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Seasonal Changes in Total Carotenoid Contents of Some Fish and Crustaceans Inhabiting the Eastern Mediterranean Sea

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Abstract: This study was conducted to determined the total carotenoid concentration of different body parts of green tiger shrimp (*Penaeus semisulcatus*), kuruma shrimp (*Penaeus japonicus*), speckled shrimp (*Metapenaeus monoceros*), blue crab (*Callinectus sapidus*), red porgy (*Pagrus pagrus*) and red mullet (*Mullus barbatus*) caught from the coast of the Eastern Mediterranean in February and October. The carotenoid levels of head, shell and whole body of all animals were higher in October than those in February while their muscle carotenoids were not affected by seasons, except for kuruma shrimp and speckled shrimp. Amounts of carotenoids were highest in the head, decreasing in shell (or skin) and muscle. Average total carotenoid concentration among body parts of species ranged from 16.19-55.44 mg kg^{-1} in green tiger shrimp, 16.77-74.92 mg kg^{-1} in kuruma shrimp, 17.24-66.36 mg kg^{-1} in speckled shrimp, 6.41-14.34 mg kg^{-1} in blue crab, 1.37-28.77 mg kg^{-1} in red porgy and 1.30-39.26 mg kg^{-1} in red mullet. These values are quite high compared to other sea foods. Since, coloration plays an important role in consumer preference, it would be advantageous to fish these animals in October rather than February.

Key words: Shrimp, crab, red porgy, red mullet, carotenoid, seasonal changes

INTRODUCTION

Colour is one of the most important quality parameters in seafoods such as shrimp, crab and reddish fish and it affects market demand and value of these products (Gormley, 1992; Rounds *et al*., 1992; Sigurgisladottir *et al*., 1994; Skonberg *et al*., 1998). Carotenoids, especially astaxanthin are pigments responsible for typical reddish colour of these animals (Katayama *et al*., 1971, 1972; Tanaka *et al*., 1976; Okada *et al*., 1994). Their deficiency in shrimps leads a disease called blue colour syndrome (Howell and Matthews, 1991). Besides having a role in pigmentation as precursors of vitamin A and have strong antioxidant effects, carotenoids are also know to have some significant benefits to human health such as protective role against cancer and cardiovascular diseases (Ziegler, 1989; Gaziano and Hennekens, 1993; Mayne, 1996). Therefore, the carotenoids are considered important in the preparation of diet formulations and protocols for human nutrition.

Green tiger shrimp (*Penaeus semisulcatus*), kuruma shrimp (*Penaeus japonicus*), speckled shrimp (*Metapenaeus monoceros*), blue crab (*Callinectus sapidus)*, red porgy (*Pagrus pagrus*) and red mullet (*Mullus barbatus*) are among the most popular and most expensive fish and crustaceans in the Eastern Mediterranean sea. Their reddish colour is important for consumer preference towards these species. Yet, knowledge about the carotenoid content in these seafoods are limited to the data reported from the culture conditions. The carotenoid content of shrimps fed on artificial diets under culture conditions was reported to be 10-46 mg kg^{-1} in muscle, 69-107 mg kg^{-1} in head and 37-74 mg kg^{-1} in shell (Iwamato *et al*., 1990; Yamada *et al*., 1990; Chien and Jeng, 1992; Negre-Sadargues *et al*., 1993; Menasveta *et al*., 1993). On the other hand, in the studies carried out on wild shrimp species (*Metapenaeus affinis*, *M. dopsoni*, *M. monoceros*, *Penaeus indicus*, *P. monodon*, *P. vannamei*, *P. japonicus* and *Parapenaeopsis stylifera*) this content was reported as 10-17 mg kg^{-1} in muscle, 51-153 mg kg^{-1} in head and 59-104 mg kg^{-1} in shell (Gopakumar and Nair, 1975; Latscha, 1989; Yanar *et al*., 2004; Sachindra *et al*., 2005a). However, the above studies have not dealt with the same species we used in the study and have not been interested in seasonal samplings as well. Yanar *et al*. (2004) have been the first to study seasonal carotenoid changes in shrimp but the study was limited to only two shrimp species (*P. semisulcatus* and *M. monoceros*) and

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their data was collected from only muscle tissue. Similarly, few studies are available on wild crab and fish species and these studies were also utilized single seasonal sampling sand certain body parts. Total carotenoid content was measured as 4.63 mg kg^{-1} in the muscle of blue crab (Felix-Valenzuela *et al*., 2001) and 53.99 mg kg^{-1} in the skin of red porgy (Kalinowski *et al*., 2007). However, no data has been reported about the carotenoid content of red mullet.

