

## Fatty Acids, $\alpha$ -Tocopherol and Proximate Composition of Four Red Macroalgae in the Sinop Bay (Turkey)

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**Abstract:** The present investigation was to study the nutritional value, fatty acid composition and  $\alpha$ -tocopherol of the red macroalgae Rhodophyta (*Antithamnion*, *Ceramium*, *Corallina* and *Laurencia* sp.) in the Sinop Bay from Black Sea. The highest level of 20:5 (n-3) was 27.18% of Dry Weight (DW) for *Antithamnion* sp., however, the lowest level was 11.90% DW for *Ceramium* sp. The  $\alpha$ -tocopherol content in samples showed marked variation among the four types of algae and it was max;  $21.2 \pm 0.2$  and min;  $8.5 \pm 0.3 \mu\text{g g}^{-1}$  DW. The highest level of total carotenoids was determined in the *Antithamnion* sp., contained as  $1.69 \pm 0.46 \text{ mg g}^{-1}$  DW. Nevertheless, the highest level of chlorophyll a was observed in the *Laurencia* sp. ( $0.26 \pm 0.01 \text{ mg g}^{-1}$ ). The crude protein content of the *Antithamnion* sp. ( $28.1 \pm 0.3\%$ ) and *Ceramium* sp. ( $28.5 \pm 0.5\%$ ) was higher than the *Corallina* and *Laurencia* sp. As a result, *Antithamnion* sp., is a good source of lipids with a good level of EPA, total carotenoids and  $\alpha$ -tocopherol and *Ceramium* sp., evidenced to be an excellent protein source. In Europe, the development of novel foods, such as functional foods could be a new possibility for the use of these macroalgae, especially for the protein, fatty acids and  $\alpha$ -tocopherol-rich species in human nutrition. This present study results showed that these red macroalgae in the Sinop Bay could be utilized as functional ingredients for the valuable nutritional properties for seafood industries.

**Key words:** Macroalgae, fatty acids, tocopherol, Black Sea, Rhodophyta

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### INTRODUCTION

Marine macroalgae have been used since ancient times as animal and human food, fodder and fertilizer and as sources of medical drug (Sanchez-Machado *et al.*, 2004) because they contain high amount of protein, fatty acids and minerals (Fleurence, 1999; Orhan *et al.*, 2003; Wong and Cheung, 2000). Currently, macroalgae are attracting increasing interest in view of their low calorie content and high vitamin, mineral and dietary fiber contents, making them attractive to both consumers and the food industries. In nature,  $\alpha$ -tocopherol is the most abundant form of vitamin E and it has the greatest nutritional significance. Moreover, 20:5 (n-3) and 22:6 (n-3) were shown to have several beneficial effects, such as preventing coronary heart diseases, hypertriglyceridemia, blood platelet aggregation and lowering blood cholesterol, thus reducing the risk of arteriosclerosis, inflammation and several carcinomas (Guerrero *et al.*, 2001). It has also been reported that the

fatty acids of certain macroalgae have antiviral activity (Kamat *et al.*, 1992) and vitamin E is able to protect membrane lipids from oxidative damage (Huo *et al.*, 1997). It is commonly believed that some kinds of macroalgae have high economic value to be used for human and animal nutrition. Having this into account, it has been suggested that its use as a supplement or nutraceutical can have a positive impact on health (Carballo-Cardenas *et al.*, 2003).

The nutrient contents of macroalgae vary greatly and demonstrate a dependence on such factors as geographical location (Dawczynski *et al.*, 2007), season and temperature (Kaehler and Kennish, 1996; Sanchez-Machado *et al.*, 2004). Although, the Black Sea region have many macroalgae species, there is scarce information on the fatty acids  $\alpha$ -tocopherol, chlorophyll a and carotenoid for some macroalgae located in Sinop Bay (Southern Black Sea, Turkey) and there is hardly any report about chemical composition and fatty acid profile studies for some kind of macroalgae in the

mentioned area. The purpose of the present investigation was to contribute to a deep knowledge on the nutritional value, fatty acid composition and  $\alpha$ -tocopherol of some macroalgae informs Sinop Bay.

