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Antioxidant radical scavenging capacity and total carotenoid content of narrow-clawed crayfish (*Pontastacus leptodactylus*, Eschscholtz, 1823) in Atikhisar Reservoir (Çanakkale, Türkiye)

by

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Abstract

In this study, antioxidant radical scavenging capacity and total carotenoid content in the meat and shells of *Pontastacus leptodactylus* were investigated. Concerning the antioxidant scavenging effect, the highest IC_{50} values were found to be 388.77 mg g^{-1} and 155.53 mg g^{-1} for females and males in July and March, respectively. The mean IC_{50} values of the meat were calculated as 239.83 mg g^{-1} and 105.21 mg g^{-1} for females and males, respectively. The mean total carotenoid content in the meat was found to be 14.35 and 12.78 µg g^{-1} for females and males, respectively. The results indicated that crayfish meat had antioxidant radical scavenging capacity and was rich in carotenoid content.

Key words: Crayfish, *Pontastacus leptodactylus*, antioxidant, carotenoid, Çanakkale

1. Introduction

The narrow-clawed crayfish, formerly *Astacus leptodactylus* Eschscholtz, 1823, called Turkish crayfish, Danube crayfish, Galician crayfish or marshland crayfish was reclassified as '*Pontastacus leptodactylus*' by the revision made in 2017 (Crandall & De Grave 2017). The species is naturally distributed across 27 countries, including Türkiye, Ukraine, south-west Russia and in the canal systems of the rivers which flow into the Baltic and Caspian seas as well as in Kazakhstan, Belarus, Slovakia, Bulgaria, Romania and Hungary. Moreover, it is also known to have been grafted through lakes and canals in Czechia, Poland, Germany, Finland, Denmark, the Netherlands, Britain, Lithuania, Latvia, Spain and Italy (Skurdal & Taugbøl 2002). The species is distributed across Thrace, north, central and west Anatolia in Türkiye (Holthius 1961; Geldiay & Kocataş 1970; Berber et al. 2012, 2019, 2020; Berber & Kale 2018; Berber 2020; Kale et al. 2020, 2021). Turkish records on crayfish production date back to 1965, mentioned as 270 tons in total, while the highest crayfish harvest was 7937 tons in 1984. Over the last decade fluctuations in catches have been observed and total crayfish production was recorded to be 2022 tons in 2021. Kale & Berber (2020) compared different trend analysis methods and forecasting models to evaluate the trends in freshwater crayfish production in Türkiye. The authors noted that freshwater crayfish production tended to decrease during the study period (1909-2018), and it is predicted that it will continue to decrease in the future period.

Antioxidants can be defined as molecules that have the ability to prevent oxidising chain reactions caused by reactive free radicals from starting or spreading and interact with them prior to bioactive molecules of vital importance in living metabolism being affected (Ismail et al. 2004; Sirivibulkovit et al. 2018). Antioxidants can inhibit free radical reactivity through a variety of mechanisms, including hydrogen support, singlet oxygen quenching, and radical scavenging capacity (Sirivibulkovit et al. 2018; Antolovich et al. 2022). Antioxidants play an important role as a protective factor for human health. Scientific research and associated evidence indicate that antioxidants tend to reduce the risk of numerous chronic diseases, especially cancer and heart disease (Tailor & Goyal 2014). The method of 2.2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging is used as a fast simple and inexpensive process to calculate the antioxidant capacity of foods (Kirtikar & Basu 2006; Tailor & Goyal 2014).

Carotenoids are one of the classes of organic compounds widely used in the food industry for their colouring power and growth potential (Mezzomo & Ferreira 2016). Carotenoids are known as tetraterpene pigments, which are usually coloured yellow, orange and red (Maoka 2020). Commonly found in nature, carotenoids are found in almost all colourful fruits and vegetables/plants. Carotenoids currently used in industries can be chemically synthesised by extracting only a small percentage of those taken from plants or algae. Considering that consumers' preferences and demands for natural compounds are high, it can be said that there is a general tendency towards products with natural ingredients (Mezzomo & Ferreira 2016). Photosynthetic bacteria can be found in some species of fungi, algae, plants and animals. Despite their general skeleton of 40 carbons, carotenoids are composed of eight units of isoprene. Their structures consist of a polyene chain with nine conjugated double bonds and final groups on both ends (Maoka 2020). Aquatic organisms may contain different types of carotenoids, which can be obtained nutritionally from algae and other different organisms and can be modified by metabolic reactions. Most of the carotenoids found in aquatic organisms can be cited as β-carotene, fucoxanthin, peridinin, diatoxanthin, alloxantin and astaxanthin (Matsuno 2001; Maoka 2009, 2011, 2020).