Carotenoid amount in head, shell or skin of fish and crustaceans which form colouration play an important role in the consumer preference increasing the visual appeal of the product. Therefore, researchers need knowledge about which season the animals display intensive colouration that dictates the price of the captured products. In the present study, researchers determined the total carotenoid contents of head, shell (or skin), muscle and whole body of wild green tiger shrimp, kuruma shrimp, speckled shrimp, blue crab, red porgy and red mullet collected from the Eastern Mediterranean sea in February and October.

MATERIALS AND METHODS

All the species used in this study were obtained from the trawlers fishing off the coast of Karatas and Yumurtalik of Turkey along the Eastern Mediterranean sea in February and October of 2011. The samples were kept on ice until they arrived at the laboratory and then stored in a freezer (-20°C) until total carotenoid analyses. The main body weights of species caught in February and October were measured as 42.76±1.22 and 38.23±1.26 g for green tiger shrimp, 16.86±0.52 and 16.41±0.66 g for speckled shrimp, 26.37±0.91 and 22.46±0.82 g for kuruma shrimp, 113.32±2.54 and 121.26±2.61 g for blue crab, 203.31±2.46 and 179.00±1.82 g for red porgy and 17.62±0.74 and 16.19±0.55 g for red mullet, respectively. Samples were stored 2-3 weeks before analysis.

Carotenoid analysis: Spectrophotometric Method was used to determine the total carotenoid amount of the samples. Three animal individuals were used for each carotenoid analysis which were run in triplicate. Dissection of animals and extraction of carotenoid content of samples were conducted by modifying the procedure described by Torrissen and Naevdal (1984). Total carotenoid analyses were carried out in head, shell, muscle and whole body in shrimp, shell and muscle in crab as well as skin and muscle in fish for each season. Fish were filleted by removing the muscle on both sides between the pelvis and anus region of animals and the muscle samples were subsequently cleared of skin and fat layer. Muscle samples of crab and shrimp were collected as viscera-free from their abdominal region. Skin samples of fish were collected from both sides between their abdominal and dorsal regions and then the fat layer was removed from the skin. Shell samples of crab and shrimp were obtained from their exoskeleton. Extraction was conducted in a dark and cool room. Samples of 1-2 g body parts of animals to be analysed were taken and weighed transferring to 10 mL pre-weighed glass tubes. After anhydrous acetone was added to the tubes, the samples were ground thoroughly several times with a homogenizer (Ultraturrax, IKA-WERK, Staufen, Germany). About 1-2 g of anhydrous sodium sulphate was also added into the tube containing the tissues. In order to prevent the effects of light on carotenoids, tubes were wrapped with aluminium foils and were stored for 3 days at 4°C in a refrigerator. During this time, extractions were repeated several times until no more colour of samples could be obtained and the extractions were made >10 mL with acetone. After the solutions were centrifuged (Hettich, Rotina 420R) at 5000 rpm for 5 min, their absorptions were measured at 475 nm with a spectrophotometer (Thermo 60 S, US). Total carotenoid concentration of the samples was determined spectrophotometrically in acetone using extinction coefficients (E_1%, 1 cm) of 1900 for astaxanthin (Foss *et al*., 1984) at 475 nm.

