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Determination of Free Radical Scavenging Activity in Some Economic Bivalve Species

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Abstract**—** *In this study, free radical scavenging activity, alpha tocopherol and total carotenoid contents of extracts of Mytilus galloprovincialis, Ruditapes philipinarum, Donax trunculus and Chamelea gallina bivalve species collected from Denizkent in the Marmara Sea are determined. As a result of the monthly samplings, the amount of alpha tocopherol is quite high in all species, whereas the total amount of carotenoids is highest in Donax trunculus (2435 µg/g), then in Mytilus galloprovincialis (2189.6 µg/g), and in the other two species parallel results are obtained. When the free radical scavenging activity is evaluated, on average, Ruditapes philipinarum (22.8%) is the highest, Mytilus galloprovincialis (16.75%) and Chamelea gallina (17.98%) are close to each other, 11% in Donax trunculus, it has been determined that 85. In addition, other parameters obtained in this study were examined by considering statistical evaluations*

Keywords**— *Chamelea gallina, Donax trunculus, Mytilus galloprovincialis, Ruditapes philipinarum, Scavenging activity, Total carotenoid, α-Tocopherol***

I. INTRODUCTION

Bivalves are invertebrates that are included in the Mollusca phylum and are distributed in both marine and freshwater ecosystems. The production of bivalve species in Turkey is mainly done by hunting from natural stocks. However, especially in recent years, production has started by aquaculture in our coasts [1]. It is known that bivalves are creatures that live in coastal areas of aquatic ecosystems and feed by filtering water. They have important economic value with their nutritional value.

Bivalve organisms feed by filtering particles from the water. As nutrients, they generally take in phytoplankton and organic particles suspended in water. For this reason, the amount of chlorophyll-a and organic matter in the aquatic environment is important in the nutrition of bivalves [2,3]. It has been reported that bivalves accumulate carotenoids in their tissues due to their feeding on phytoplanktonic organisms and that carotenoids help bivalves to provide tolerance and adaptation to various stressful conditions [4,5,6]. There is β carotene in some forms and α carotene in some forms as carotene in photosynthesizing phytoplankton forms. Carotenoids have high antioxidant potential and are reported to be used in the prevention of free radical-induced diseases, including atherosclerosis, cataracts, and age-related muscle degeneration [7]. In addition, Tocopherols are antioxidant compounds that can be found in algae [8]. Tocopherols that cannot be synthesized in some living organisms have to be taken from outside. The antioxidant property of vitamin E is due to its inhibition of lipid peroxidation initiated by free radicals [8,9].

In this study, it was tried to determine free radical scavenging activity, total carotenoid, and vitamin E content for healthy consumption by humans of some bivalve species, which are an important commercial source.

II. MATERIAL METHOD

The study was carried out monthly between November 2017 and October 2018. Samples of *Mytilus galloprovincialis*, *Ruditapes philipinarum*, *Donax trunculus*, and *Chamelea gallina* were collected from production areas open to commercial fishing on the southern shores of the Marmara Sea ($40^0$19' 57" N–$27^0$24'58"E; $40^0$19'44" N–$27^0$25'29" E). Samples were collected from 0–5 m depth contour from sandy bottom structures by scuba diving and hand dredge. The samples were brought to the laboratory by cold chain method on the same day. The samples separated from their shells were cut into small pieces and then homogenized. DPPH (2,2-diphenyl-1- picrylhydrazil) was used to determine the free radical scavenging activity in the samples. Free radical scavenging power was calculated by measuring the ability of DPPH antioxidant, which is a stable and synthetic radical, to capture free radicals [10]. Samples were extracted with dilute MeOH solution. DPPH solution (6x 10^{-5} M) was added to the diluted sample (1 mg/ml), then left in the dark for 30 minutes for the reaction to take place. Then, the absorbance of the samples was measured at 515 nm in a Thermo Aquamate UV-VIS spectrophotometer. DPPH radical scavenging activity was calculated according to the following formula [10]; DPPH = [(A control –A sample)/A control].

where, A is absorbance.

To determine the total carotenoid amount in bivalve samples, 5 gr of tissue samples were weighed, and 20 ml of acetone was added to it. The absorbance values of the samples, which were extracted with the help of a mixer, were measured at 450nm. The carotenoid content was calculated using the following equation [11]:

$A = \alpha.c.l$

where, A is the absorbance at 450 nm, α is the specific absorbance coefficient of the solvent, c is the concentration of the carotenoids in µg/g extraction, and l is the path length of the cuvette (1 cm) [11].

In the determination of α-Tocopherol analysis; Its 200µl MeOH extract consists of a mixture of C-18 Nova pack HPLC column (5.0nm, 4.6mm x 25cm) and a mobile phase (moving) methanol: hexane (72:28) (V/V). It was also measured at 292 nm. The flow rate of the mobile phase is 1ml/min, the flow time is 2.95±0.03min. [12].

