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Variation in radical antioxidant capacity and the total amount of carotenoids in razor clams, *Ensis marginatus* (Pennant, 1777), from the Çanakkale Strait (Abidealtı), Turkey

by

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Abstract

Metabolic activities such as breathing and digestion, resulting from natural functions of the body through oxidation, lead to the formation of free radicals that cause cancer, premature cardiac aging and some chronic diseases. Antioxidants are substances that remove free radicals and prevent cell damage. Seafood significantly contributes to the elimination of free radicals, especially owing to its high quality nutrient content. In this context, the objective of the study was to determine the radical antioxidant capacity and the total amount of carotenes in razor clams. The IC_{50} (mg g^{-1}) value of the DPPH radical sweeping effect varied over the months ($p < 0.05$), showing the highest value in June, gradually decreasing from September and reaching the lowest level in February. The total amount of carotenoids also varied, with the highest value in September ($p < 0.05$). The total amount of chlorophyll ranged from 6.15 µg g^{-1} in August to 66.71 µg g^{-1} in December.

Key words: razor clam, *Ensis marginatus*, antioxidant, carotenoid, chlorophyll, Çanakkale Strait

1. Introduction

The species *Solen dactylus*, *Tagelus plebeius*, *Ensis marginatus*, *E. macha* and *E. argunatus* from the Solenidae family in the Bivalvia class are consumed by humans and are therefore of economic value. These species, also known as razor clams, live in intertidal and subtidal zones where they burrow into the seabed sediment. The depth at which clams are buried varies depending on their age and length (Hayward et al. 1996). Like other bivalve species, razor clams are filter-feeding organisms that feed by filtering the seawater in which they live. Since the species belonging to this group are characterized by their distinctive flavors, they are popularly consumed in Eastern and Far Eastern countries, particularly in Chile, Japan, Argentina and Spain (Diaz et al. 2011). Razor clams are harvested by hand during free diving, extracted from the sea sand by hand using salt, or by shoveling and sifting the sand. However, fishing for razor clams is not governed by any official regulations. The products collected are generally used as bait, because they cannot easily come off the hook when angling. On the other hand, their consumption by humans is uncommon and limited to coastal regions of Turkey, as are many bivalve species. The total world fishery production of Solenidae is 9473 t. The major razor clam fisheries are in the Netherlands – 6109 t (*Ensis ensis*), followed by Pakistan with 589 t (Solenidae) and England with 559 t (*Solen* spp.). As for aquaculture, it totals 19.43 t, with *Solen* spp. accounting for 11.63 t in Portugal, followed by *Ensis ensis* in Spain with 7.8 t (FAO, Fishstat 2020).

Antioxidants are composed of numerous compounds and bioactive molecules obtained from a variety of sources (Reddy et al. 2011). Those from natural sources play an important role in the neutralization of oxidative stress (Sasikumar et al. 2009). Antioxidants are bioactive compounds that prevent the reaction of compounds or molecules with oxygen or free radicals (Abdel-Satter et al. 2007). Bivalve species are filter-feeding organisms that feed by filtering the seawater. In other words, they feed on organic and inorganic matter and microalgae (Gosling 2003). Microalgae are an important source of antioxidants (Goiris et al. 2012). Recently conducted research aimed at obtaining valuable algal metabolites from algae. Phycobiliproteins (anticancer agents) extracted from red algae and cyanobacteria, beta carotenes from *Spirulina*, *Chlorella* and *Dunaliella*, xanthophylls from diatoms, PUFA (EPA, DHA, ARA, LA) from diatoms and cryptophytes, and astaxanthin from *Haematococci* are valuable algal metabolites. The fact that these compounds produced by microalgae have a strong and positive antioxidant effect in humans and other organisms adds to their value (Gökpınar et al. 2006).

