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Determination of total Carotene and Chlorophyll contents of tellina (*Donax trunculus* Linnaeus, 1758) using UV spectrophotometer in Black Sea

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Abstract

Present study investigates monthly changes of the total carotene and chlorophyll a content of tellina (*Donax trunculus* Linnaeus, 1758) from Kefken territory on the Black Sea coast between 2013 and 2014. The total carotene and chlorophyll analysis was determined by UV spectrophotometer. Three different calculation methods were used for total carotenoid calculation. The methods that have been chosen are comparable with the available methods in the literature. The highest carotene amount was determined in March about 78.91 µg/g as Car_2 (secondary carotene calculation method). Furthermore, the lowest carotene amount was determined in November. The highest and the lowest amount of chlorophyll-a was detected in July about 84.90±2.60 µg/g and June about 8.11±0.39 µg/g, respectively. It is possible to say that tellina can be consumed as food. In respect of the detections in this study, it can be said that tellina can be conveniently consumed between February and July depending on the territory.

Keywords: *Donax trunculus*; Carotenoids; Chlorophyll.

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1. Introduction

Due to the rise in population worldwide, food consumption increases at the same time. Within seafood bivalves are one of the increasingly consumed food resource in our country too. Carotenoids are one of the most important food and antioxidant compounds. Carotenoids take place in the oxidative metabolism of cell (Goodwin, 1952; Isler, 1971). Generally, animals do not synthesise anew carotenoid and those that take place in animals are either accumulated by food or partly modified by metabolic reactions. Therefore, the model of carotenoid in animals is has a key role for not only metabolic way but also food chain (Liaaen-Jensen, 1998; Suckling *et al*., 2020). In a study it is stated that molluscs can be divided into three groups in respect of tolerance for environmental pollution considered with the decrease in dissolved oxygen concentration and the increase of toxic materials; additionally, the species in the second and the third groups significantly vary in carotenoid concentration in their bodies. It is stated that low concentration of carotenoids is characteristic for the second group and their population decreases when the pollution increases (Karnaukhov *et al*., 1977). It is found that black mussels collected from Sevastopol Bay involve high carotenoid intent. The value of carotenoid is found as 500 µg/100g (w/w) wet meat (Posleslova and Nekhoroshev, 2003). Stancheva *et.al.* (2017) analyzed the carotenoid values and found that β-carotene value of black mussels cultivated in Varna was 107, 117, 221, 481 µg/100g (w/w); whereas, it was 70 µg/100g (w/w) as wet meat in the mussels collected from nature. In the studies of Maoka *et al*. (2005), *C. japonica*, *C. sandai*, ve *Corbicula* sp.'nin (Chinese Freshwater Corbicula Clam) on carotenoid content, it is found that total carotenoid content was 5.3, 2.6 and 0.3 mg/100g as wet meat respectively. It is stated that carotenoid content of *C. Japonica* and *C. Sandai* was more than the study of Matsuno and Hirao (1989); moreover, it was added that carotenoid content of China fresh water sand mussels was lower than *C. Japonica* and *C. Sandai*. Maoka *et al*. (2010) found that carotenoid content of *R. philippinarum* and *M. petechialis* was 1.15 and 0.5 mg/100g as wet meat, respectively. β-carotene, fucoxanthin, fucoxanthin 3-ester, fucoxanthinol, fucoxanthinol 3-ester, crassostreaxanthin A, crassostreaxanthin A 3-acetate, crassostreaxanthin B, crassostreaxanthin B 3-acetate, halocynthiaxanthin, halocynthiaxanthin 30-acetate, alloxanthin, diatoxanthin, diatoxanthin 3.6-epoxide, diadinoxanthin, and heteroxanthin carotenoids were detected in both of the species.

This study examines the convenient consumption time of tellina from Kefken, and the changes of its total carotenoids due to seasonal temperatures.

2. Materials and methods

2.1. Collection of the Samples

Samples of Tellina (*Donax trunculus* Linnaeus, 1758) were collected in depths of 1-2 meters from the coast by dredge with a mesh width of 1.6 cm. The samples were monthly provided from the Kefken station between November 2013 and October 2014.

2.2. Determination of Carotenoids

In order to assess total carotenoid, the extraction method (Yanar *et al*., 2004; Zheng *et al*., 2010) was used. The sampled dried by freeze-dryer was extracted serially a few times with acetone. Then, the extracted sample solutions were centrifuged and measured by UV spectrophotometer. Spectrophotometric measurements were carried out by using Lichtenthaler and Buschmann (2001) with 450 nm absorbance according to Oliveira *et al*. (2010); and 470 nm absorbance according to Biehler *et al*. (2010). Three different calculation methods were used for total carotenoid calculation using following formulas which the first one according to Oliveira *et al*. (2010) is Car_1, the second one Car_2 is according to Biehler *et al.* (2010), and the third one Car_3 is according to Lichtenthaler and Buschmann (2001). Moreover, one of the samples chlorophyll a (Chl_a) was calculated according to Lichtenthaler and Buschmann (2001).