Statistical analysis: Data were expressed as mean value±SE (n = 3). Student's t-test was used to determine the significant differences ($p<0.05$) in the total carotenoid amounts between the two seasons. Statistical procedures were performed by using the SPSS 12.0 Software for Windows. Experiments were conducted according to the European Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

RESULTS AND DISCUSSION

The present results have shown that total carotenoid contents of animals were affected by season (Table 1). The carotenoid contents of head, shell (skin in fish) and whole body of all animals were higher in October than in February ($p<0.05$) while their muscle carotenoid contents were not affected by season ($p>0.05$) except in kuruma and speckled shrimp. Only one study is available about the effects of season on carotenoid contents of sea foods (Yanar *et al*., 2004) however, these researchers used only two shrimp species (green tiger shrimp and speckled shrimp) in their study and the data was gathered from the muscle only. The carotenoid levels measured in muscle

Table 1: Total carotenoid contents of fish and crustaceans caught in October and February

Animal species	Sampling seasons	Total carotenoid concentration (mg kg^{-1}) of different body parts of animals in different seasons				
		Head	Muscle	Shell	Skin	Whole body
P. semisulcatus	February	53.04±0.63^a	15.46±0.60^a	41.28±0.28^a	-	28.37±0.07^a
(Green tiger shrimp)	October	57.84±0.79^b	16.93±0.98^a	46.89±0.36^b	-	31.58±0.64^b
	Mean	55.44±1.16	16.19±0.61	44.08±1.27	-	29.97±0.77
P. japonicus	February	71.50±0.93^a	14.06±0.33^a	58.57±0.73^a	-	34.39±1.40^a
(Kuruma shrimp)	October	78.34±0.68^b	19.47±0.41^b	65.26±0.30^b	-	43.87±1.04^b
	Mean	74.92±1.61	16.77±1.23	61.91±1.54	-	39.13±2.25
M. monoceros	February	66.98±0.47^a	16.11±0.55^a	55.38±0.85^a	-	36.57±1.01^a
(Speckled shrimp)	October	65.75±1.40^a	18.36±0.38^b	59.54±0.96^b	-	37.01±1.34^a
	Mean	66.36±0.72	17.24±0.59	57.46±1.09	-	36.79±0.76
C. sapidus	February	-	6.30±0.27^a	11.55±0.44^a	-	-
(Blue crab)	October	-	6.52±0.71^a	17.13±0.26^b	-	-
	Mean	-	6.41±0.35	14.34±1.27	-	-
P. pagrus	February	-	1.39±0.21^a	-	25.64±0.49^a	-
(Red porgy)	October	-	1.35±0.09^a	-	31.91±0.80^b	-
	Mean	-	1.37±0.10	-	28.77±1.46	-
M. barbatus	February	-	1.27±0.04^a	-	35.07±0.27^b	-
(Red mullet)	October	-	1.52±0.11^a	-	43.43±0.56^a	-
	Mean	-	1.39±0.08	-	39.25±1.89	-

Data are expressed as means value±SE (n = 3). Means marked with different superscript letters for each animal species in each column are significantly different ($p<0.05$) from each other

tissues of both shrimp species by Yanar *et al.* (2004) were also higher in October than in January, similar to the results of the study. The current data have demonstrated that seasonal effects on carotenoid content were most significant in shell of crustaceans and skin of fish while muscle tissue was least affected. It is known that during the periods close to propagation time, carotenoids are transferred to ovary in female and skin in male individuals in rainbow trout (Tacon, 1981). Hence, reproductive stage might have also affected the carotenoid levels in different body parts of the crustaceans and fish in the study. Animals do not have capability to synthesize carotenoids and have to take them in their diets (Goodwin, 1984). It is know that red colour formation of animals in aquatic environment is depended on algae which is the main producer of carotenoids. Thus, it may be postulated that the main reason why total carotenoid levels of crustaceans and fish species show seasonal differences might be related to the algae levels in their surroundings. In fact, a study carried out in the same region as the study (Polat *et al.*, 2000) reported that the presence of algae is more intensive in autumn than in winter. On the other hand as astaxanthin is better synthesized by algae under intense light (Kobayashi *et al.*, 1992), pigmentation quality of alga would be better in more sunny seasons. For this, another reason for higher carotenoid contents of the seafood caught in autumn could be attributed to higher synthesis of carotenoids by alga which affects the fish and crustacean species directly or indirectly through food chain, under more intensive light.