Carotenoids are a family of natural compounds in living organisms. Some studies correlate diets rich in carotenoids with minimizing the risk of several chronic and degenerative diseases, including cancer (Nishino 1998), cardiovascular disorders (Sesso et al. 2004) and age-related macular degeneration (Zeegers et al. 2001). Carotenoids are a group of yellow-orange pigments, mostly divided into two general classes: oxidized derivatives known as hydrocarbon carotenes and xanthophylls. More than 650 different natural carotenoids are found in bacteria, fungi, plants and animals (Matsuno 2001). Related studies showed that carotenoids are abundant in some mollusks such as Polyplacophora (Peebles et al. 2017), Gastropoda (Wei et al. 2019), Bivalvia (Borodina 2016) and Cephalopoda (Navarro et al. 2014). Thalassic carotenoids have different structures and most of them can be obtained from β-carotene, fucoxanthin, peridinin, diatoxanthin, alloxanthin and astaxanthin (Maoka 2011). In general, carotenoids give the skin and muscles of some fish and the soft tissues and shells of mollusks a certain glow and clarity (Li et al. 2010; Peebles et al. 2017). The total content of carotenoids (TCC) ranges from 10 to 140 µg/100 g depending on the species and tissue of mollusks. Mollusks do not synthesize carotenoids by themselves but obtain them through feeding on algae and accumulate them in their bodies. Some of the carotenoids are metabolized into retinol derivatives in the mollusk tissues (Kantha 1989). Carotenoids are responsible for tissue pigmentation, especially in aquatic animals (García-Chavarría & Lara-Flores 2013). Some carotenoids are also precursors of vitamin A (Miki et al. 1982). Carotenoids are essential for the growth and maturity of gonads and a high rate of fertilization (Sánchez et al. 2016). Carotenoids accumulated in mollusks through food intake are passed on to fish and humans who eat them (Czeczuga 1980).

2. Materials and methods

The study was performed at the location referred to as Abidealtı on the Gallipoli Peninsula, the Çanakkale Province, Turkey (40°03′02″N; 26°12′54″E), between May 2011 and April 2012 (Fig. 1). The study area is 0.5–2 m deep, with the bottom structure being granular and sandy, covered sporadically with the seagrass *Posidonia oceanica*.

After being cleaned, razor clams were transported to the laboratory. Soft tissues were completely removed from shells and freeze dried.

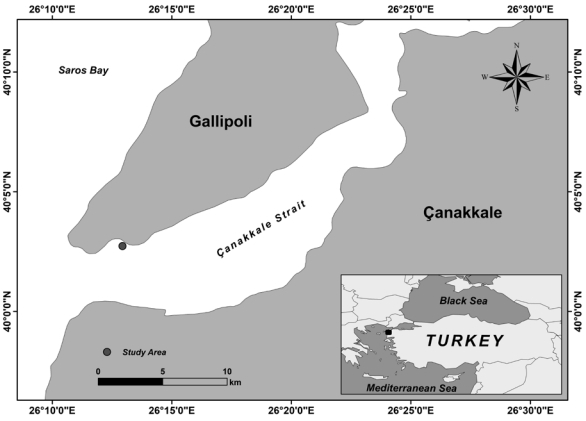

Figure 1

Map showing the sampling area – Çanakkale Strait (Gelibolu-Abidealtı), Turkey

2.1. Determination of Free Radical Scavenging Activity (DPPH)

Samples were extracted using methanol to determine free radical scavenging activity using DPPH (2,2-diphenyl-1-picrylhydrazyl). This is one of the most widely used methods to determine the antioxidant capacity by calculating the free radical scavenging effect. DPPH solution is a dark purple colored substance. It turns transparent when the extract solutions react with the DPPH solution. The absorbance of the DPPH reaction with the antioxidant substance at 515–517 nm is measured (Brand-Williams et al. 1995). For DPPH analysis, 30 min after mixing a given amount of DPPH with sample solutions, the absorbance at 515 nm is read and calculated using the following formula:

