hypocholesterolaemic properties of this invasive species.

Further analysis is needed to confirm the sustainability of lipid quality of this species in annual cycle.

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Conflict of interest

Authors declare no conflict of interest.

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