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Seasonal Changes in the Quality and Fatty Acid Composition of Meat in Rapa Whelk (*Rapana venosa*) from the Bulgarian Black Sea Coast

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Abstract

The study was conducted to evaluate the seasonal variations in the quality and lipid profile of rapa whelk meat (*Rapana venosa*) harvested in the Bulgarian coast of Black Sea. The trial period lasted from June to October and the sampling was carried out in the area of Varna Bay. Technological quality of the whelks was determined by measuring the water holding capacity (WHC), as well as cooking losses. Further determination of the chemical composition, fatty acid analysis and total aerobic plate count were done. Strong seasonal influence on the meat quality characteristics in rapa whelk was observed in the study. The live weight of the whelks was the highest in October ($P<0.001$). Consequently, the content of meat and the other body parts (gut and operculum content) were highest in the early autumn as well. The increased content of meat was accompanied by lower WHC ($P<0.001$). The chemical composition of the rapa whelks differed significantly between the months of fishing. Both moisture and lipid had highest content in July, while proteins and ash increased in October. Similarly, lipid profile was significantly affected by the season. Most favourable fatty acid composition and related nutritional indices of lipid healthy value were found in October.

Key words: Chemical composition, lipid profile, meat, rapa whelk, seasons

Introduction

The marine invertebrates highly appreciated and mainly consumed are crustaceans, bivalves and to a lesser extent gastropods (Mohammad and Yusuf, 2016). In many countries edible bivalves and gastropods represent non-traditional and relatively cheap protein supply, and hence they might be considered as a promising food source. The veined Rapa whelk (*Rapana venosa*) is a marine snail native to marine and estuarine waters in the Western Pacific from the Sea of Japan, Yellow Sea, East China Sea and the Bohai Sea (Richerson and Benson, 2015). It was found in the Novorosiisk Bay in 1946 (Sahin et al., 2009) and like the most exotic species it is supposed to be carried in ballast

waters and introduced (Belicelik, 1975). The species has fast growth rate and tolerance to low salinity, high and low temperatures, water pollution and oxygen deficiency. Known to be highly voracious predator, rapa whelk have exerted dramatic impact on both natural and cultivated populations of mussels and other molluscs, and significant negative changes in the ecosystem. However, as reported by Saglam and Duzgunes (2016), there are positive effects of the rapa whelk in socio-economical life of the fishermen communities. According to Merdzhanova et al. (2014), since the 1980s rapa whelk has become a valuable commercial resource, since its meat is exported to Japan, South Korea and China and also

included in the diet of those native to the Black Sea area. In 2015, the rapa whelk production in the Bulgarian coast of Black Sea reached 4092 tones (Agricultural report 2016). Despite this fact, however, rapa whelk is underexploited source of nutrients in Bulgaria and so far the information available from the literature on its nutritional characteristics and their seasonal variations remain scarce. Therefore, the aim of this study was to assess the seasonal changes in the quality, chemical composition and fatty acid profile in rapa whelk meat (*Rapana venosa*) harvested in the Bulgarian coast of Black Sea.

Materials and Methods

Collection of rapa whelk and sample preparation

The study was carried out from late spring to early autumn (June to October) 2016 when fishing of the rapa whelks from the wild population has its peak. Three batches weighing 6 kg each were collected in the area of Varna Bay at 1 mile distance from the shore (Figure 1) by divers at 10–15 m depth. The samples were immediately transported to the laboratory of the Department of Biology and Aquaculture in Trakia University under refrigeration ($3 \pm 1^\circ\text{C}$), where they were brushed, washed and processed on the same day. A total of 6 pooled samples containing 20 whelks were prepared from each batch for the necessary analyses.