$$DPPH(\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100$$

2.2. Determination of the total amount of carotenoids and chlorophyll

To determine the total amount of carotenoids, the extraction method by Yanar et al. (2004) and Zheng et al. (2010) was used. Freeze-dried samples were exposed to a serial extraction by acetone; the procedure was repeated three times. The extracted samples were then centrifuged and measured using a UV spectrophotometer. A review of the available literature shows that different calculation methods are used to determine the total amount of carotenoids. In order to make the results comparable with the literature data, three most common carotenoid calculation methods were used in this study: 1) carotene calculation (Car 1; Formula 1) by Oliveira et al. (2010), 2) carotene calculation (Car 2; Formula 2) by Biehler et al. (2010) and 3) carotene calculation (Car 3; Formula 3) by Lichtenthaler & Buschmann (2001). Chlorophyll *a* (Chl-*a*; Formula 4) and chlorophyll *b* (Chl-*b*; Formula 5) were calculated according to Lichtenthaler & Buschmann (2001):

$$Car1\left(\frac{\mu g}{g}\right) = \frac{A450 \times V(ml) \times 10^4}{A1cm1\% \times W(g)} \quad \text{Formule 1}$$

$$Car2\left(\frac{\mu g}{g}\right) = \frac{A450 \times V(ml) \times Ma \times d \times 10^3}{135310 \times W(g)} \quad \text{Formule 2}$$

$$Car3\left(\frac{\mu g}{g}\right) = \frac{Car(x+c) \times V(ml)}{W(g)} \quad \text{Formule 3}$$

$$Car(x+c)\left(\frac{\mu g}{ml}\right) = \frac{1000 \times A470 - 1.90 \times Ca - 63.14 \times Cb}{214} \quad \text{Formule 3a}$$

$$Ca\left(\frac{\mu g}{ml}\right) = 11.24 \times A662 - 2.04 \times A645 \quad \text{Formule 3b}$$

$$Ca\left(\frac{\mu g}{ml}\right) = 20.13 \times A645 - 4.19 \times A662 \quad \text{Formule 3c}$$

$$Chl_a\left(\frac{\mu g}{g}\right) = \frac{C_a \times V(ml)}{W(g)} \quad \text{Formule 4}$$

$$Chl_b\left(\frac{\mu g}{g}\right) = \frac{C_b \times V(ml)}{W(g)} \quad \text{Formule 5}$$

where A450, 662 and 645 is the absorbance at 450, 662 and 645 nm; Ma (548 g mol^{-1}) is the average molecular weight for carotenes; A1cm1% is the absorption coefficient; V (ml) is the volume of the extraction solution; W (g) is the weighed amount of a sample; d is the path length of the cuvette in cm, usually 1 cm; Car (x+c) is xanthophylls and carotenes in an extract of plant material containing carotenoids; Ca and Cb is the value of chlorophyll *a* and *b* in samples in µg ml^{-1}.

The normal distribution of data was analyzed using the Kolmogorov–Smirnov normality test ($p > 0.05$). Pearson's correlation analysis was applied to determine the degree of association between carotenoids, chlorophyll *a* and chlorophyll *b* concentrations. Monthly data on carotenoids,

chlorophyll *a*, chlorophyll *b*, IC_{50} and inhibition (%) were also analyzed with one-way ANOVA. The difference between the groups was determined by the nonparametric Kruskal–Wallis test. All statistical analyses were performed using the SPSS program version 23.0 for windows.

3. Results

The radical elimination effect and the total amount of carotenes in the dried and dehumidified samples of razor clams were determined and compared. The DPPH method was used on razor clam samples to study the radical elimination effect. Figure 2b shows the IC_{50} value and its capacity for the radical elimination effect. The better the radical elimination effect, the lower the IC_{50} value. The lowest IC_{50} value of 4.04 mg g^{-1} was observed in February, while the highest value ranged between 32.76 mg g^{-1} in June and 32.82 mg g^{-1} in September with inhibition (%) values ranging from 81% to 95% (Fig. 2a). The IC_{50} value of the DPPH radical sweeping effect varied over the months ($p < 0.05$), being the highest in June, gradually decreasing after September and reaching its lowest level in February.

