Turkish Journal of Agriculture - Food Science and Technology, 7(sp1): 150-153, 2019
DOI: https://doi.org/10.24925/turjaf.v7isp1.150-153.2790

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology

Antioxidant Activity of *Mytilus galloprovincialis* and *Ruditapes philippinarum*[#]

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ARTICLE INFO

[#]This study was presented as an oral presentation at the 4th International Anatolian Agriculture, Food, Environment and Biology Congress (Afyonkarahisar, TARGID 2019)

Research Article

Received : 30/06/2019
Accepted : 04/09/2019

Keywords:
Antioxidant Activity
Mytilus galloprovincialis
Ruditapes philippinarum
Total Carotenoids
DPPH

ABSTRACT

This study reports on the evaluation of the antioxidant of ethanolic extracts of *Mytilus galloprovincialis* and *Ruditapes philippinarum* collected from the Marmara Sea. The antioxidant activity of the two bivalve species was evaluated using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. The total carotenoid contents were also determined. In the DPPH method, all extracts exhibited low radical scavenging activity when compared to the standards used, i.e. α-tocopherol and butylated hydroxytoluene (BHT) with the inhibition percentage in the range of 35.87±0.05 – 36.27±0.02%. In particular, *M. galloprovincialis* exhibited the highest radical scavenging activity with an IC_{50} value of 29.55±0.02 mg/g Ext. However, there are no statistically significant differences in the IC_{50} values of two species. The highest total carotenoid content was found in *M. galloprovincialis* as 1.13±0.02 µg/g sample. The statistically significant differences were found in total carotenoid contents of two bivalves. In this study in *M. galloprovincialis* was also found to be high in carotenoid content, while *R. philippinarum* was found to be almost nonexistent. More detailed studies are needed to determine relationship between the bioactive compounds of crustaceans and their antioxidant activities, distributed in our country.

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Introduction

Bivalve molluscs are harvested commercially and are of considerable significance for aquaculture. They are important food resources that contribute considerable economic value to the world's fisheries (Leiva and Castilla 2002). It has been recognized as a high- quality nutritious food and is considered a delectable. The bivalves are among the most harvested species in the world, as well as in Turkey (FAO, 2009). In recent years in Turkey, bivalves were harvested, which represents 5% of the total fisheries production (TUIK 2010). The main production areas of bivalves are the west Black Sea and the Marmara Sea in Turkey.

M. galloprovincialis called Mediterranean mussel is distributed in the Mediterranean Sea and the Black Sea, and the Atlantic coasts (Mathiesen et al., 2017). It lives on all coasts that have hard substrates within sheltered harbors and estuaries and on rocky shores of the open coast (FAO, 2019). According to the official statistics of the National Agency for Fishery and Aquaculture (TUİK, 2010), natural beds of the Mediterranean mussel is abundance in our coastal zones and it is the only molluscs species cultivated in Turkey. The venerid clam *Ruditapes philippinarum* (Japanese carpet shell) inhabits sandy and muddy bottoms of seas and it is usually found buried 2–3 cm below the surface in the intertidal zone (Moschino et al.,2012). Natural populations of this species are distributed along the coast of the Pacific and Atlantic Oceans, as well as the coastlines of the Adriatic and Aegean Seas (Jensen et al., 2004), and along the coast of the Mediterranean and Marmara Seas (Albayrak, 2005). *R. philippinarum* is one of the most economically important molluscs in the world and their commercial production comes from both fishing of natural stocks and cultivation (Çolakoğlu and Palaz, 2014).

The considerable amount of literature has been published on the nutritional composition of bivalves (Mooney et al. 2002; Orban et al., 2002; Dridi et al. 2007;

Lorenz, 2010). They contain useful functional lipids, particularly proteins, and bioactive components. Especially mussels are rich on carotenoids, which act as antioxidants (Orban et al., 2002; Lorenz, 2010). In recent years, these aspects are classified as more valuable than most animal foods and therefore they are included in the category of “functional foods” (Alasalvar and Taylor, 2002; Shahidi and Naczk, 2004). Due to these facts, the importance of marine mussels as a source for bioactive substances with anti-inflammatory, antimicrobial, and lowering cholesterol level agents is increasing rapidly (Merdzhanova et al., 2017).

