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Relative changes of retinol, α-tocopherol, β-carotene and astaxanthin in cooked edible mollusks from the Black Sea

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Molluscan species are a major component of global seafood production. The Mediterranean mussel (*Mytilus galloprovincialis*) and veined rapa whelk (*Rapana venosa*) are marine mollusks with high socio-economic value in the Black Sea region. Seafood is not only a good source of digestible proteins, furthermore delivering a healthy portion of vitamins and carotenoids. However, domestic cooking (high temperatures, exposure to air, etc.) inevitably alters the amounts of these bio-antioxidants. The aim of the present study was to explore changes in retinol, α-tocopherol, β-carotene and astaxanthin occurring in black mussel and veined rapa whelk after culinary treatment. The fat-soluble components were analyzed simultaneously using high-performance liquid chromatography with ultraviolet and fluorescence detectors (HPLC-UV/FL). *Rapana venosa* contained higher amounts of retinol, α-tocopherol, β-carotene and astaxanthin compared to black mussel. The concentrations of all analytes decreased significantly following temperature treatment with the exception of astaxanthin in *Mytilus galloprovincialis* – 55.7 $\mu g.g^{-1}$ lipid in raw to 42.1 $\mu g.g^{-1}$ lipid in cooked tissue. Vitamin A accounted 80.7 $\mu g.g^{-1}$ lipids in raw *Mytilus galloprovincialis* and 13.8 $\mu g.g^{-1}$ lipids in raw *Rapana venosa.* After cooking it decreased to 17.2 $\mu g.g^{-1}$ lipids in *Mytilus galloprovincialis* and was not detected in cooked *Rapana venosa*. Beta-carotene decreased three-folds in *Mytilus galloprovincialis* and two-folds in *Rapana venosa.* Although susceptibility of vitamins and carotenoids to heat and oxygen, seafood remains a significant source of natural antioxidants, even after culinary treatment. Further studies are needed to investigate the changes of the mentioned analytes in marine mollusks during various cooking processes and prolonged storage.

Keywords: vitamins, carotenoids, shellfish, Black Sea, cooking

INTRODUCTION

One of the most valuable marine derived antioxidants are carotenoids, especially β-carotene and astaxanthin. Their antioxidant properties directly emerge from their molecular structure. Beta-carotene has important roles in the human organism mainly as an antioxidant in different tissue as skin and others [1]. Being provitamin A, its bioavailability and conversion depend strongly on type of food sources. Moreover, β-carotene presented the highest provitamin A activity compared to other carotenoids. Astaxanthin has a beneficial effect on the human health due to its high antioxidant activity - 10 times higher than β-carotene and 100 times higher than vitamin A [2]. Other interesting lipid soluble antioxidants presented in marine organisms are vitamin E and vitamin A [3]. It is known, that marine lipids originated from shellfish contain appreciable quantities of described above biologically active compounds.

Bearing in mind that seafood could cause food-borne illnesses, cooking is a good method to improve food safety and decrease the risk for infection from contaminated products. On the other hand, food processing (high temperatures, exposure to air, etc.) inevitably alters the amounts of fat-soluble bio-antioxidants, such as vitamins and carotenoids [4-7]. Thermal stress produce significant oxidative degradation of β-carotene. Some researchers supposed that β-carotene has pro-oxidant properties and presents lower stability than astaxanthin during thermal treatments [8].

The Mediterranean black mussel (*M. galloprovincialis*) and veined rapa whelk (*Rapana venosa*) are traditionally consumed by the population from the Black Sea region. These mollusks species have important socio-economic effect for the Black Sea countries and comprise a valuable source of nutrients with bio-antioxidant properties in the human diet. Information about the changes in the nutritional quality based on fat-soluble vitamins and carotenoids of bivalves from the Bulgarian Black Sea waters is very limited. The aim of the present study was to explore changes in retinol, α-tocopherol, β-carotene and astaxanthin occurring in black mussel and veined rapa whelk after domestic culinary treatment.

MATERIAL AND METHODS

Sample preparation

Live mollusk samples were purchased in the autumn of 2017 from a mussel farm (*Mytilus galloprovincialis*) and local fishermen (*Rapana venosa*) near Kavarna village, Bulgaria. Live specimens were transported to the laboratory in ice boxes and immediately processed. One hundred animals were randomly chosen, washed and brushed

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and their shell lengths were measured. Each species was divided into two groups. Average length of mussels shell was 45.5 ± 1.5 mm. Half of the mussels (n=50) were shucked and the whole tissue was taken for immediate analysis. The other half (n=50) was processed by steaming (90 ± 5 ºC) for 6 min.

Mean shell length of *Rapana venosa* was 62.7 ± 9.0 mm. Samples of *Rapana venosa* were divided into two groups: raw (n =5 0) and cooked (n = 50). Cooking method used was boiling. Mollusks were placed in an autoclave at 90 ºC and boiled for 20 min.