Statistical analysis was performed using SPSS 22.0 (IBM SPSS Statistics 22) package program. Means and standard deviations were determined to evaluate the results. Whether the data showed normal distribution or not was analyzed with the Shapiro-Wilk test. In addition, when the skewness and kurtosis tests were performed on the obtained data, it was determined that the independent variables were not normally distributed (-1 to + 1), so nonparametric tests were used in the analysis of the data [13]. In this context, the Kruskal-Wallis test and Dwass-Steel-Critchlow-Fligner test were used in the comparison of more than two groups of quantitative variables that did not show the normal distribution in the analysis of the data. Statistical significance was accepted as $p<0.05$.

III. RESULT AND DISCUSSION

In this study, total carotenoid, vitamin E (αTocopherol) and free radical scavenging activity were measured for the first time in bivalve species (*Mytilus galloprovincialis*, *Donax trunculus*, *Ruditapes philippinarum*, *Chamelea gallina*).

Carotenoids found in algae, which are the food source of bivalves, are powerful biological antioxidants [14]. Antioxidants are also considered by researchers as a source for dietary supplements. In this study, the free radical scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrazil) in bivalve mollusc species was investigated.

The DPPH free radical scavenging capacities of bivalve extracts are presented in Table 1 and Table 2. In addition, since the p-value was determined as <0.05 by the Shapiro-Wilk test, the data did not show a normal distribution, but also among the skewness and kurtosis coefficients; Since the skewness coefficient/own standard error and kurtosis coefficient/own standard error were outside the range of +1.96 and -1.96, it was seen with 95% confidence level that the data did not fit the normal distribution. This situation is similar to the obtained histogram graphs (Fig. 1). In the current study, the antioxidant activities of four bivalve species extracts were evaluated. The results showed that the bivalve species tested in this study had low antioxidant activity.

Table 1. Observed results from the Shapiro-Wilks test, skewness and excess kurtosis for the IC 50

Species	Mean IC 50	95% Confidence		Shapiro-Wilk		Kurtosis	Skewness
		Upper limit	Lower limit	W	p		
Mytilus galloprovincialis	12,42±5,81	15,71	9,14	0,68	< .001	8,06±1,23	2,56±0,64
Ruditapes philipinarum	9,5 ± 1,45	10,32	8,68	0,80	0,009	-0,37±1,23	-1.10±0,64
Donax trunculus	8,32 ±2,87	9,94	6,70	0,74	0,002	-0,84±1,23	1.03±0,64
C. gallina	10,11±2,27	11,39	8,83	0,81	0,012	-01,45±1,23	-0.65±0,64

Table 2. Observed results from Shapiro-Wilks test, skewness and excess kurtosis for the Inhibition

Species	Mean % Inhibition	95% Confidence		Shapiro-Wilk		Kurtosis	Skewness
		Upper limit	Lower limit	W	p		
Mytilus galloprovincialis	16,75 ± 10,98	22,96	10,54	0,85	0,042	-1,18±1,23	0,33±0,64
Ruditapes philipinarum	22,83 ± 7,40	27,02	18,64	0,68	< .001	0,49±1,23	1,46±0,64
Donax trunculus	11,85 ± 3,39	13,77	9,94	0,87	0,058	-0,88±1,23	-0.57±0,64
C. *gallina*	17,98 ± 13,22	25,46	10,50	0,74	0,002	-1,91±1,23	0.51±0,64

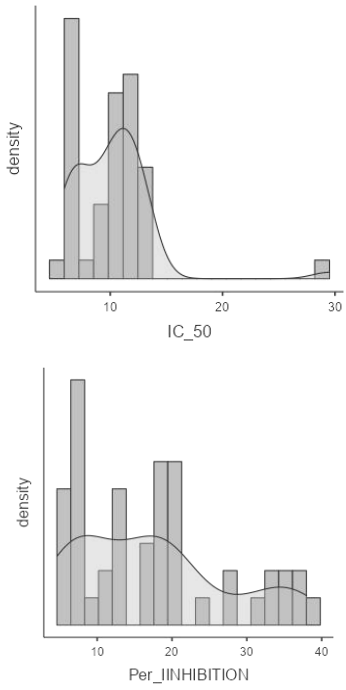

Fig.1. Histogram of the IC50 and Inhibitions extracts studied.

When the difference in both IC50 and per inhibition values between bivalve species is examined, there is a difference between species since p<0.05 according to the data obtained (Table 3). In the paired comparison test performed to determine this difference, it was determined that there was a difference in Per-inhibition rates only between *Ruditapes philipinarum* and *Donax trunculus* species. Since no difference could be detected between both IC 50 and per-inhibition among other species, it can be considered as a similar level (Table 4).