Car_1 (µg/g) =
$[A_{450} \times V\ (mL) \times 104] / [A_{1cm}^{1\%} \times W\ (g)]$
Car_2 (µg/g) =
$[A_{450} \times V\ (mL) \times Ma \times d \times 103] / [135310 \times W\ (g)]$
Car_3 (µg/g) = $Car_{(x+c)} \times V\ (mL) / W\ (g)$
$Car_{(x+c)}$ (µg/mL) =
$(1000 \times A_{470} - 1{,}90 \times C_a - 63{,}14 \times C_b)/214$
Chl_a (µg/g) = $C_a \times V\ (mL) / W\ (g)$
C_a (µg/mL) = $11.24 \times A_{662} - 2.04 \times A_{645}$
C_b (µg/mL) = $20{,}13 \times A_{645} - 4{,}19 \times A_{662}$

where;
$A_{1cm}^{1\%}$ = The absorption coefficient (2592)
$Car_{(x+c)}$ (µg/mL) = Amount of xanthophylls (x) and carotenes (c) in the extract of the sample
Ma = Average Molecule Weight for Carotenoids (548 gr)
V = Volume (mL)
W = Sample weight (g)
d = The path length of the cuvette in cm
C_a = µg/mL value of chlorophyll-a within the samples
C_b = µg/mL value of chlorophyll-b within the samples
A_{450} = Absorbance in 450 nm
A_{662} = Absorbance in 662 nm
A_{645} = Absorbance in 645 nm
A_{470} = Absorbance in 470 nm

PG Instruments T80+ model UV/VIS spectrophotometer device, which takes place in the Feed and Food Analysis Laboratory of Faculty of Marine Sciences and Technology at Çanakkale Onsekiz Mart University, was used for carotenoid measurements.

2.3. Statistical analyses

Data were given as mean ± SD for each of the measured variables. Kruskall-Wallis test was used for determining the differences in non-normal data (Kruskall & Wallis, 1952). In the correlation graph, the boxes that the columns and lines coincide show the relation of the variates. As the number of the stars and the type size of the numbers in the box increases, the relevancy increases too. Statistical significance ($p<0.05$) of Carotene and chlorophyll concentrations in mussels were tested using (PCA) R software package (V. 2.13.1). Pearson's correlation coefficients were used to describe the relationships between nutrient ingredients (Freedman *et al*., 2007).

3. Results

Carotene and chlorophyll content of the species which obtained from Kefken station between November 2013 and October 2014 is given in Table1 and the amount of carotenoid per month is given in Figure1. As it is seen, carotene content of tellina is Car_1 between 21.88 µg/g

(November) and 77.96 µg/g (March); and Car_2 values are between 22.15 µg/g (November) and 78.91 µg/g (March), and Car_3 values are between 11.58 µg/g (November) and 51.76 µg/g (March). Chlorophyll-a values are between 8.11 µg/g (June) and 84.90 µg/g (July).

Table 1. Carotene and chlorophyll content of *D. trunculus* (µg/g), Kefken station (November 2013 to October 2014)

	Car_1 (µg/g)	Car_2 (µg/g)	Car_3 (µg/g)	Chl_a (µg/g)
November	21.88±0.50	22.15±0.52	11.58±0.51	16.63±0.51
December	34.88±1.24	35.31±1.26	22.35±0.53	28.47±0.88
January	59.23±1.88	59.96±1.92	36.16±1.31	50.58±1.39
February	76.65±2.13	77.59±2.19	48.11±1.99	60.93±2.72
March	77.96±2.21	78.91±2.26	51.76±1.46	28.86±0.90
April	35.50±1.27	35.94±1.30	17.64±0.26	28.11±0.86
May	36.50±1.33	36.95±1.36	27.44±0.82	22.97±0.57
June	36.34±1.32	36.79±1.35	35.03±1.25	8.11±0.39
July	68.11±1.65	68.94±1.70	30.57±1.00	84.90±2.60
August	36.50±1.33	36.95±1.36	30.85±1.01	13.25±0.46
September	52.04±2.21	52.68±2.25	38.73±1.46	31.71±1.06
October	37.42±1.38	37.88±1.41	24.86±0.67	24.10±0.63