This paper is the first attempt to report the carotenoids and their radical scavenging properties of *P. leptodactylus,* vital for human health and nutrition, from the Atikhisar Reservoir (Çanakkale, Türkiye). The more suitable period for consumption was also determined by investigating whether this property changes according to the month of the year. A review of international literature showed that research on the species has focused primarily on unsaturated fatty acids, and there were significant gaps. In this regard, we tried to provide additional data on the subject in this study.

2. Materials and methods

2.1. Study area and sampling

The present study was carried out in Atikhisar Reservoir (Çanakkale, Türkiye) (Figure 1) which is constructed on the Sarıçay Stream. Atikhisar Reservoir provides drinking water for the local people inhabiting Çanakkale (Kale 2019) and is the only source of potable water for the people in the region (Kale & Acarlı 2019a; Kale et al. 2020). In addition, the reservoir provides water for both agricultural activities and domestic use (Kale & Acarlı 2019a, 2019b).

Crayfish samples were collected monthly from the reservoir using fyke nets between July 2020 and

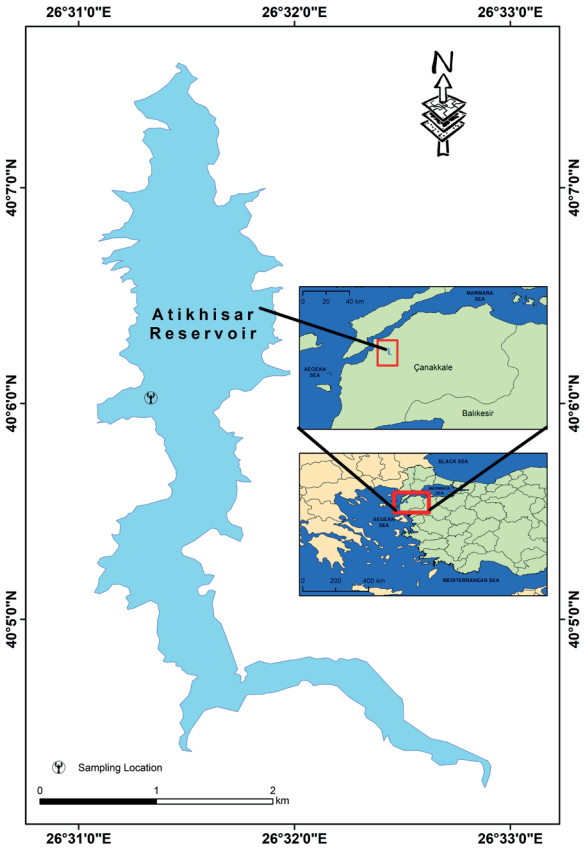

Figure 1

Study area, Atikhisar Reservoir (Çanakkale, Türkiye)

June 2021. The samplings were conducted during the daytime. The fyke nets were left at the bottom of the reservoir and collected after 3 days. The monthly sample size was 30 individuals for both females and males. The collected specimens were secured in styrofoam boxes and transferred to the Biochemistry Lab of the Faculty of Marine Sciences and Technology at Çanakkale Onsekiz Mart University (Çanakkale, Türkiye) for the laboratory experiments and analyses. The shell and meat of female and male crayfish were extracted and homogenised separately according to sex.

2.2. Antioxidant radical scavenging capacity (DPPH)

The samples were extracted by methanol to find out the antioxidant radical scavenging capacity. Determining the free radical scavenging capacity requires using DPPH (2,2-Difenil-1-Pikrilhidrazil) as a stable and synthetic radical, and the antioxidant activity is determined by measuring the ability of the antioxidant to catch free radicals. DPPH is a dark purple coloured radical and its colour can be lightened after its reduction by antioxidant matter, which is the most widely used test method to measure the absorbance of the reaction of DPPH with the antioxidant at 515-517 nm (Brand-Williams et al. 1995; Huang et al. 2005). The analysis of DPPH entailed a mixture of a given amount of DPPH solution with the sample solution, reading the absorbance at 515 nm after 30 minutes and calculated according to the following formula (Equation 1):

$$DPPH(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

IC_{50} is the value to represent 50% of the inhibition concentration of those concentrations shown by the DPPH radical scavenging effect.