The carotenoid levels in various body parts of both crustacean and fish species were found to be quite different from each other. In the decreasing order, the highest carotenoid contents were determined in head, shell and muscle in shrimp; shell and muscle in crab and skin and muscle in fish. Considering the mean levels of both seasons, the carotenoid contents demonstrated a wide variation between 16.19 and 55.44 mg kg^{-1} in green tiger shrimp, 16.77 and 74.92 m kg^{-1} in kuruma shrimp and 17.24 and 66.36 mg kg^{-1} in speckled shrimp. Yet, muscle carotenoid contents ranged from 16.19-17.24 mg kg^{-1} and were relatively similar between the shrimp species sampled in this study ($p>0.05$). Yanar *et al.* (2004) reported the mean seasonal muscle carotenoid content as 16.9 mg kg^{-1} for wild speckled shrimp and 14.1 mg kg^{-1} for wild green tiger shrimp which are close to the results of the present study. A rather low value was reported (4.2 mg kg^{-1}) for wild speckled shrimp by Gopakumar and Nair (1975). The data on muscle carotenoid content of kuruma shrimp cultured in ponds vary greatly between 10 and 46 mg kg^{-1} (Iwamato *et al.*, 1990; Yamada *et al.*, 1990; Chien and Jeng, 1992; Negre-Sadargues *et al.*, 1993). These values ranged between 10 and 17 mg kg^{-1} for some wild shrimp species (*M. dopsoni*, *P. indicus*, *P. monodon*, *Parapenaeopsis stylifera*) (Gopakumar and Nair, 1975; Sachindra *et al.*, 2005a). While the muscle carotenoid contents were found to be quite similar, the data gathered from head and shell demonstrated a wide variation among the shrimp species. The carotenoid levels in the head and shell were 74.92 and 61.91 mg kg^{-1} for kuruma shrimp, 66.36 and 57.46 mg kg^{-1} for speckled shrimp and 55.44 and 44.08 mg kg^{-1} for green tiger shrimp. No data is available for wild forms of these species but carotenoid content was reported as 69-107 mg kg^{-1} in head and 37-74 mg kg^{-1} in shell of cultured kuruma shrimp (Chien and Jeng, 1992).

These values were 35.8-58.4 mg kg^{-1} in head and 59.8-86.6 mg kg^{-1} in shell of some other wild shrimp species (*P. monodon*, *P. indicus*, *M. dobsoni* and *Parapenaeopsis stylifera*) (Sachindra *et al*., 2005a). Considering the whole body, the highest carotenoid level was determined in kuruma shrimp (39.13 mg kg^{-1}) followed by speckled shrimp (36.79 mg kg^{-1}) and green tiger shrimp (29.97 mg kg^{-1}) in the study. A close value (37 mg kg^{-1}) was reported for cultured kuruma shrimp by Yamada *et al*. (1990) while a higher value (44 mg kg^{-1}) was reported for wild speckled shrimp by Latscha (1989).

In the present study, the mean carotenoid content of two seasons in blue crab was 6.41 mg kg^{-1} in muscle and 14.34 mg kg^{-1} in shell. A quite lower value was reported (4.63 mg kg^{-1}) for muscle carotenoid content of wild blue crab by Felix-Valenzuela *et al*. (2001). The carotenoid levels measured in different crab species elsewhere were also lower than the findings of the study. For instance, these levels were measured as 3.4-11 mg kg^{-1} in *Charybdis crusciata* and 4.1-6.8 mg kg^{-1} in *Potamon potaman* (Sachindra *et al*., 2005a, b). In the present study, the mean carotenoid contents measured in muscle and skin of red porgy were 1.37 and 28.77 mg kg^{-1}, respectively while these values were 1.39 and 39.25 mg kg^{-1} in red mullet.

No data is available in the literature about the carotenoid content of red mullet and the studies with red porgy are only limited to cultured forms. Total carotenoid content in skin of wild red porgy was reported as 53.99 mg kg^{-1} (Kalinowski *et al*., 2007) which is quite higher than the findings of the present study. The levels in skin of the same species under culture conditions ranged from 14-68.8 mg kg^{-1} (Kalinowski *et al*., 2007; Tejera *et al*., 2007, 2010; Doolan *et al*., 2008).

CONCLUSION

This study shows that the total carotenoid contents in various body parts of both crustaceans and fish species were found to be generally higher in October than in February. The body parts mostly affected in term of pigmentation was head and shell or skin which are the most appealing organs during the marketing phase of these products. As red colour is known to increase the consumer preference, fishing these species in autumn would be more advantageous than in winter season.

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