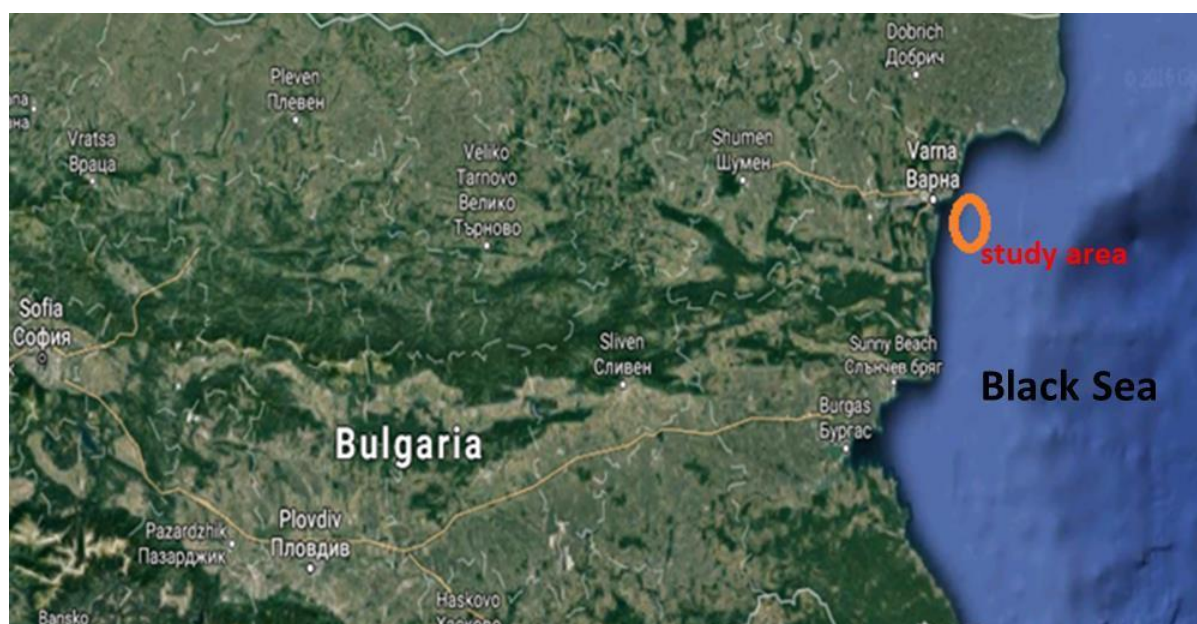


Figure 1. Study area

Weight and dressing percentage determination

Live weight was determined by digital scale. Further, the tissue was removed from the shell, and the operculum and guts were removed and weighed. The weight of meat was also recorded. The meat yield was determined as follows:

$$\text{Meat yield} = [\text{Meat content (g)} / \text{Live weight (g)}] \times 100$$

Water holding capacity (WHC) and cooking losses

WHC was determined according to the method of Grau and Hamm (1952). The cooking methods applied were boiling and roasting. Weight losses due to boiling were measured after treating of the samples in plastic bags in boiling water for 15 min until the temperature in the sample reached 75°C . The losses due to roasting were determined while roasting the samples in

conventional oven at 150°C for 10 min until the temperature in the sample reached 75°C .

Chemical composition of meat and fatty acid analysis

The samples were minced and subjected to analysis of the chemical composition including determination of lipid, protein, moisture and ashes content (AOAC 2004).

The analysis of lipid composition was performed in the Laboratory of Lipid analysis in the Institute of Animal Science – Kostinbrod. Total lipids were extracted according to the method of Bligh and Dyer (1959). Methyl esters of the total lipids, isolated by preparative thin layer chromatography were obtained using 0.01% solution of sulfuric acid in dry methanol for 14 h as described by (Christie, 1973). The fatty acid composition of total lipids was determined by gas -

liquid chromatography (GLC) analysis using a chromatograph C Si 200 equipped with a capillary column (DM-2330:30 m x 0.25 mm x 0.20 µm) and hydrogen as a carrier gas. The oven temperature was first set to 160 °C for 0.2 min, then raised until 220 °C at a rate of 5°C/min. The temperatures of the detector and injector were 230 °C. Methyl esters were identified through a comparison to the retention times of the standards. Fatty acids are presented as percentages of the total amount of the methyl esters (FAME) identified (Christie, 1973). The amount of each fatty acid was used to calculate the indices of atherogenicity (AI) and thrombogenicity (TI) as proposed by Ulbricht and Southgate (1991):

$$AI = (4 \times C14:0 + C16:0) / [MUFA + \Sigma(n-6) + \Sigma(n-3)]$$

$$TI = (C14:0 + C16:0 + C18:0) / [0.5 \times MUFA + 0.5 \times (n-6) + 3 \times (n-3) + (n-3) / (n-6)]$$

The h/ H ratio was calculated as suggested by Santos-Silva et al. (2002): $(C18:1 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0)$.