2.3. Total carotenoid content

To determine the total carotenoid content, we used the extraction method used by Yanar et al. (2004) and Zheng et al. (2010). Freeze-dried samples were exposed to sequential extraction with extracted samples being centrifuged to finally measure them by UV spectrophotometer. Spectrophotometric measurements were performed according to Oliveira et al. (2010) and Biehler et al. (2010) and Lichtenthaler & Buschmann (2001) by 450 and 470 nm, respectively. Three different calculation methods (Car_1, Car_2, Car_3) were used to calculate carotenoid as given below (Equations 2-4) according to Oliveira et al. (2010), Biehler et al. (2010), and Lichtenthaler & Buschmann (2001), respectively.

$$Car_1(mg \times g^{-1}) = \frac{A_{450} \times V(ml) \times 104}{A_{1cm}^{1\%} \times W(g)} \quad (2)$$

$$Car_2(mg \times g^{-1}) = \frac{A_{450} \times V(ml) \times Ma \times d \times 103}{135310 \times W(g)} \quad (3)$$

$$Car_3(mg \times g^{-1}) = \frac{Car(x+c) \times V(ml)}{W(g)} \quad (4)$$

where A_{450} is absorbance measurements at 450 nm; $A_{1cm}^{1\%}$ is the absorption coefficient of β-carotene in petroleum ether (2592); *d* is the length of measurement cover (1 cm); *Ma* means molecular weight for carotenoids (548 g); *V* is the volume (ml) and *W* is the weight (g).

2.4. Data analysis

The normal distribution of data was analysed using the Kolmogorov-Smirnov test of normality ($p > 0.05$). Pearson correlation analysis was performed to determine the relationship between the carotenoid analysis method and IC_{50}. Monthly data of carotenoid and IC_{50} were also analysed by one-way ANOVA. The difference between the groups was determined by a non-parametric Kruskal-Wallis test by considering the results of the Levene's test. In addition, a chi-square test was used to determine the difference between females and males. All statistical analyses were performed using the SPSS program 23.0 version for Windows.

3. Results

The radical scavenging effect was studied in meat samples of female and male crayfish with the DPPH method, and the relevant results are presented in Table 1. The IC_{50} value indicates the radical scavenging capacity. The better the radical scavenging effect, the lower the IC_{50} value. The IC_{50} value was the lowest in May with 122 mg g^{-1} in female individuals, while the highest value was in July with 388 mg g^{-1}. On the other hand, male individuals showed the lowest and highest values of IC_{50} in July and November, respectively.

The study measured the total carotenoid content in the meat and shells of crayfish individuals. Table 2 illustrates the total carotenoid content in the samples collected over 12 months and calculated by different methods. The highest and the lowest values in female meat samples were found to be 21.26-3.38 µg g^{-1} (Car_1) with a mean of 14.35 µg g^{-1}. Consequently, it can be said that a mean value of 14.35 µg g^{-1} in the sample is a moderately good total carotenoid content in female individuals. Meat samples from the male individuals were calculated as 12.78 µg g^{-1} and it was concluded that there was no difference between female and male individuals. However, the carotenoid content in the shells of female and male individuals varied between 23 and 25 µg g^{-1}. Comparison of the carotenoid of the shell with that of the meat indicates an almost two-fold difference between them – therefore, more carotenoids in the shell than in the meat samples.

The highest carotenoid content was observed in the meat of female and male individuals in November and September, and in the shells of female and male individuals in October and August, respectively (Table 2). While relatively higher carotenoid content values were observed in the meat in August and September compared to other months, it was found in shells in November and September.

Total carotenoid content in the shell and meat samples varied by month ($p \leq 0.05$) (Table 2). No correlation was observed between the carotenoid values and IC_{50} values in the meat of both female and male individuals ($p > 0.05$) (Table 3). However, the results of the Pearson correlation analysis revealed a strong correlation between the calculations ($p \leq 0.05$) (Tables 3-4).

