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Original article

BLACK SEA RAPANA VENOSA – A PROMISING SOURCE OF ESSENTIAL LIPIDS

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ABSTRACT

Background: A diet rich in seafood has been linked to a variety of health benefits. While worldwide overfishing results in declining fish stocks, the growing demand for alternative sources of marine lipids has been expected. *Rapana venosa* (veined Rapa whelk) has become valuable seafood with nutritional and economic importance in the Black Sea region.

Purpose: The aim of the present study was to provide knowledge about biologically active lipids in Black Sea *Rapana venosa*, harvested in the region of Varna.

Material/Methods: Lipid classes were separated and purified by column and thin-layer chromatography. The saponifiable lipid fraction was derivatized into fatty acid methyl esters (FAMEs) and analysed by gas chromatography–mass spectrometry (GC-MS). Non-saponifiable lipids were identified by high pressure liquid chromatography coupled with UV/Vis and fluorescence detectors (HPLC-UV-FL).

Results: *Rapana venosa* was characterized by low lipid content (0.50 $g.100g^{-1}$ ww) with beneficial PUFA/SFA and n-6/n-3 ratios and high content of vitamin D_3 and astaxanthin. Lipids comprised mainly of polar lipids. Polyunsaturated fatty acids represented more than 50% of total fatty acids, most abundant being from the omega-3 series. Sum of EPA and DHA accounted at 40.8% of total fatty acids. Lipid quality indices indicated the good anti-atherogenic and atni-trombogenic properties (AI and TI < 1) of rapana meat.

Conclusions: The study revealed that *Rapana venosa* from the Black Sea is a good source of high quality marine lipids and presents a high potential for developing functional foods and/or dietary supplements with beneficial health effects.

Keywords: Rapana venosa, bioactive lipids, polyunsaturated fatty acids, vitamins, carotenoids,

INTRODUCTION

Seafood consumption has been linked to a variety of health benefits, which has led to intensive research over the past three decades. Seafood is à rich source of polyunsaturated fatty acids (PUFA), phospholipids, carotenoids, vitamins (vitamin D and B_{12}), various micronutrients and essential amino acids. Marine organisms could provide the necessary intake of very long-chain omega-3 (VLC n-3) PUFA, having a protective role on cardiovascular health. Moreover, omega-3 (n-3) PUFAs from seafood (fish, crustaceans, mollusks) are considered more effective than those of landcorp origin [1]. Marine oils, rich in fatty acids bound to phospholipids (shellfish, crustaceans, algae) have many advantages compared to fish oils since they are much more stable to oxidation. In addition, dietary phospholipids act as natural emulsifiers, which facilitate and improve the digestion of nutrients in the intestine [2].

Most of the researches in the literature discuss the composition of fish, crustaceans and cephalopods, yet information on the nutritive value of shellfish is generally scattered. Marine mollusks are the second major phylum of marine invertebrates. Veined Rapa whelk (*Rapana venosa*) is a marine origin gastropod that is recognized as one of the worst invader species worldwide [3]. Nowadays, veined Rapa whelk now has become valuable seafood with nutritional and economic importance in the Black Sea region. Over the past decade, there has been considerable fishing particularly in Bulgaria and Turkey as it is widely consumed in East Asian cuisine [4]. Studies have reported that *Rapana venosa* consumption improves the lipid profiles and antioxidant capacities in the serum of rats fed on atherogenic diet [5].

To our knowledge, there are limited studies on lipid composition, fatty acids composition, fat-soluble vitamins and carotenoids, lipid quality indexes of Rapana venosa from the Bulgarian Black Sea coast. The aim of the present study is to provide new information on the lipid content, lipid classes, fatty acid profiles, fat-soluble vitamins, carotenoids (beta-carotene, astaxanthin) and cholesterol content of Rapana venosa meat.

MATERIAL AND METHODS

Sampling

Live samples of *Rapana venosa* were purchased from a local enterprise for fish and seafood processing near Varna, Bulgaria in the spring of 2017. Animals were transported to the laboratory in wet tissue towels in an ice box. They were washed and processed immediately.

Lipid extraction, separation and purification

Total lipids were extracted by the method of Bligh and Dyer (1959) [6]. They were subsequently separated into neutral lipids (NL) and phospholipids (PL) by column chromatography 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 – with methanol. The amounts of total lipids and lipid classes were determined gravimetrically. The purity of each fraction was tested by thin-layer chromatography, using Silica gel F254 plates (thickness = 0.25 mm; Merck, Darmstadt, Germany).