Extraction and HPLC analysis

Astaxanthin β-carotene, all-trans-retinol and alpha-tocopherol were extracted by alkaline saponification, following the method of Dobreva *et al.* [9]. Briefly, one gram of mollusc tissue (±0.005 g) was weighed and homogenized with 5 mL 0.5M methanolic potassium hydroxide. The saponification was performed in a water bath at 50ºC for 30 min. L-ascorbic acid in methanol was used as an antioxidant. Analytes of interest were extracted twice with a mixture of n-hexane: dichloromethane = 2:1 (v/v) and simultaneously analyzed by high performance liquid chromatography. Separation was achieved on a reversed-phase analytical column (Synergi 4µ Hydro-RP 80A pore 250x4.6 mm). Flow rate was 1.1 mL/min in a gradient consisting of: Solvent A – methanol:water (93:7); solvent B – acetonitrile; and solvent C – 2-propanol. All solvents were of HPLC grade purity purchased from Sigma-Aldrich Chemie GmbH. Astaxanthin (λ = 474 nm) and β-carotene (λ= 450 nm) were monitored by ultraviolet detection, while concentrations all-trans-retinol (at λex = 334 nm and λem = 460 nm) and α-tocopherol (at λex = 288 nm and λem = 332 nm) were measured by fluorescence detection. The identification and quantification were achieved by the external calibration method. Analytical standards of all-trans-retinol, α-tocopherol and β-carotene were purchased from Sigma-Aldrich Chemie GmbH and astaxanthin – from Dr. Ehrenstorfer™.

Statistical analysis

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

RESULTS AND DISCUSSION

Fat-soluble vitamins A and E presented different amounts in analyzed raw species. Retinol was observed in significantly higher content in mussel tissues (80.7 ± 3.4 µg.g^{-1} lipid), whereas in rapana was found only 13.8 ± 1.4 µg.g^{-1} lipid. Being a metabolite of β-carotene, retinol levels are strongly related to species-specific conversion of this provitamin A. Black mussel contained two times lower β-carotene (147.4 ± 3.4 µg.g^{-1} lipid) and we can suppose that during sampling period this species had more active retinol metabolism compared to *Rapana venosa* (319.7 ± 10.5 µg.g^{-1} lipid). Another interesting carotenoid with higher antioxidant activity than β-carotene found in both species is astaxanthin. According to Venugopal *et al.* [10] astaxanthin is among the major carotenoids in shellfish, which can protect mollusk body tissue from oxidative damage of UV-radiation. Borodina *et al.* [11] reported that rapa whelk can synthesize and accumulate astaxanthin from β-carotene via metabolic pathway. Analyzed black mussel (55.9 ± 3.0 µg.g^{-1} lipid) and rapana (50.9 ± 3.0 µg.g^{-1} lipid) presented similar levels of astaxanthin. Observed higher levels of β-carotene and lower for astaxanthin may be result from slower carotenoid metabolism in rapana. Analyzed black mussel presented significantly lower contents of β-carotene and similar levels of astaxanthin compared to rapana.

Phytoplankton is the major source of carotenoids such as β-carotene. According to Shulman and Soldatov [12] the domination of diatoms in the Black Sea ecosystem leads to an increase in β-carotene, whereas astaxanthin is species-specific for phytoplankton species (e.g. dinoflagellates). The Black Sea mussel is filter-feeding molluscan and can accumulate carotenoids directly via dietary microalgae, whereas rapa whelk is a carnivores species feeding mainly on mussels and clams. This could be one possible reason for the observed higher β-carotene content in rapa whelk tissue compared to the black mussel. Regardless of the observed differences, we can classify both mollusc species as good sources of carotenoids.

Changes in retinol, β-carotene and astaxanthin after cooking of mussel and rapana are presented on Figures 1 and 2.

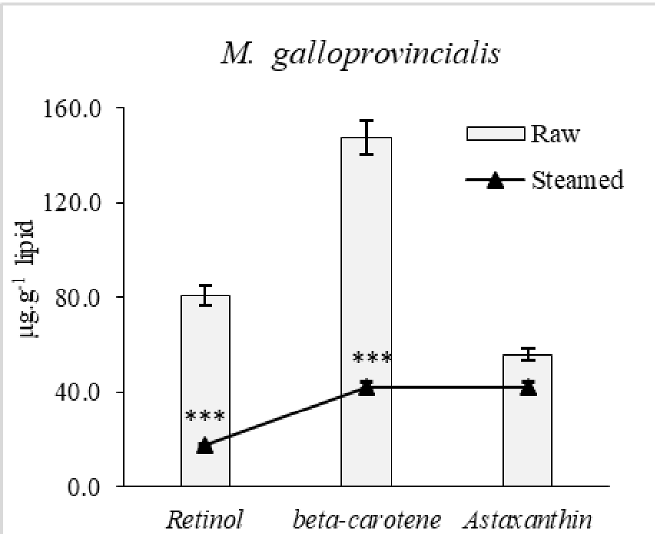

Fig. 1 Changes in retinol, β-carotene and astaxanthin content in mussel after steaming.