Table 1

IC_{50} values (mg g^{-1}) for the DPPH scavenging effect in the meat of female and male crayfish

Month	IC_{50} (mg g^{-1})	
	Female	Male
July	388.77	41.94
August	291.18	62.49
September	264.05	58.19
October	334.00	157.33
November	259.21	257.71
December	231.95	134.89
January	222.46	75.77
February	236.40	56.45
March	160.67	155.53
April	232.14	63.16
May	122.65	113.31
June	134.51	85.77
Mean	239.83	105.21
SD	74.26	59.78

SD is the standard deviation

4. Discussion

IC_{50} (mg g^{-1}) is used to determine antioxidant capacity via/through the DPPH method (Kizilkaya et al. 2021). The antioxidant mechanism of carotenoids is often known as radical scavenging, a method based on measuring the ability of the antioxidants to scavenge radicals. Now that the process is simple, easy, fast and can be used even with a small-scale sample, it is an in-vitro method frequently preferred when considering antioxidant activity, which requires DPPH to be used only without adding any substrate, otherwise free radicals tend to attach directly to it (Fawwaz et al. 2020). It can be said that the IC_{50} found is inversely proportional to the antioxidant capacity (Manuguerra et al. 2020). The lowest values IC_{50} found were 122.65 mg g^{-1} and 41.94 mg g^{-1} in the meat of female and male crayfish individuals, respectively. The highest values of IC_{50} were seen in July as 388.77 mg g^{-1} and 257.71 mg g^{-1} for female and male individuals, respectively. This refers to the lowest radical scavenging effect. In other words, the highest antioxidant capacity

Table 2

Monthly variation of carotenoid content in the meat and shells of female and male crayfish

Month	Car_1 (μg g^{-1})		Car_2 (μg g^{-1})		Car_3 (μg g^{-1})	
	Female	Male	Female	Male	Female	Male
Meat						
July	6.76 ± 0.06	5.32 ± 0.40	6.84 ± 0.06	5.39 ± 0.40	7.29 ± 0.27	4.91 ± 0.53
August	20.68 ± 0.85	22.78 ± 2.91	20.94 ± 0.86	23.06 ± 2.95	15.08 ± 1.55	21.51 ± 1.25
September	20.62 ± 1.39	21.98 ± 1.78	20.88 ± 1.40	22.25 ± 1.80	25.13 ± 1.38	24.45 ± 1.56
October	14.42 ± 0.48	11.28 ± 0.85	14.60 ± 0.49	11.42 ± 0.86	17.44 ± 0.76	11.32 ± 0.08
November	21.26 ± 1.73	15.42 ± 1.22	21.53 ± 1.75	15.61 ± 1.23	26.76 ± 2.39	16.55 ± 1.18
December	3.38 ± 0.03	4.84 ± 0.40	3.42 ± 0.03	4.90 ± 0.40	3.73 ± 0.10	3.22 ± 0.20
January	10.04 ± 1.87	12.54 ± 1.22	10.17 ± 1.89	12.70 ± 1.23	10.01 ± 0.15	11.27 ± 1.62
February	11.28 ± 0.06	9.98 ± 0.20	11.42 ± 0.06	10.10 ± 0.20	14.45 ± 2.59	8.64 ± 1.76
March	13.20 ± 2.09	12.60 ± 2.04	13.36 ± 2.12	12.76 ± 2.06	13.71 ± 0.14	11.61 ± 0.48
April	15.82 ± 1.10	13.50 ± 2.23	16.02 ± 1.12	13.67 ± 2.26	17.36 ± 0.34	14.26 ± 2.98
May	18.10 ± 3.82	11.62 ± 2.12	18.33 ± 3.87	11.77 ± 2.15	17.45 ± 0.23	9.71 ± 1.00
June	16.64 ± 1.13	11.46 ± 2.01	16.85 ± 1.15	11.60 ± 2.03	16.60 ± 1.12	7.53 ± 1.26
Mean	14.35	12.78	14.53	12.94	15.42	12.08
SD	5.69	5.43	5.76	5.50	6.55	6.30
Min	3.38	4.84	3.42	4.90	3.73	3.22
Max	21.26	22.78	21.53	23.06	26.76	24.45
Shell						
July	23.30 ± 2.69	25.52 ± 2.15	23.59 ± 2.72	25.84 ± 2.18	26.53 ± 3.07	30.60 ± 3.38
August	25.92 ± 3.45	31.44 ± 1.75	26.24 ± 3.49	31.83 ± 1.78	23.96 ± 1.91	37.40 ± 0.63
September	31.14 ± 3.59	25.76 ± 2.04	31.53 ± 3.64	26.08 ± 2.06	36.11 ± 5.35	29.83 ± 2.72
October	38.96 ± 1.81	17.94 ± 3.14	39.45 ± 1.83	18.16 ± 3.18	44.09 ± 2.17	18.15 ± 1.72
November	37.98 ± 1.27	17.58 ± 3.54	38.45 ± 1.29	17.80 ± 3.58	43.40 ± 1.09	18.00 ± 2.35
December	24.30 ± 0.25	24.52 ± 4.58	24.60 ± 0.26	24.83 ± 4.64	26.54 ± 1.51	26.63 ± 2.53
January	18.32 ± 1.30	21.28 ± 0.85	18.55 ± 1.32	21.55 ± 0.86	20.17 ± 1.38	21.32 ± 3.21
February	19.38 ± 1.90	18.80 ± 0.23	19.62 ± 1.92	19.03 ± 0.23	21.09 ± 1.73	20.69 ± 0.79
March	18.70 ± 0.65	23.86 ± 0.31	18.93 ± 0.66	24.16 ± 0.32	20.94 ± 0.67	26.95 ± 1.41
April	20.54 ± 1.33	27.72 ± 0.57	20.80 ± 1.35	28.07 ± 0.57	22.31 ± 0.71	32.61 ± 1.12
May	24.08 ± 0.57	22.78 ± 2.69	24.38 ± 0.57	23.06 ± 2.72	26.57 ± 0.36	22.93 ± 3.35
June	25.86 ± 3.37	25.98 ± 2.57	26.18 ± 3.41	26.30 ± 2.61	27.04 ± 6.57	29.72 ± 2.18
Mean	25.71	23.60	26.03	23.89	28.23	26.24
SD	6.98	4.16	7.07	4.22	8.40	6.10
Min	18.32	17.58	18.55	17.80	20.17	18.00
Max	38.96	31.44	39.45	31.83	44.09	37.40