Fatty acid derivatization and analysis

Lipid fractions were methylated using 2% H_2SO_4 in anhydrous methanol and n-hexane [7]. Fatty acid compositions of TL, NL and PL were determined by gas chromatography with masspectrometry (GC/MS) of the corresponding fatty acid methyl esters (FAME). Chromatographic separation was performed by Thermo Scientific FOCUS Gas Chromatograph on a TR-5 MS capillary column (30 m, 0.25 mm i.d.). For identification and quantification of FAME peaks, authentic standards (SUPELCO FAME Mix C4-C24) were used.

Fat-soluble vitamins and carotenoids analysis

Retinol, cholecalciferol, cholesterol, astaxanthin and β-carotene were extracted from tissue by alkaline hydrolysis and simultaneously analyzed by high performance liquid chromatography as previously described [8].

Nutrition quality indices (NQI)

Several indices and ratios were employed to estimate the quality of Rapa whelk lipids: omega-6/omega-3 (n-6/n-3) and polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA) ratios, indices of atherogenicity (AI) and thrombogenicity (TI), and cholesterolemic index (h/H).

Statistical analysis

Student's t-test was employed to estimate the significance of values. Statistical significance was indicated at $p<0.05$.

RESULTS AND DISCUSSION

Lipids and fatty acid composition

Spring samples of *Rapana venosa* (April 2017) showed low lipid content: 0.50 $g.100g^{-1}$ ww. Polar lipids (phospholipids, PL) predominated, accounting 63 %, while neutral lipid fraction was 30 % of total lipids. The results for the fatty acid composition of total lipids and lipid classes as well as nutrition quality indices are listed in Table 1.

Table 1. Fatty acid composition of lipid classes and nutrition quality indices of *Rapana venosa* from the Black Sea coast

	Total lipids		Neutral lipids		Phospholipids	
Fatty acid groups	% of TFA	$mg.100g^{-1}$ EP	% of TFA	$mg.100g^{-1}$ EP	% of TFA	$mg.100g^{-1}$ EP
SFA	29.3	122.0	32.2	48.8	31.1	98.8
MUFA	11.6	48.3	11.3	17.1	9.2	29.2
PUFA	59.2	246.8	56.5	85.7	59.7	190.1
n-6	10.2	42.5	5.8	8.8	11.0	34.9
n-3	49.0	204.3	50.7	76.9	48.8	155.2
DHA + EPA	***40.8***	***170.1***	***32.8***	***49.7***	***37.37***	***118.9***
Ratios and indexes						
PUFA/SFA	***2.02***		***1.76***		***1.92***	
n-6/n-3	***0.21***		***0.12***		***0.23***	
AI	***0.37***		***0.53***		***0.37***	
TI	***0.16***		***0.18***		***0.18***	
h/H	***2.73***		***1.94***		***2.25***	

**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);*
TFA – total fatty acids; EP – edible portion

The values for FA in the present study are reported as a percentage of total fatty acids and as $mg.100g^{-1}$ edible portion due to discrepancies in expressing the values only in percentage since the latter could be inaccurate for estimating the nutritive content. The FA profile of total lipids and lipid fractions presented similar distribution: PUFA>SFA>MUFA. It is well known that animals can synthesize SFA and MUFA *de novo*. In addition, the World Health Organization (WHO) recommended the replacement of high SFA intake with PUFA or MUFAs, preferable from seafood origin [9]. Thus, the information for alternative sources of unsaturated FAs, especially phospholipids PUFAs are very important for consumers and pharmacists. Although FAs composition of marine mollusks depends on the environmental factors, such as temperature, salinity, pollution and diet, most of the studies reported same pattern (PUFA>SFA>MUFA) for *Rapana venosa* lipids from the Black Sea [10-11].