Determination of total aerobic plate count

Total aerobic plate count was determined as described by De Witte et al. (2014). Ten grammes of rapa whelk meat from each batch were collected aseptically and weighed in a Stomacher® 400 Circulator bag. Ninety ml sample diluent were added, and samples were homogenised at 256^{-rpm} for 1 min. Tenfold

dilutions were prepared in tubes with 9 ml sample diluent. Thereafter, 0.1 ml of each dilution was inoculated on two plates with Plate Count Agar. Plates were incubated at 20 °C for 72 hours. The colony counts were evaluated on a counter. All dilutions and inoculations were done in a microbiology box. The results are presented as log cfu/g.

Statistical analysis

Data were analysed by one-way ANOVA to determine the effect of the season on the various examined traits and fatty acid composition. Further, when appropriate, Tukey post-hoc test was performed to compare means. Statistical analysis was performed using JMP v. 7 software package.

Result and Discussion

Meat quality characteristics

The weight and meat content of the rapa whelks varied between 56.44-110.02 g and 11.98-23.27 g, respectively, and followed similar pattern of changes during the months of the study. Both parameters had lowest values in June and increased constantly until October where they showed their maximum. Similarly, both operculum and guts exhibited significant increase during the study ($P < 0.05$), however, the changes of the meat yield did not show the same tendency as all of the above mentioned traits. In June, meat yield of the rapa whelk was the lowest and increased in July after which slowly decreased in October (Table 1).

Table 1. Live weight, meat content and meat yield (mean ± SEM)

Item	June	July	October	Sig.
Live weight (g)	56.44±9.96 ^a	76.08±6.34 ^a	110.02±9.66 ^b	***
Meat content (g)	11.98±2.34 ^a	19.72±2.22 ^b	23.27±1.75 ^b	**
Operculum (g)	0.64±0.14 ^a	0.69±0.06 ^{ab}	0.99±0.10 ^b	*
Gut (g)	7.12±1.03 ^a	9.25±0.77 ^a	19.20±4.71 ^b	*
Meat yield (%)	20.75±0.94 ^a	25.52±1.44 ^b	20.08±0.42 ^{ab}	*

Significance of the factor season * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; Means connected by different superscripts are significantly different ($P < 0.05$)

Table 2. Water holding capacity, cooking loss and roasting loss (mean ± SEM)

Item	June	July	October	Sig.
WHC (%)	6.74±0.71 ^a	12.92±0.86 ^b	22.08±0.42 ^c	***
Cooking loss (%)	21.43±0.45	21.83±0.53	21.43±0.38	NS
Roasting loss (%)	26.29±0.62 ^b	21.34±0.72 ^a	21.30±0.39 ^a	***

Significance of the factor season *** $P < 0.001$, NS- non significant; Means connected by different superscripts are significantly different ($P < 0.05$)

The water holding capacity showed differences between the months of fishing ($P < 0.001$) and was the highest in June. In July, however, it decreased twice and showed its

minimum in October. The loss measured after roasting was highest in June and decreased until the end of the study period while surprisingly cooking loss showed no significant differences due to the month of fishing (Table 2). Water-holding

capacity is an important quality parameter closely associated to the sensory properties of meat such as juiciness, tenderness and the overall palatability. It also determines the appropriate technological approach for further processing of meat. It is often considered that losses at cooking are good indicator of WHC, as decreased cooking loss means high WHC (Skipnes et al., 2007; Zhao et al., 2015; Bowker and Zhuang, 2015). Although good relationship between WHC and losses after cooking and roasting were observed in our study on black mussel, such we failed to observe in the rapa whelk, as presented above. Moreover, the decrease of the WHC detected in the period June–October was accompanied by decrease in the losses due to roasting, and no changes in the cooking losses.