SD is the standard deviation; Min is the minimum value; Max is the maximum value

Table 3

Pearson correlation between IC_{50} and radical DPPH calculated by different equations for the carotenoid content in the meat of female and male crayfish

♀	IC_{50}	Car_1	Car_2	Car_3	♂	IC_{50}	Car_1	Car_2	Car_3
IC_{50}	1				IC_{50}	1			
Car_1	-0.206	1			Car_1	0.018	1		
Car_2	-0.206	1.000**	1		Car_2	0.018	1.000**	1	
Car_3	-0.148	0.959**	0.959**	1	Car_3	0.005	0.971**	0.971**	1

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

Table 4

Pearson correlation between radical DPPH calculated by different equations for the carotenoid content in the shells of female and male crayfish

♀	Car_1	Car_2	Car_3	♂	Car_1	Car_2	Car_3
Car_1	1			Car_1	1		
Car_2	1.000**	1		Car_2	1.000**	1	
Car_3	0.976**	0.976**	1	Car_3	0.984**	0.984**	1

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

was observed in female crayfish in March, April, May and June. Although antioxidant capacity varies continuously in male individuals, it can be said that it is higher in the summer months. During the study, it was determined that female individuals lost their granule-like appearance due to the increase in the size of the eggs in the body in July, August, September and October. Consumption or storage of antioxidants in the bodies of crustaceans is associated with whether they are in the reproduction period or hormonal activity, seasonal changes and the quality and quantity of food they eat (Valgas et al. 2020). It has been observed that female individuals tried to consume more energy to mature their eggs, particularly during the summer period of the study. Therefore, the IC_{50} value was high in this period whereas antioxidant capacity remained low due to increased energy consumption. Male individuals require less energy to produce sperm than females do for oogenesis, which could cause a lower IC_{50} value in males than females during the study. Marine carotenoids show strong antioxidant and reparative, antiproliferative and anti-inflammatory effects and can be used as nutraceutical/cosmeceutical agents for photoprotection of the skin from solar UV radiation or for the prevention of diseases caused by oxidative stress (Nichols & Katiyar 2010; Berthon et al. 2017). Diets rich in carotenoids have been attributed to the reduction in risks of some chronic and degenerative diseases such as cancer, cardiovascular disorders (Nishino 1998), cardiovascular disorders (Sesso et al. 2004) and age-related molecular degeneration (Zeegers et al. 2001). Carotenoids are a group of yellow-orange pigments divided into two general classes known as hydrocarbon carotenoids and xanthophylls in the form of oxidised derivatives. There are more than 650 carotenoids found naturally in bacteria, fungi, plants and animals (Matsuno 2001). Carotenoids from marine animals have different structures, most of which can be obtained from β-carotene, fucoxanthin, peridinin, diatoxanthin, alloxantin and astaxanthin (Maoka 2011). Studies have shown that carotenoids are abundant in edible species of molluscs, crustaceans and echinoderms among marine organisms (Kantha 1989; Matsuno 2001; Maoka 2011; Wade et al. 2017; Kizilkaya et al. 2021). Carotenoids are strong antioxidants, so they have the capacity to scavenge free radicals (Miki 1991). Carotenoids are generally synthesised by photosynthetic algae and plants, fungi and bacteria while other organisms obtain the carotenoids they need directly from foods or carotenoid precursors in their diets by modifying