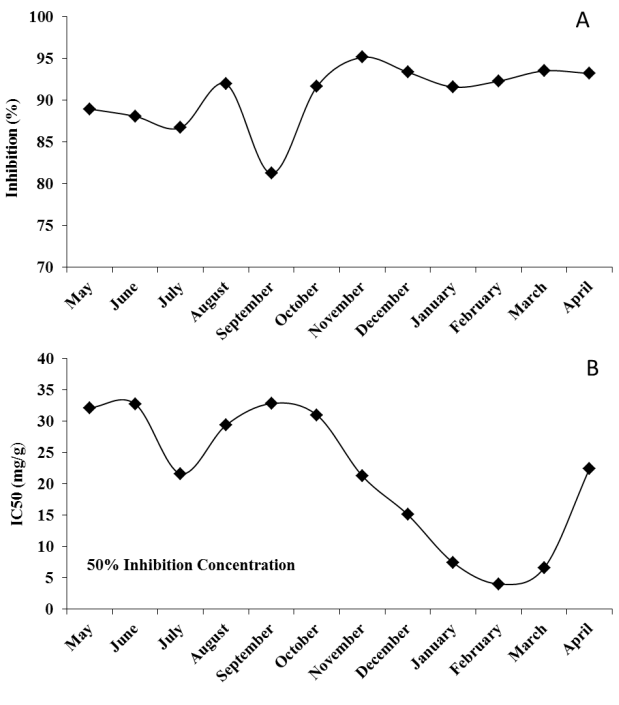

Figure 2

Inhibition values of the DPPH (%) radical sweeping effect (A) and IC_{50} (mg g^{-1}) (B) for the collected samples

Different UV wavelengths were used to study the total amount of carotenoids and the chlorophyll effect. The results are presented in Table 1, which shows three different carotenoid amounts calculated by three different calculation methods. The results are presented as µg g^{-1}. A strong correlation was found between Car 1, Car 2 and Car 3 (Table 2). The first two calculations showed that the total amount of carotenoids varied from 5.16 to 32.19 µg g^{-1}. The total amount of carotenoids varied during the year, with the highest value in December ($p < 0.05$).

Moreover, the amount of chlorophyll *a* (Chl-*a*) and chlorophyll *b* (Chl-*b*) varied throughout the months ($p < 0.05$), with the total chlorophyll levels ranging from 6.14 µg g^{-1} (August) to 66.71 µg g^{-1} (December).

4. Discussion

Marine invertebrates have long been studied for their pharmacological effects or other related bioactive properties used, e.g., in the production of cosmetics, food processing etc. As natural products of marine origin have become more attractive due to their potential use in the pharmaceutical industry, it is important to redefine new sources of such products (Pachaiyappan et al. 2014). Oxidative stress caused by an imbalance between prooxidants and antioxidants has been widely recognized as the main cause of chronic diseases (Urquiaga & Leighton 2000). As a result of potential cell and tissue damage caused by ROS, marine and other organisms compensate for the production of these radicals using a variety of cellular defensive mechanisms (Correia et al. 2003).

Therefore, antioxidants are very important due to their positive effects in the treatment of atherosclerosis, numerous types of cancer, cardiovascular diseases and in the anti-ageing therapy. Studies showed that the mollusk species *Tapes decussatus* has certain antioxidant properties (Passi et al. 2002). Studies on bivalve species such as *Paphia malabaricanın* (Pawar et al. 2013), *Perna viridis* (Jena et al. 2010), *Crassostrea* spp., *Placuna plasenta* and *Polymesoda erosa* (Shenai et al. 2012) demonstrated that they affect radical elimination processes. Moreover, antioxidant peptides of *Meretrix casta* (Nazeer et al. 2013) and glycoprotein of *Paphia undulate* (Yanan & Cuiping 2008) were found to have a radical sweeping effect. Another study showed 56.77% scavenging activity at 039 mg ml^{-1} for *Galatea paradoxa* and 79.77% at 0.39 mg ml^{-1} for *Patella rustica* using the DPPH method (Borquaye et al. 2015).