All the meat chemical components showed seasonal fluctuations as most affected were moisture, protein and ash content ($P < 0.001$) (Table 3). Moisture varied within the range from 70.89% to 76.24%. It showed the highest value in July, and decreased in October. Protein followed the opposite pattern of changes, with the lowest value detected in July. Contrary to the protein, lipid

percentage was the lowest in June, increased in July and decreased again in October. Ash content showed constant increase from the beginning to the end of the study. The trends in the seasonal changes in the moisture and protein that we observed in this study coincided with those of (Celik et al., 2014). However, they showed only slight decrease in lipids throughout the study period until autumn. The minimal protein content in July suggests strong reproductive activity during the summer time. This is in agreement with Bi et al. (2016) who observed peak of spawning in July in *Rapana venosa* from the Northern coast of China. The results of the chemical composition of the rapa whelk meat from the Black Sea coast of Bulgaria in this study indicate that it has good nutritional value with high protein and low lipid content and can be included as a component of a healthy human diet. The protein content of the rapa whelk in the study varied from 18.62 to 24.09% of fresh tissue. Similarly, high protein in predatory gastropods has been reported by Zarai et al. (2011) in *Hexaplex trunculus*-47.79% and Periyasamy et al. (2011), in *Babylonia spirata*-53.86%.

Table 3. Chemical composition (mean \pm SEM)

Item	June	July	October	Sig.
Moisture (%)	73.71 \pm 0.20 ^b	76.24 \pm 0.63 ^c	70.89 \pm 0.52 ^a	***
Protein (%)	21.64 \pm 0.26 ^b	18.62 \pm 0.85 ^a	24.09 \pm 0.60 ^c	***
Lipids (%)	0.58 \pm 0.04 ^a	0.85 \pm 0.12 ^b	0.59 \pm 0.03 ^a	*
Ashes (%)	2.03 \pm 0.06 ^a	2.24 \pm 0.08 ^a	2.70 \pm 0.14 ^b	***

Significance of the factor season: * $P < 0.05$, *** $P < 0.001$; Means connected by different superscripts are significantly different ($P < 0.05$)

Rapa whelk is characterised by low lipid content. In this study, it was 0.58-0.85% and is in line with the previously reported by Merdzhanova et al. (2014). According to Celik et al. (2014), *Rapana venosa* appears to have the best nutritional characteristics when compared to other molluscs (*Ostrea edulis*, *Mytilus galloprovincialis*, *Ruditapes decussatus*, *Ruditapes philippinarum*)

Fatty acid composition

Fatty acid composition is presented in Table 4. The predominant saturated fatty acid in *Rapana venosa* is C16:0, followed by C18:0. The content of C16:0 was affected by the month of fishing ($P < 0.01$) showing significant increase between June and July while the percentage of C18:0 remained unchanged. Similar to C16:0, C14:0 showed significant increase between June and July and decreased in October.

The season of fishing had significant impact on the content of C18:1 Δ 9 ($P < 0.001$). It was the highest in June and dramatically decreased in July

and October.

With the exception of C22:5n-3, all the polyunsaturated fatty acids underwent seasonal fluctuations, however, in different directions. Both C18:2n-6 and C18:3n-3 showed decreased content from June to July. In October, they reached their maximum. C20:4n-6 followed the same pattern of changes as C18:2n-6 and C18:3n-3. C20:5n-3 and C22:6n-3 showed opposite changes. The former decreased from June until the autumn season while C22:6n-3 increased and had exhibited the highest content in October.