them via metabolic reactions (de Carvalho & Caramujo 2017). Crustaceans do not synthesise carotenoids, but instead obtain them directly or partially from foods by modifying them through metabolic reactions (Castillo et al. 1982).

The total carotenoid content in freshwater crayfish varies according to the species and the organ in which it is stored (Czeczuga & Czeczuga-Smeniuk 1999), reproductive activity (Sagi et al. 1995), growth and, finally, nutrient abundance and diversity (D'Abramo et al. 1983). The present study revealed that carotenoid content in the meat and shells of female and male individuals varied according to months, this can be attributed to the variable consumption of carotenoid reserves during periods of active feeding, oogenesis in females and, finally, mature individuals shedding their skin. Seasonal differences in the total amount of carotenoids could be influenced by the quality and quantity of ingested food, as well as direct and indirect nutrition/feeding (Yanar et al. 2004). In the present study, the total carotenoid content was found to be 3.38 μg g^{-1} in females and 4.84 μg g^{-1} and males in meat samples, in the winter when the water temperature is low. Freshwater crayfish tend to abandon active feeding and retreat to their nests at temperatures below 10°C, when they do not ingest food from outside during this period. Carotenoids are stored in various organs (muscle, carapace, hepatopancreas, etc.) of the body (Sagi et al. 1995; Czeczuga & Czeczuga-Smeniuk 1999). The stored carotenoid is used when feeding activity is low to optimise health during developmental processes or in response to environmental stress such as pollution or parasites (Barim & Karatepe 2010; Caramujo et al. 2012; Wade et al. 2017). Czeczuga & Czeczuga-Semeniuk (1999) compared the total carotenoid contents in the meat and carapaces of *Astacus leptodactylus, Pacifastacus leniusculus, Oconectes limosus* and *Astacus astacus* and found that other species except *Astacus leptodactylus* stored more carotenoids in their carapace. Su et al. (2018) compared the total carotenoid content in different organelles in shrimp species produced under aquaculture conditions in China and reported that their shells contained significantly higher carotenoids than abdominal meat. Similarly, the present study determined that the shells of female and male individuals contained much more carotenoids. Cilbiz (2010) reported that the carotenoid contents in the meat and shells of *P. leptodactylus* in Eğirdir Lake (Türkiye) in October were 3.59 mg kg^{-1} and 19.43 mg kg^{-1} in females and 5.22 mg kg^{-1} and 22.20 mg kg^{-1} in males, respectively. The body coloration in crustaceans depends on specific pigments (carotenoids are the most important) present in the major layer of the exoskeleton and subepidermal chromatophores (Su et al. 2018; Stachowiak & Szulc 2021). The abundance and quality of food directly affect the storage of carotenoids (D'abroma et al. 1983; Wang et al. 2021). It can be clearly concluded that the carotenoid content is higher in the shell. As reported by Hansson (2004), Tlusty et al. (2009) and Wade et al. (2017), it can be suggested that the high amount of carotenoids in the shell is due to photoprotection, which protects sensitive tissues from the oxidizing effects of sunlight, especially from the need for protection from UV radiation, and the desire to defend against predators. In this context, it can be argued that the higher amount of carotenoids in the shell in the summer when the daylight is longer compared to the winter is due to the photoprotection behaviour.