The IC_{50} (mg g^{-1}) value is a value that measures the effect of antioxidant capacity by the DPPH radical

Table 1

Monthly variation in the total carotenoid and chlorophyll content in razor clams (μg g^{-1})

	Car 1	Car 2	Car 3	Chl-*a*	Chl-*b*	Chl-*a*+*b*
May	7.29 ± 0.64	7.38 ± 0.64	3.82 ± 0.30	6.77 ± 0.53	4.98 ± 0.42	11.75
June	5.73 ± 0.16	5.80 ± 0.16	3.36 ± 0.22	4.27 ± 0.78	3.98 ± 0.49	8.25
July	14.49 ± 0.64	14.67 ± 0.64	5.91 ± 0.24	13.62 ± 0.75	11.82 ± 0.88	25.44
August	5.16 ± 0.11	5.22 ± 0.11	3.18 ± 0.11	3.31 ± 0.08	2.83 ± 0.38	6.14
September	7.74 ± 0.25	7.84 ± 0.26	3.51 ± 0.05	6.88 ± 0.52	5.23 ± 0.15	12.11
October	25.41 ± 0.78	25.73 ± 0.79	10.83 ± 0.27	25.86 ± 1.38	17.19 ± 1.13	43.05
November	22.78 ± 0.99	23.06 ± 1.00	9.24 ± 1.53	29.12 ± 1.63	12.58 ± 3.26	41.70
December	32.19 ± 1.54	32.59 ± 1.56	12.00 ± 0.90	39.41 ± 4.24	27.29 ± 3.42	66.71
January	12.78 ± 0.28	12.94 ± 0.29	5.53 ± 0.26	9.97 ± 0.01	8.87 ± 0.14	18.84
February	9.80 ± 0.20	9.92 ± 0.20	4.07 ± 0.39	8.12 ± 0.65	8.21 ± 1.54	16.33
March	9.76 ± 0.11	9.84 ± 0.18	3.60 ± 0.04	10.54 ± 0.12	10.85 ± 0.15	21.39
April	8.31 ± 0.04	8.47 ± 0.12	2.72 ± 0.34	9.10 ± 0.08	9.64 ± 0.18	18.74

Table 2

Pearson's correlations for the relationship between carotenoid formula 1 (Car 1), carotenoid formula 2 (Car 2), carotenoid formula 3 (Car 3), chlorophyll *a* (Chl-*a*), chlorophyll *b* (Chl-*b*), total chlorophyll (Chl-*a*+*b*), IC50, inhibition (%) (Inh)

	Car 1	Car 2	Car 3	Chl-a	Chl-b	Chl-a+b	IC50	Inh
Car 1	1							
Car 2	1.000**							
Car 3	.953**	.952**	1					
Chl-a	.982**	.982**	.899**	1				
Chl-b	.929**	.930**	.789**	.949**	1			
Chl-a+b	.973**	.973**	.865**	.993**	.980**	1		
IC50	−.142	−.141	−.004	−.124	−.294	−.194	1	
Inh	.374	.374	.309	.392	.414	.412	−.427	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

sweeping effect. The lowest IC_{50} value was found in February, which was 4.04 mg g^{-1} and had the highest radical sweeping effect. The highest IC_{50} value, i.e. 32.82 mg g^{-1}, was recorded in September, which indicates the lowest radical sweeping effect.

The lowest antioxidant capacity was observed in May, June, August, September and October. It can therefore be concluded that the low level of radical antioxidants in these months is due to seasonal changes as well as quality and quantity of food. The fact that IC_{50} decreased particularly after October shows an increase in radical antioxidants. Inhibition (%) is a value that measures the strength of antioxidant capacity by the DPPH radical sweeping effect. High inhibition (%) suggests that the radical sweeping effect is effective. In this sense, the highest inhibition value of 93.51% in March and the lowest IC_{50} value of 92.29% in February appear to be similar. The availability of food is an important factor for energy reserve and gametogenesis (Acarli et al. 2018a). Lipids and carbohydrates are used as the main energy sources in the reproduction cycle of bivalve species (Dridi et al. 2007; Pogoda et al. 2013; Acarli et al. 2018b). The spawning period of *Solen marginatus* was observed from May to June, from June to August and from May to July at three different locations in Spain (Remacha-Triviño & Anadón 2006). Hmida et al. (2010) reported that gametogenesis of *Solen marginatus* in Tunisia occurred between November and March, while maturation and spawning between April and May. Like other bivalves, razor clams also convert energy into their metabolic activities (gametogenesis and growth). We may suggest that there is an interaction between the presence of food in the environment, its quality and antioxidant capacity, and reproduction activity. The data obtained should be evaluated accordingly. Carotenoids of marine origin show a strong antioxidant effect, repairing anti-proliferative and anti-inflammatory effects and can be used as nutraceutical/cosmeceutical substances to prevent oxidative stress-related diseases or to protect the skin against solar UV radiation (Berthon et al. 2017).