Table 3. Kruskal-Wallis test results for species comparison

	χ^2	df	p	ε^2
IC_50	8.03	3	0.045	0.17
Per_IINHIBITION	8.17	3	0.043	0.17

Tablo4. Dwass-Steel-Critchlow-Fligner pairwise comparisons between species

Pairwise comparisons - IC_50		**W**	**p**
Mytilus galloprovincialis	*Ruditapes philipinarum*	-3.10	0.125
Mytilus galloprovincialis	*Donax_trunculus*	-3.10	0.125
Mytilus galloprovincialis	*C_Gallina*	-1.55	0.691
Ruditapes philipinarum	*Donax_trunculus*	-2.53	0.278
Ruditapes philipinarum	*C_Gallina*	1.51	0.709
Donax_trunculus	*C_Gallina*	1.96	0.508
Pairwise comparisons - Per_INHIBITION			
Mytilus galloprovincialis	*Ruditapes philipinarum*	0.82	0.939
Mytilus galloprovincialis	*Donax_trunculus*	-0.98	0.900
Mytilus galloprovincialis	*C_Gallina*	1.55	0.692
Ruditapes philipinarum	*Donax_trunculus*	-5.88	< .001
Ruditapes philipinarum	*C_Gallina*	-1.80	0.582
Donax_trunculus	*C_Gallina*	0.49	0.986

Depending on the sampling time of the bivalve species, DPPH free radical scavenging activities were also low due to low total carotenoid contents in autumn and winter seasons. Because the total amount of carotenoids increases the formation of Chlorophyll-a and chlorophyll b in living things because of collecting light by sea creatures such as algae. Since carotenoids also have electronic inferreactions, they have destructive effects of reactive oxygen species (ROS), ie free radical scavenging capacity [15]. Therefore, seasonally, DPPH free radical scavenging activity % inhibition and total carotenoid were lower than summer periods. Due to the increase in phytoplanktonic activity in the summer period, free radical scavenging activity is expected to be higher as carotenoid compounds increase. The results of this study also support this situation (Table 5).

When we make a comparison according to the months in the study, the amount of alpha-tocopherol is at the highest level in all species in November, December, and January. The lowest alpha-tocopherol levels were detected in May. This coincides with the breeding season of these living species. However, during this breeding period, total carotenoids are at their highest level in the *Mytilus galloprovincialis* and *Donax trunculus* species. On the other hand, it was detected at the lowest level in *Ruditape*s *philipinarum* and *Chamelea gallina.*

The period in which the free radical scavenging activity was highest according to the % inhibition rates was determined as May for *Mytilus galloprovincialis* and *Donax trunculus* species, and January for *Ruditapes philipinarum* and *Chamelea gallina*. The lowest levels of % Inhibition rates are; August for Mytilus galloprovincialis, December for *Donax trunculus*, May and June for *Ruditapes philipinarum*, May, June, July, August, and September for *Chamelea gallina*.

The % Inhibiton ratios correspond to the time when the total carotenoid contents for *Chamelea gallina* and *Ruditapes philipinarum* are at their lowest. Therefore, free radical scavenging activities are also at the lowest level compared to other species. This situation changes depending on the metabolic activities of the species.

Table 5. α-Tocopherol (α-Toc.) and Total Carotenoid (TC.) (µg/g) results in bivalve species

		Mytilus galloprovincialis	*Donax trunculus*	*Ruditapes philippinarum*	*Chamelea gallina*
Nov.17	**α-Toc.**	561,654	390,721	689,623	x
	TC.	938,6	1067,8	410,4	x
Dec.17	**α-Toc.**	304,577	243,476	558,504	626,999
	TC.	932,9	684	452,2	524,4
Jan.18	**α-Toc.**	1206,201	1990,493	1002,32	1150,276
	TC.	1375,6	1400,3	361	330,6
Feb.18	**α-Toc.**	73,36	84,283	65,32	95,878
	TC.	1968,4	1263,1	817	461,3
Mar.18	**α-Toc.**	72,969	76,106	61,254	75,369
	TC.	1991,2	1901,5	634,3	0
Apr.18	**α-Toc.**	70,231	102,475	71,88	137,947
	TC.	1094,4	2470	459,8	225
May.18	**α-Toc.**	0	82,884	70,258	87,687
	TC.	2189,6	2435	0	0
Jun.18	**α-Toc.**	120,625	49,582	85,369	85,241
	TC.	1417,4	1972,2	0	0
Jul.18	**α-Toc.**	336,546	44,707	98,752	98,654
	TC.	1108,8	2012,5	926,4	0
Aug.18	**α-Toc.**	135,357	45,32	0	0
	TC.	827,6	1566,4	292,6	0
Sep.18	**α-Toc.**	49,963	50,808	173,226	152,639
	TC.	858,8	1120,2	1200,8	0
Oct.18	**α-Toc.**	42,398	51,745	879,268	159,875
	TC.	1276,8	1796,6	691,6	263

IV. CONCLUSION

As a result of the data obtained, % inhibition values were determined at the highest rate in all bivalve species sampled due to the high levels of alpha-tocopherol and total carotenoid values in January. On the other hand, the reason why the % inhibition value was the lowest in May is the low alpha-tocopherol amounts of *Ruditapes philipinarum* and *Chamelea gallina* in May and June. These months also coincide with the breeding periods of bivalve species.

In winter months when alpha-tocopherol and total carotenoid contents are high for all species, the consumption of these species are more beneficial for human health, and free radical scavenging activity are more effective in winter, thus increasing resistance to diseases.

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