Free radicals are high-energy reactive molecules containing one or more unpaired electrons, formed as a result of natural body functions such as respiration and digestion. It is known that the free radicals formed are associated with cancer, atherosclerosis, Alzheimer, Parkinson, premature ageing and some chronic diseases. Antioxidants are capable of scavenging free radicals and preventing cell damage. In recent years, interest in aquatic products, which have an important place in terms of healthy nutrition, has been increasing and attracting great attention in healthy diets. Crustaceans have high levels of carotenoids in their waste as well as in their soft tissues. *P. leptodactylus* is one of the most economically important crustacean species in the freshwater ecosystem. However, studies on the nutritional value of this species are limited. Therefore, more studies should be carried out.

Growth and reproduction activities in crayfish differ depending on seasonal changes (McLay & van den Brink 2016). The reproduction activity of *P. leptodactylus* is mostly dependent on environmental factors. However, it is interesting to note that the process usually starts in the autumn when the water temperature begins to decrease. Mating occurs in October and November when the water temperature is 7–12°C, followed by spawning within 4 or 6 weeks when the water temperature is 6–11°C. *A. leptodactylus* reproduces only once a year with low fertility and long embryonic development (6–9 months) under natural conditions (Reynolds et al. 1992). Its energy reserves generally tend to decrease during the process of reproduction and moulting. Muscle and hepatopancreas are organelles where energy is stored, especially in lipids (Harrison 1990; Moore et al. 2000; Berber et al. 2014; Mazlum et al. 2019) and protein would be needed and provided

for ovogenesis/egg formation (Harlıoğlu et al. 2021). Oliveira et al. (2007) reported that the *Parastacus defossus* species exhibited an increased energy demand related to gamete production in summer, incubation and spawning in autumn and winter, and parental care in the spring and summer months. Generally, changes in metabolic requirements during the life cycle lead to different ROS proliferation/production and potentially affect (its) susceptibility to oxidative stress. In other words, the high energy demand for reproduction causes more ROS formation. Endocrine, behavioural and antioxidant mechanisms help crustacean species to manage environmental influences and oxidative reactions as long as they live (Fanjul-Moles & Gonsebatt 2011).

Marine invertebrates have long been investigated for their pharmacologic activities or other bioactive characteristics used in the biomedical field. As natural products of marine origin are becoming increasingly attractive as a result of current and potential processes in the pharmaceutical industry, the identification of new sources of those materials is of great importance (Pachaiyappan et al. 2014). It has been widely accepted that oxidative stress caused by an imbalance between prooxidants and antioxidants in an organism is mainly attributed to the root cause of chronic diseases (Urquiaga & Leighton 2000). Oxidative stress occurs due to the imbalance between the proliferation of Reactive Oxygen Species (ROS) and the antioxidant capacity of the organism. ROS could also occur during immune system reactions caused by external factors such as UV radiation. The proliferation of ROS occurs during normal psychological processes in a variety of cellular divisions as well (Zglińska et al. 2022). As a consequence of potential damage caused by this ROS reactivity to cells and tissues, marine organisms and other living things balance the proliferation of these radicals through various cellular antioxidant defensive mechanisms (Correia et al. 2003). Antioxidants consist of a large number of compounds and bioactive molecules based on a spectrum of sources (Reddy et al. 2011). Those obtained from natural sources play an important role in the neutralisation of oxidative stress (Sasikumar et al. 2009). Therefore, antioxidants have gained more importance and popularity due to their positive effects in the treatment of cardiovascular deficiencies, atherators, atherosclerosis, many types of cancer, ageing and related geriatric processes.