The carotenoids possess the high antioxidant potential and some studies report their use in preventing free-radical-initiated diseases including atherosclerosis, cataracts, and age-related muscular degeneration (Perera and Yen, 2007). By predominantly feeding on aquatic plants, molluscs can accumulate carotenoids in their tissue. Many studies showed that carotenoids have also been linked to tolerance and adaptation to several stressful conditions in mussels (Bendich and Olson, 1989; Hill and Johnson, 2012; Anbazahan et al., 2014). Natural antioxidants as free radical scavengers are widely used in the food industry to enhance the sensory, health-promoting, or keeping quality of foods.

The aim of the present study is to determine the antioxidant activity and carotenoid of extracts obtained from *Mytilus galloprovincialis* and *Ruditapes philippinarum* collected from the Marmara Sea.

Materials and Methods

Sample Collection

Mytilus galloprovincialis and *Ruditapes philippinarum* were collected from natural beds located at the Southern Coast of Marmara Sea, Turkey. Collected samples were transported with ice after transportation of samples to the laboratory, each sample was inspected; dead, or damaged specimens were eliminated. Mean height and weight of samples were measured as 5.47±0.14 g and 80.76±44 mm, 20.32±0.21 and 37.01±0.14, respectively. The samples were immediately frozen at −20°C and stored in a fridge. All shucked mussels were cut into small pieces and they were homogenized at 800 rpm for 5 min, using a Molineux blender.

Standards and Reagents

All chemicals were purchased from Sigma-Aldrich (USA), SPA (Milan, Italy), Merck (Germany), and Fluka Chemie (Switzerland).

DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging activity of the sample extract was measured according to the procedure described by Brand-Williams et al. (1995). Methanolic extracts were prepared in dilution series (0.25 to 1 mg/ml). 0.1 ml of each dilution was added to 3.0 ml of a 6×10^{-5} M methanolic solution of DPPH followed by vortexing. The mixture was shaken vigorously and then it allowed standing in the dark at room temperature for 30 min. The absorbance of the solution was measured spectrophotometrically (UV-Vis. Spectrophotometer, Thermo Aquamate) at 515 nm against methanol. The DPPH radical scavenging activity (%) was calculated using the following equation:

DPPH = $[(A_{control} - A_{sample})/A_{control}]$ is the absorbance of the control.

Butylated hydroxytoluene (BHT) and α-Tocopherol (Vitamin E) was used as a positive control. A lower value of IC_{50} indicates a higher antioxidant activity.

The Analysis of Total Carotenoid Content

The carotenoid content was determined spectrophotometrically (UV-Vis. Spectrophotometer, Thermo Aquamate) and the absorbance was measured at 450 nm according to Biehler et al. (2010). The average carotenoid concentrations (mol/L) were calculated with the following equation:

$$C(Mol/L) = A_{450} \times Fd / 135310$$

The A_{450} and Fd can be defined as the absorbance and the dilution factor at 450 nm. (1cm). The average concentrations (C mol/L) were turned as micrograms per grams (µg /g). 135310 value is an average molar absorption coefficient to be calculated for ethanol solvent by Biehler et al. (2010).

Statistical Analysis

The significance of differences between IC50 values and % Inhibition was determined by one-way analysis of variance (ANOVA) and the significance of differences between total carotenoid contents was measured by Student’s *t*-test. Before ANOVA or Student’s *t*-test, all data were checked for homogeneity of variance and normal distribution. A significant difference was considered at the level of $P<0.05$.

Results and Discussion

Free radicals are inevitably produced in biological systems and encountered exogenously. They are known to cause various degenerative disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances, and aging (Singh and Singh 2008). The importance of the antioxidant potential of carotenoids to human health derives from their potential to reduce the oxidative stress linked to various ROS related disorders and/or diseases (Voutilainen et al., 2006; Fiedor and Burda, 2012). Antioxidants are the compounds, which neutralize the free radicals by intervening at any one of the three major steps of the free radical-mediated oxidative process, viz., initiation, propagation, and termination (Cui et al., 2004). These antioxidants are also produced by a biological system and occur naturally in many foods and taken from food compounds to the body (Halliwell, 1996).