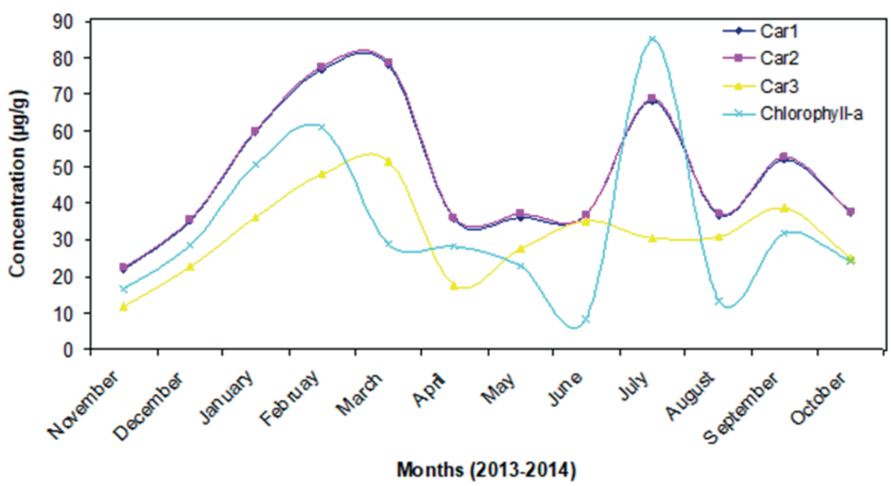

Figure1. Carotenoid content of tellina per month

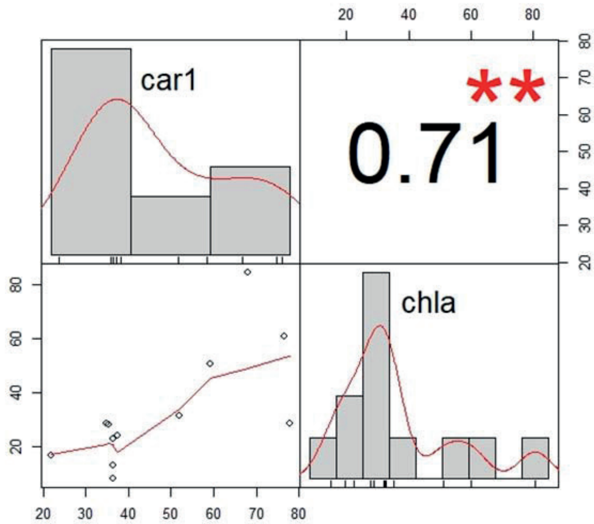

Figure 2. The correlation between carotene (car_1) and chlorophyll-a

The Figure 2 shows the correlation between carotenoid and chlorophyll-a that represents there is a positive correlation between these two factors and these factors are directly proportionate to each other considerably ($p<0.05$).

4. Discussion

Carotenoids have an important role in the oxidative metabolism of the cell, but they cannot be synthetized by animals. They are taken from outside to the body and stored by the change via biochemical reactions. Therefore; carotene contents of the living creatures are related to the dietary habit (Goodwin, 1952; Isler, 1971; Liaaen-Jensen, 1998). In this study, carotene values are found as between 21.88 µg/g and 77.96 µg/g. Posleslova and Nekhoroshev (2003) found that black mussels involve high amount of carotene content. Carotene content of C. Sandai, C. japonica, and Corbicula sp. are found as 2.6, 5.3, and 0.3 (mg/100 g wet meat) (Maoka *et al*., 2005). In this study; the highest amount of carotene content was found in March and July and the lowest one was found in November. Total amount of carotenoid and chlorophyll-a for tellina ascended in November and December, but in July this amount rose rapidly. In the reproduction period of tellina that corresponds to April, May, and June; it can be said that the values of carotene and chlorophyll-a remained stable relatively with the rise of gonad production. In July, it is thought that the rise of alga activity due to the increase of water temperature and the acceleration of living metabolism caused a sever increase in the amount of carotene and chlorphyll-a. In Turkey, it is declared that commercial hunting of D. trunculus, which is the subject of this study, is prohibited in Gulf of Saros and in Çanakkale by Ministry of Agriculture and Forestry according to the declaration published in the Official Gazette of Republic of Turkey (Number 4/1, Declaration of the Settings on Commercial Hunting of Sea Food, Issue: 2016/35). It is also stated that the length of mesh of the sieves must not be smaller than 1.6 cm. Provided that the individuals smaller than 1.6 cm hunted, new generations cannot replace adult individuals and it may threaten the population and even existence of tellina. The legal limits determined for sustainable hunting provide continuity of the species. Therefore, the hunters can only hunt in the procurement areas due to the legal limits stated by OSİB (2019).

Conclusion

Bivalves have a rich nutritional content of high quality. Even though it is consumed worldwide, this consumption is too low in Turkey. It is known that the bivalve consumption in Turkey did not take place much because of Turkish feeding habits and cultural prejudices since ancient times. *D. trunculus*, which is studied in this research, is one of these species of high quality that can be found in the shores of Turkey surrounded by three different seas (Black Sea, Aegean Sea and Mediterranean). Furthermore, it was represented that proteins, carbohydrates, fatty acids and amino acids, which are crucial for a healthy life, involve abundantly in tellina.

Conflict of Interest

The authors declare that there is no conflict of interest. This article was written within the scope of Evren Tan's thesis.

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