The main chemical components of crayfish shell waste are calcium carbonate, proteins, and chitin (Nguyen 2021). Among these components, chitin is considered to be the most valuable biomass material from crayfish shells (Shamshina et al. 2019). Efficient treatment and use of crayfish shell waste is critical for achieving sustainable development. Therefore, the extraction of chitin from crayfish shell waste is beneficial for solving the problem of crayfish shell waste accumulation, as well as further improving the value-added utilisation of crayfish shell waste (Li et al. 2023). Crayfish shells are a source of chitin, an antioxidant, and a potential source of beneficial dietary fiber (Bai et al. 2023). Chitin is a valuable biomaterial because of its antioxidant and antimicrobial properties, biocompatibility, non-toxicity, and biodegradability (Benhabiles et al. 2012). It is commonly used in food, water treatment, pharmacy medicine, and textiles (Felse & Panda 1999; Rinaudo 2006; Aranaz et al. 2009; Muzzarelli 2011; Kaya et al. 2015; Laribi-Habchi et al. 2015). Harish Prashanth & Tharanathan (2007) noted that chitin shows promise in a wide range of application, such as food and nutrition (food preservation and antioxidants), medical science (drugs and pharmaceuticals), microbiological (antibacterial), immunological (gene therapeutics and immune potentiator), and material science (packaging films and polymeric membranes). Chitosan is also broadly used in many sectors, mainly in food packaging, environmental protection, pharmaceutical, cosmetics, medicine, dentistry, textile, agriculture, veterinary, chemistry, and biotechnology (Kucukgulmez et al. 2016; Tekelioglu et al. 2017; Mirtajaddini et al. 2021; Farivar et al. 2022; Kadak et al. 2023). Moreover, carotenoids and carotenoproteins are responsible for most of the colour found in the exoskeletons and shells of crayfish and other crustaceans (Schuster 2020). Therefore, crayfish shells can be used to produce astaxanthin pigments used in the trout farming industry by colouring eggs and flesh. On the other hand, crayfish can be used as a biological indicator of exposure to both organic and inorganic pollution in aquatic systems (Schilderman et al. 1999). Crayfish are suitable biomarkers of heavy metal contamination of freshwater ecosystems due to their rapid bioaccumulation and long retention times (Kouba et al. 2010). Refaat Mohamed Morsi et al. (2023) used crayfish chitosan composite modified film, prepared from the exoskeleton of *Procambarus clarkii*, in treating water copper toxicity. Lu et al. (2023) used magnetic crayfish shell biochar to remove Sr(II) in an aqueous solution. Recently, carbon dots, as a new class of zero-dimensional carbon nanomaterials, have been widely used as fluorescent probes in the field of contaminant analysis due to their tunable photoluminescence, excellent solubility, low toxicity, chemical inertness, and good biocompatibility. Chen et al. (2023) used crayfish shells as precursors to prepare a new type of carbon dots and the proposed sensor was successfully applied for the detection of

4-NP in fresh crayfish meat and aquatic water samples. He & Du (2023) detected tartrazine with fluorescence sensor from crayfish shell carbon quantum dots.

In conclusion, this study investigated antioxidant radical scavenging capacity and total carotenoid content in the meat and shells of the narrow-clawed crayfish (*P. leptodactylus*) from the Atikhisar Reservoir in Çanakkale, Türkiye. The highest IC_{50} values for scavenging of free radicals were found to be 388.77 mg g^{-1} and 155.53 mg g^{-1} for female and male individuals in July and March, respectively. The IC_{50} value showed statistically significant variation in female and male individuals ($p \leq 0.05$). The mean IC_{50} values of the meat were calculated as 239.83 mg g^{-1} and 105.21 mg g^{-1} in females and males, respectively. This indicates that the IC_{50} value is high but the radical scavenging effect is low in females. It is thought that this is due to the fact that female individuals actively consume most of the body biocomponents as broodstock. On the other hand, it is predicted these biocomponents are stored in male individuals. The mean total carotenoid content in the meat was found to be 14.35 and 12.78 µg g^{-1} for female and male individuals, respectively. It was 25.71 and 23.60 µg g^{-1} in the shells of female and male individuals, respectively. The results indicated that the meat of the narrow-clawed crayfish has antioxidant radical scavenging capacity and is rich in carotenoid content.

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Statements and Declarations

Conflict of interest

No potential conflict of interest was reported by the authors.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

Not applicable

Consent to participate and consent to publication

Not applicable

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