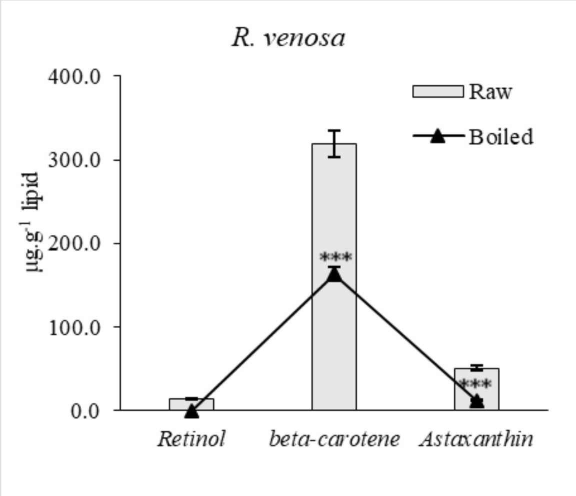

Fig. 2 Changes in retinol, β-carotene, and astaxanthin in rapana content after boiling.

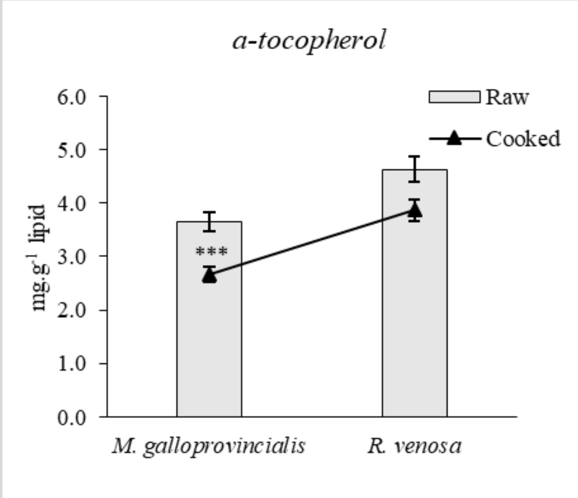

Fig. 3 Changes in α-tocopherol in mussel and rapana after cooking

In this study, the most unstable fat-soluble antioxidant in both species was vitamin A. Retinol decreased to 21% after mussel steaming, while boiling of rapana resulted in 100% loss of this vitamin. There is a limited information in the literature about thermal degradation of vitamin A in mollusk from the Black Sea coast. Our previous study for cooked rapana [3] and Black Sea mussel [13] presented similar results for low retinol stability. One previous study on nutritional quality of New Zeeland Green mussels presented information for fat soluble vitamins in raw and steamed samples [6]. Author reported that vitamin A is very stable and its content is well preserved during steaming process of mussel tissues. This discrepancy of our results can be explained by various factors, including culinary preparation, as temperature, duration, etc.

Beta-carotene and astaxanthin losses during thermal processing differ in between species, even when the same procedure and conditions are applied [8]. The presented results confirm this statement. Black mussel tissue show lower β-carotene susceptibility to degradation since it decreases up to 28% (42.3 ± 5.0 $\mu g.g^{-1}$ lipid), while astaxanthin remains stable (42.4 ± 4.0 $\mu g.g^{-1}$ lipid) compared to rapana. Papaioannou *et al.* [14] reported different results for soft clam *Callista chione* from Aegean Sea, Greece. Raw clams contained significantly higher astaxanthin, which levels increased after boiling (with 70%), whereas β-carotene showed similar amounts and decreased after boiling (with 25%). Discrepancies of our results can be explained by different sources and quantity of feeding, with species-specific metabolism and cooking procedure. *R. venosa* presented opposite trends: astaxanthin content was reduced to 23.5% (12.0 ± 0.7 $\mu g.g^{-1}$ lipid), whereas β-carotene decreased with 49% compared to raw samples. Similar results were reported in our previous studies for rapana [3] and mussels [13]. Yang *et al.*, [15] found that astaxanthin hydrolyzes and decomposes during thermal processing of Pacific white shrimp (*L. vannamei*). Total astaxanthin loss is significantly high (57%) compared to our results. Swastawati [5] reported significant decrease (up to 62%) of astaxanthin content in boiled tiger prawn (P. monodon) from Indonesia. The author supposed that boiling treatment may provoke denaturation and partial solubilization of the carotenes protein complex.