Rapana venosa meat contains only 0.122 g SFA per 100 g edible portion, thus can be classified as low-saturated fat food (containing less than 1.5 g per 100 g) [12]. PUFAs accounted for more than 50 % of fatty acids in all lipid fractions. One hundred grams of rapana meat contained 246.8 mg of PUFA, two-thirds of them in the form of polar lipids. It is important, since phospholipids act as natural emulsifiers, easing digestion and absorption of nutrients in the gastrointestinal tract. *Rapana venosa* lipids are rich of very long-chain PUFA – eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in particular. These PUFAs can reduce the platelet adhesion and aggregations, have blood pressure reducing properties and thus influencing positively cardiovascular diseases (CVD). DHA plays structural and functional roles in brain and retina tissues. Therefore DHA consumptions is important to ensure optimum neural and visual functions [13]. EPA and DHA represented 40 % of TFA or almost 70 % of PUFA in TL. From this point of view, *Rapana venosa* is a very good source of these two fatty acids, as more than 70% of these FAs have a phospholipidic origin, which significantly increases its bioavailability. Sum of EPA and DHA found in this study was 170.1 mg per 100 g EP. In the past decades, the Black Sea sprat species (*Sprattus sprattus L.*) and freshwater rainbow trout (*Oncorhynchus mykiss W.*) are the most consumed fish in our country. According to previous studies, 100 g EP of Black Sea sprat delivers between 620 mg and 780 mg EPA and DHA [14], while 100 g EP rainbow trout provides 660-790 mg EPA+DHA [15]. Although fish is considered the main source of EPA and DHA, rapana meal consumption could contribute to enhanced intake of these biologically active fatty acids, nevertheless maintaining low-saturated fat levels (only 29.3% of TFA). Moreover, 75% of omega-3 fatty acids in rapana tissues are bonded to phospholipids, which facilitates and increases their absorption and bioavailability. For that reason, the inclusion of Black Sea molluscs in the diet may be beneficial to resident population, increasing the intake of essential omega-3 fatty acids [16].

In the past decades, a higher intake of SFA and n-6 PUFAs is a typical dietary pattern in European countries, which results in a high and unsafety n-6/n-3 FA ratios. The WHO recommends that the n-6/n-3 ratio should not exceed 10 in a diet and its decrease in the human diet is essential to help prevent coronary heart disease by reducing the plasma lipids. Moreover, in all lipid classes, n-3 PUFAs remained the dominant one, especially in PL ($155.2mg\ 100g^{-1}$ EP) and low and beneficial n-6/n-3 ratios (below 0.23). PUFA/SFA ratio is another indicator of nutrition quality assessment, supposed as a measure of the tendency of the diet to affect the incidence of CVD [13]. In this study, the PUFA/SFA ratio was found lower than 4.0 (Table 1) in all lipid fractions, which is within the recommendations of the Department of Health (1994) [17]. Calculated lipid quality indices are used to measure of the ability of the Rapana lipids to reduce blood lipids (AI), platelet activity (TI) and functional effect of long chain PUFAs on cholesterol metabolism (h/H). In this study, low AI and TI, and high h/H levels were found in both PL and NL fractions (Table 1), which can classify Rapana edible tissue as beneficial for human consumptions. In addition, *Rapana venosa* lipid fractions analyzed in the present study showed indices values more favorable compared to those reported by Prato *et al* [13] for commercial scallop species from the Ionian Sea.

Fat-soluble vitamins and carotenoids

The results obtained for vitamins A, D_3, carotenoids – beta-carotene and astaxanthin, and cholesterol are presented in Table 2.

Table 2. Fat soluble vitamins and carotenoids content of *Rapana venosa* from the Black Sea coast

	$\mu g.100g^{-1}$ EP
Vitamin A	16.4 ± 0.1
Vitamin D_3	18.3 ± 0.8
Astaxanthin	20.73 ± 0.01
Beta-carotene	10.3 ± 0.1
Cholesterol ($mg.100g^{-1}$ EP)	19.8 ± 0.9

Carotenoids are important metabolites, essential for the normal growth, metabolism and reproductive cycle of mollusks. They exhibit high antioxidant activities. *Rapana venosa* species is able to synthesize and accumulate astaxanthin from beta-carotene by oxidative metabolic pathway [18]. In a previous study [19], autumn samples of *Rapana venosa* presented significantly higher amounts of carotenoids. Vitamin D_3 content in this study accounted for 18.3 μg per 100 g of rapana meat, which supplies more than 100% of the recommended daily intake [20]. Hence, *Rapana venosa* from the Black Sea can be regarded as a good source of this vitamin, while presenting low cholesterol content (19.8 mg per 100 g).

CONCLUSION

The present study reveals that *Rapana venosa* from the Black Sea is characterized by high amounts of marine bioactive lipids – long-chain PUFA (EPA and DHA), carotenoids with antioxidant properties (astaxanthin) and vitamin D_3. This Black Sea gastropod contained very low amounts of SFA, but high omega-3 PUFAs and could assist to implement dietary recommendations for replacement of high SFA intake with PUFA or MUFAs, if possible with seafood origin.

Although oily fish is considered the main source of omega-3 PUFA and vitamin D_3, rapana meal consumption could contribute to the enhanced intake of these nutrients, whilst maintaining low-saturated fat and cholesterol levels. Moreover, the inclusion of Black Sea molluscs in the diet may be beneficial to the resident population, increasing the intake of essential omega-3 fatty acids.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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