In the present investigation, we discuss the level of total carotenoids and radical scavenging activated level in shellfishes. In our study, it was found that these two molluscs species showed antioxidant properties and the carotenoid content was high. The scavenging activity of *M. galloprovincialis* and R. *philippinarum* on DPPH free radical are summarized in Table 1. These two bivalves have same IC_{50} values and they showed the low antioxidant potential (29.55±0.02 mg/g Ext.). Our results are significantly lower than two commercial antioxidants tested, BHT and Vitamin E ($P<0.05$). The antioxidant activities of two shellfish were not significantly different ($P>0.05$), and % inhibition values ranged from 35.87±0.05 to 36.27±0.02 mg/g of extract. Thus, both *M. galloprovincialis* and *R. philippinarum* showed medium potential DPPH radical scavenging activity.

The total carotenoid contents in the *M. galloprovincialis* and *R. philippinarum* are summarized in Table 2. The total carotenoid content varied between 0.03±0.07 to 1.13±0.02 μg/g sample. The highest carotenoid content was detected in *M. galloprovincialis*. There is a significant difference between the total carotenoid content of two bivalves ($P<0.05$). Photosynthetic algae and plants, fungi and bacteria synthesize carotenoids while other organisms must either obtain the necessary carotenoids directly from the diet or modify the dietary carotenoid precursors through metabolic reactions. Carotenoid pigments are abundantly present in marine plants. Molluscs, such as bivalves, accumulate carotenoids obtained in their body tissues either directly from their dietary microalgae (e.g., fucoxanthin, diatoxanthin, diadinoxanthin, and alloxanthin) or after modification through metabolic reactions (Kantha, 1989; Liaaen-Jensen, 1998; Maoka and Akimoto, 2008). Metabolites derived from fucoxanthin like mytiloxanthin or crasssostreaxanthin are widely distributed in marine mussels and oysters (Matsuno, 1989; Liaaen-Jensen, 1998; Maoka and Akimoto, 2008). Astaxanthin is the main pigment in the body and eggs of crustaceans (van Nieuwerburgh et al., 2005).

Table 1 DPPH values of the *M. galloprovincialis* and R. *philippinarum*. Different lowercase letters show the significant differences between the groups according to the ANOVA results ($P<0.05$).

Species	[1]IC_{50}	[2]%
M. galloprovincialis	29.55±0.02^a	35.87±0.05^c
R. philippinarum	29.55±0.07^a	36.27±0.02^c
Butylated hydroxytoluene	1.33±0.01^c	99.00±0.11^a
α-tocopherol	1.48±0.02^b	96.00±0.15^b

[1]Inhibition values (mg/g Ext.), [2]Inhibition

Table 2 Total Carotenoid values of the *M. galloprovincialis* and R. *philippinarum*. Different lowercase letters show the significant differences between the groups according to the Student's t-test results ($P<0.05$).

Species	Total carotenoid values (μg/g sample)
M. galloprovincialis	1.13±0.02^a
R. philippinarum	0.03±0.07^b

In organisms, especially shellfish, carotenoids, in the food web benefit from their protection against ultraviolet (UV) radiation, their antioxidant properties against reactive oxygen species (ROS) and free radicals (Maoka, 2011; Caramujo et al., 2012). They play an important role as precursors of transcription regulators and in the immune system (Bendich and Olson, 1989; von Schantz et al., 1999; Hill and Johnson, 2012; Anbazahan et al., 2014). Some of the carotenoids are metabolized into vitamin A derivatives in the molluscan tissues (Kantha, 1989). In this study in *M. galloprovincialis* was also found to be high in carotenoid content, while *R. philippinarum* was found to be almost nonexistent. Carotenoids are colored compounds that affect the color of food. They are responsible for shellfish for the yellow, orange, or red color, but there are many non-carotenoid pigments in them (Tanaka and Katayama, 1979). The data obtained in our study coincide with this finding. In *M. galloprovincialis*, the color of the flesh is visually darker, and the gonads are usually orange. *The R. philippinarum* is lighter in color. The fact that antioxidant activity obtained in *R. philippinarum* is equivalent to *M. galloprovincialis* data shows that this property is not only related to total carotenoid content. However, both species have moderate free radical scavenging activity**.**

Conclusion

Seafood offers important bioactive molecules that have advantages on the human body. They are used in many fields such as the drug, cosmetic, and food industries. Functional foods are developed from marine products since they are widely available, and they have the ability to prevent certain diseases and cure some illnesses. Various kinds of seafood especially shellfish are consumed as a nutritionally beneficial food. Consequently, more detailed studies are needed to determine relationship between the bioactive compounds of crustaceans and their antioxidant activities, distributed in our country.

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