In general, marine lipids contain low to moderate levels of tocopherols [4]. Nevertheless, bivalves contain higher concentrations than other meats (chicken or poultry). One possible reason is relation to adaptation of seawater organisms to cold-water environment. Moreover, cell membrane homeostasis is influenced by synthesizes of highly unsaturated fatty acids, which are susceptible to oxidation, and antioxidants, as vitamin E may react as defenders and improve membrane permeability and fluidity [4]. In this study vitamin E presented low levels in both mollusks, as rapana contained 4.6 ± 0.5 $mg.g^{-1}$ lipid, and mussel – 3.7 ± 0.5 $mg.g^{-1}$ lipid. (Fig.3). Alpha-tocopherols with animal origin showed a higher antioxidant potency compared to vegetable oils. Moreover, the presence of carotenoids and phospholipids in marine lipids increased its antioxidant potency [4].

There was limited data for vitamin E content of mollusk species. Square [6] found significantly lower content of vitamin E in Greenshell mussels compared to Black Sea mussel and Rapana and no content in Pacific oysters from New Zealand. Shulman and Soldatov [12] reported similar levels of vitamin E for *M. galloprovincialis* from Northern Part of Black Sea (Sevastopol). Regardless of the observed minimal changes of vitamin E contents of both mollusk species, they can be classified of moderate sources of this vitamin.

Carotenoids are highly lipophilic compounds, which results in decreased bioavailability in humans. Modern research is focused on different type of lipid-based formulations [16]. Black Sea mussel and rapana are characterized by high content of polar lipids [3,13] which could have a beneficial effect on the absorption of carotenoids [17].

CONCLUSION

In the present study, stability of dietary antioxidants such as retinol, α-tocopherol, β-carotene and astaxanthin after culinary treatment of Black Sea mussels and rapana was assessed. Raw mussels *M. galloprovincialis* showed higher content of retinol, while *R. venosa* – b-carotene and α-tocopherol. Analyzed antioxidants decreased significantly after cooking with the exception of astaxanthin in *M. galloprovincialis*. Retinol was most affected by cooking process. It decreased by 100% in cooked *R. venosa*, probably due to the extended cooking time. Although susceptibility of vitamins and carotenoids to heat and oxygen, Black Sea *M. galloprovincialis* and *R. venosa* are excellent sources of natural antioxidants, even after culinary treatment.

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REFERENCES

1. V.D. Kancheva, O.T. Kasaikina, *Curr Med Chem*, **20**, 4784 (2013)
2. Y. Naguib, *J Agric Food Chem*, **48**, 1150 (2000)
3. A. Merdzhanova, V. Panayotova, D.A. Dobreva, R. Stancheva, K. Peycheva, *Ovidius University Annals of Chemistry*, **29**, 49 (2018)
4. C. Afonso, N.M. Bandarra, L. Nunes, C. Cardoso, *Crit Rev Food Sci Nutr*, **56**, 128 (2016)
5. F. Swastawati, *IOP Conf Ser Earth Environ Sci*, **139**, 012050 (2018)
6. M. Square, in: Identification of Health and Nutritional Benefits of New Zealand Aquaculture Seafood's., Report from Grant MacDonald, MacDonald and Associates Ltd, Nelson, 2010, p. 41.
7. A. Cilla, L. Bosch, R. Barberá, A. Alegría, *J Food Compost Anal*, **68**, 3 (2018)
8. A. Zeb, M. Murkovic, *Food Chem*, **127,** 1584 (2011)
9. D.A. Dobreva, V. Panayotova, R. Stancheva, M. Stancheva, *Bulg Chem Commun*, **49(G),** 112 (2017)
10. V. Venugopal, K. Gopakumar, *Compr Rev Food Sci Food Saf*, **16,** 1219 (2017)
11. A.V. Borodina, T. Maoka, A.A. Soldatov, *J Evol Biochem Physiol*, **49**, 283 (2013)
12. E.G.E. Shulman, A.A. Soldatov, in: Black sea molluscs: elements of comparative and environmental biochemistry. Institute of Biology of Southern Seas of NASU. Sevastopol: EKOSI-Gidrofisika, 2014, p. 323.
13. A. Merdzhanova, V. Panayotova, D.A. Dobreva, R. Stancheva, Merdzhanova, *Bulg Chem Commun*, in press
14. C.D. Papaioannou, V.J. Sinanoglou, I.F. Strati, C. Proestos, V.R. Kyrana, V.P. Lougovois, *Int J Food Sci Technol*, **51,** 325 (2016)
15. S. Yang, Q. Zhou, L. Yang, Y. Xue, J. Xu, C. Xue, C. *J Oleo Sci*, **64**, 243 (2015).
16. H.V. Chuyen, J.B. Eun, *Crit Rev Food Sci Nutr*, **57**, 2600 (2017).
17. R.E. Kopec, M.L. Failla, *J Food Compost Anal*, **68,** 16 (2018)