The saturated fatty acids (SFA) accounted for 39.8% of the total fatty acids. Their changes were determined by C14:0 and C16:0 displaying the highest content in July. This corresponded to the significantly increased lipid content in the rapa whelks during this month. The increase of the content of these fatty acids in the period June–July suggests high energetic stress of the whelk, since it is known that certain fatty acids among which C14:0 and C16:0 have energetic type functions

(Freites et al. 2002). Polyunsaturated fatty acids (PUFA) were the predominant fatty acids in the meat of whelks. Their content varied between 50.23 and 58.48% while monounsaturated fatty acids (MUFA) showed the lowest percentage among the three fatty acid classes. Their amount was between 4.28 and 6.14%. The considerable fluctuations of the individual fatty acids determined those of the total content of MUFA and PUFA as the former showed marked decrease between June and July. On the other hand, PUFA showed the highest content in October. The seasonal change in the contents of SFA and PUFA observed in our study are in line with those reported by Liu et al. (2013) in *F. mutica* and Surh et al. (2003) in various types of shellfish in Korea. In addition, the latter reported that MUFA

represents the minimal content of the total FA, which is also in agreement with our results. A large part of the changes in the polyunsaturated fatty acid composition of whelk lipids can be explained by a direct transformation of algae fatty acids into neutral and polar lipids. The decrease in PUFA levels of *Rapana venosa* during June-July likely reflected reduced dietary intake of PUFA due to substantially low phytoplankton biomass in June-July. This could be confirmed by the lower contents of C18:2n-6 and C18:3n-3, which are abundant in the phytoplankton. On the other hand, the further increase in the content of these fatty acids and the long chain PUFA, especially C20:4n-6 and C22:6n-3, suggests higher elongating and desaturating activity in *Rapana venosa* in the early autumn.

Table 4. Fatty acid composition (mean \pm SEM)

Fatty acids (%)	June	July	October	Sig.
C14:0	5.16 \pm 0.62 ^a	8.72 \pm 1.07 ^b	4.21 \pm 0.25 ^a	**
C16:0	17.58 \pm 0.54 ^a	23.23 \pm 1.54 ^b	19.84 \pm 0.93 ^{ab}	**
C16:1	2.08 \pm 0.31	2.63 \pm 0.40	3.46 \pm 0.50	NS
C18:0	15.30 \pm 1.62	13.22 \pm 0.96	12.28 \pm 0.63	NS
C18:1 Δ 9	3.55 \pm 0.44 ^b	1.10 \pm 0.24 ^a	1.74 \pm 0.11 ^a	***
C18:1 Δ 7	0.52 \pm 0.06	0.55 \pm 0.19	ND	***
C18:2n-6	3.18 \pm 0.13 ^{ab}	2.31 \pm 0.28 ^a	3.90 \pm 0.35 ^b	**
C18:3n-3	0.81 \pm 0.04 ^{ab}	0.68 \pm 0.06 ^a	1.00 \pm 0.12 ^b	*
C20:4n-6	15.66 \pm 0.91 ^{ab}	12.59 \pm 1.61 ^a	17.08 \pm 0.66 ^b	*
C20:5n-3	11.74 \pm 0.85 ^{ab}	10.45 \pm 0.24 ^b	8.72 \pm 0.30 ^a	*
C22:5n-3	14.01 \pm 0.69	13.73 \pm 0.41	14.30 \pm 0.72	NS
C22:6n-3	10.39 \pm 0.36 ^a	10.47 \pm 0.48 ^a	13.45 \pm 0.75 ^b	**
SFA	38.04 \pm 1.71 ^{ab}	45.17 \pm 2.82 ^b	36.33 \pm 1.44 ^a	*
MUFA	6.14 \pm 0.58 ^b	4.28 \pm 0.53 ^a	5.21 \pm 0.44 ^{ab}	*
PUFA	55.80 \pm 2.11 ^{ab}	50.23 \pm 3.11 ^a	58.48 \pm 1.22 ^b	*

ND- not detected; Significance of factor season *P<0.05, **P<0.01, ***P<0.001, NS- non significant; Means connected by different superscripts are significantly different (P<0.05)

Table 5. Lipid indices (mean \pm SEM)