The amount of carotenoids in mollusks is affected by factors such as the stage of sexual maturity, seasonal transitions, diet, algal sources and whether they are aquaculture or fishery products (Kantha 1989). Bivalve species accumulate different amounts of carotenoids in their body segments due to their nutritional characteristics (Zheng et al. 2010). It is also mentioned that they play an important role in crucial biological processes, especially in reproductive activity (Peebles et al. 2017). Carotenes are known to be one of the important organic elements present in microorganisms such as plankton and algae. Levels of carotenes provide important data on how and where living organisms feed. When mollusks are not able to feed sufficiently in a given habitat, the amount of carotenes in their tissues is reduced (Borodina 2016). It was found that the level of carotenes in *E. marginatus* was high in October, November and December. In the remaining months, the level of carotenes in the species was mostly insignificant. Three different methods were used to calculate the amount of carotene. It was found that the amount of carotene generally ranges from 5.16 to 32.19 μg g^{-1}. Considering the obtained results, the amount of carotene varies from 0.005 μg mg^{-1} to 0.032 μg mg^{-1}. Miki et al. (1982) determined the level of carotene in wet gonadal sections of different species as 0.0156 μg mg^{-1} for *Patinopecten yessoensis*, 0.0715 μg mg^{-1} for *Chlamys nipponensis alkazara*, 0.0360 μg mg^{-1} for *C. nobilis* and 0.0578 μg mg^{-1} for *Pecten albicans*. On the other hand, Escarria et al. (1989) found 0.00002 μg mg^{-1} for *Argopecten circularis*, which can be regarded as an insignificant value compared with the values obtained in this study. However, this study considered the whole tissue. The total amount of carotenoids and part of the changes in bivalve tissues as well as seasonal transitions are related to changes in biomass and specific diversity of phytoplankton (Borodina & Soldatov 2016). Acarli et al. (2014) investigated the reproductive activity of *E. marginatus* in Abidealtı Çanakkale. The present study was carried out in a similar period of time as the study by Acarli et al. (2014). Considering both the findings of the present study and the study by Acarli et al. (2014), we believe that there may a relationship between the reproductive cycle and the amount of carotene. It can be concluded that gonadal maturity processes occur when carotene levels increase, and the partial spawning process occurs when the amount of carotene decreases. The amount of chlorophyll and carotene present in microorganisms, such as plankton and algae, provides information about the feeding pattern of living organisms. Several factors such as salinity, turbidity, pH, light, temperature, dissolved oxygen and nutrient availability affect the growth, limitation and size structure of phytoplankton (Temponeras et al. 2000; Dominiques & Barbosa Galvao 2005). These factors are therefore also related to the amount of chlorophyll. In this study, the amount of chlorophyll was calculated as chlorophyll *a* and chlorophyll *b* together. The highest level of total chlorophyll was found in December. It can be observed that especially in this period the amount of phytoplankton significantly increases depending on the environmental conditions.

Mollusca species, especially bivalves, are preferred by consumers because of their taste and for being a healthy source of energy. Depending on the quality of bivalves, the consumer also decides on the time of consumption. The optimum harvest time in aquaculture systems or wild stock is determined on the basis of meat yield, the gonadosomatic index or nutritional quality. In this study, the radical antioxidant capacity and the amount of carotenoids in *E. marginatus* were determined in relation to the nutritional quality. The data presented in this study show that the highest radical antioxidant capacity of *E. marginatus* in 12 months (May 2011 to April 2012) occurred in February and the maximum amounts of carotene and chlorophyll in December. The high carotene and chlorophyll values for *E. marginatus* in October, November and December may result from a diet rich in microorganisms. We can conclude that, considering the criteria of healthy alimentation, the consumption of the species can be recommended in October, November, December and February.

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