Index	June	July	October	Sig.
P/S	1.49 \pm 0.12 ^{ab}	1.16 \pm 0.14 ^a	1.63 \pm 0.09 ^b	*
n-6/n-3	0.50 \pm 0.01 ^{ab}	0.42 \pm 0.05 ^a	0.56 \pm 0.04 ^b	*
AI	0.62 \pm 0.01 ^a	1.10 \pm 0.15 ^b	0.58 \pm 0.09 ^a	**
TI	0.30 \pm 0.02 ^{ab}	0.39 \pm 0.04 ^b	0.29 \pm 0.02 ^a	*
h/H	2.51 \pm 0.17 ^b	1.68 \pm 0.24 ^a	2.46 \pm 0.16 ^b	**

Significance factor season **P<0.01, ***P<0.001; Means connected by different superscripts are significantly different (P<0.05)

The ratio P/S changed significantly (P<0.05) over time and corresponded to the seasonal tendencies in SFA content. Its values decreased slightly in summer and increased in October but remaining above the recommended minimal value of 0.4 throughout the period of the study. The same trend was observed in regard to the n-6/n-3 ratio. Though during the study period its values remained below 4, they were most favourable in

July (Table 5).

In an attempt to take into account the different effects of the various fatty acids, Ulbricht and Southgate (1991) proposed two indices which might better characterize the atherogenic and thrombogenic potential of the diet than the simple approaches such as the P/S ratio. Atherogenic index (AI) was significantly changes in the three months of fishing and its highest value was

observed in July corresponding to both to the highest lipid and SA content. During the period of the study AI exceeded the recommended values of 0.5. The same trend in the dynamics was detected in thrombogenic index (TI). Opposite changes were observed in the ratio h/H, and its values were most favourable in July. Low values of AI and TI are beneficial to health since there is evidence that the type of fat is more important than the total amount of fat in the quantification of cardiovascular diseases risk (Romero et al. 2013). Moreover, the higher the ratio between hypocholesterolemic and hypercholesterolemic fatty acids, the more adequate that oil or fat is for the nutrition (Santos-Silva et al. 2002).

Total aerobic plate count

According to Altug and Guler (2002) ensuring of safe rapa whelk meat is essential to human health. They indicated rapa whelk (*Rapana venosa*) as indicator organism for bacterial pollution. As presented in Figure 2, we established equal microbial load in June (5.63 log₁₀ cfu/g) and July (5.62 log₁₀ cfu/g) but the microbial density was higher in October (5.85 log₁₀ cfu/g). The data on microbiological analysis of rapa whelk (*Rapana venosa*) are scarce. Altug and Guler (2002) reported the presence of faecal coliforms, *E. coli* and *Salmonella* spp. in rapa whelk (*Rapana venosa*).

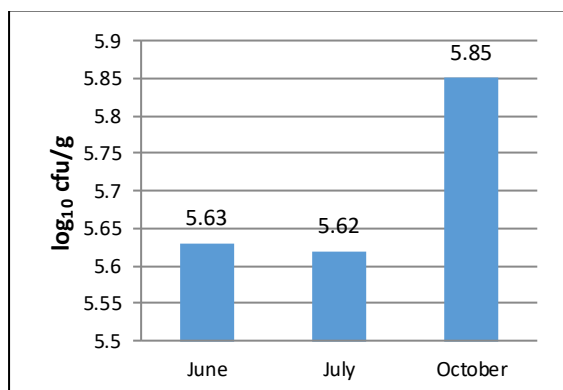


Figure 2. Total aerobic plate counts in rapa whelk meat

Conclusion

To conclude, the results of the present study show strong difference between the seasons of the peak harvest on the meat quality characteristics in rapa whelk (*Rapana venosa*). The weight of the whelks, meat and the other body parts content (gut and operculum) were the highest in October while meat yield increased in July. The increased meat content was accompanied by lower WHC. The chemical composition of the

rapa whelks showed significant differences between the months of fishing. Both moisture and lipid had highest content in July, whereas proteins and ash increased in early autumn. In addition to the chemical composition, lipid profile was significantly affected by the season. Most favourable fatty acid composition and related nutritional indices were found in October.

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