## MATERIALS AND METHODS

The samples were collected by diving in Sinop Bay (Fig. 1) which belongs geographically to the West Black Sea Region of Turkey between latitude  $42^{\circ}02'06''\text{N}$  and longitude  $35^{\circ}09'36''\text{E}$  when the temperature of seawater was about  $18^{\circ}\text{C}$ . The collected area of the samples was upper infralittoral zone of the Sinop Bay. Four red macroalgae species were identified as a belonged to Rhodophyta (*Antithamnion* sp., *Ceramium*, *Corallina* and *Laurencia* sp.).

**Preparation of the samples:** The weights of the collected sample were manually by cutting the fronds and all of them were completely washed in seawater before going to laboratory. The cooler container was used to keep their temperature with ice. The red macroalgae were rinsed in the distilled water and then they were drained. Thus, both sand and other sections containing unwanted substances were removed. The subsequent step was drying process for the samples at  $60^{\circ}\text{C}$  for 3 h by using traditional lab oven. When all samples were dried very well, they were put into the lab mixture. They were stored in plastic bags at room temperature and in the dark before using for all analyses.

**Fatty acids:** Fatty acid methyl esters were prepared according to Lepage and Roy (1986) modified by Cohen *et al.* (1988). The analysis was performed in a gas

chromatograph Varian 3800 Cx (Walnut Creek, CA) equipped with an auto-sampler and fitted with a flame ionisation detector at  $250^{\circ}\text{C}$ . Separation was done in a polyethylene glycol capillary column DB-WAX with 30 m-length 0.25 mm i.d. and  $0.25\ \mu\text{m}$  film thickness from J and W Scientific (USA). Column was subjected to a temperature program starting at  $180^{\circ}\text{C}$  for 5 min, heating at  $4^{\circ}\text{C}/\text{min}$  for 10 min and held up at  $220^{\circ}\text{C}$  for 25 min. The injector (split ratio 100:1) temperature was kept constant at  $250^{\circ}\text{C}$  during the 40 min analysis. All analytical determinations were done in triplicate.

**$\alpha$ -tocopherol:** Alpha-tocopherol was immediately analysed after freeze-drying. The extraction was carried out following a method adapted from Chen *et al.* (1998). The organic phase was injected in a HPLC JASCO Model 980 (Japan) equipped with an automatic injector JASCO Model AS-950-10 (Tokyo, Japan) and a fluorescent detector JASCO Model FP-1520 ( $\lambda_{\text{exc}} = 290\ \text{nm}$  and  $\lambda_{\text{em}} = 300\ \text{nm}$ ). The separation was carried out in a Lichrosorb Si 60-5 ( $250 \times 3\ \text{mm}$  i.d.) column from Chrompack (USA) protected by a silica pre-column S2-SS ( $10 \times 2\ \text{mm}$  i.d.) from Chrompack (USA). The mobile phase was a mixture of n-hexane and isopropanol (99.3:0.7 v/v) deaired in the Gastor Model GT-104 System (Japan) and eluted at a constant flow of  $1\ \text{mL}\ \text{min}^{-1}$ . The data was recorded and analyzed using Borwin version 1.21 chromatographic software (JMBS Developpements, France).

**Carotenoids:** Analysis of pigments of macroalgae was performed according to Gouveia *et al.* (1997). Total carotenoid content in the samples was determined by using spectrophotometric quantification (Hitachi U-2010, Japon) after extraction with acetone (Choubert and Storebakken, 1989).

**Proximate composition of the samples:** The nitrogen content of Seaweed sample was analyzed by using Kjeldahl Method. In addition, the crude protein content was calculated by multiplication of the nitrogen content by a factor of 6.25 (AOAC, 1990). Fat and ash content of the samples was determined (AOAC, 1995) in quadruplicate. All determinations were performed in triplicate and data represented on dry weight basis as Mean $\pm$ SD. All reagents except otherwise stated are from Merck (Darmstadt, Germany) and standards are from Sigma-Aldrich (USA).

## RESULTS AND DISCUSSION

All the species studied in this research were red algae (Rhodopyhta) and they were found indifferently both in

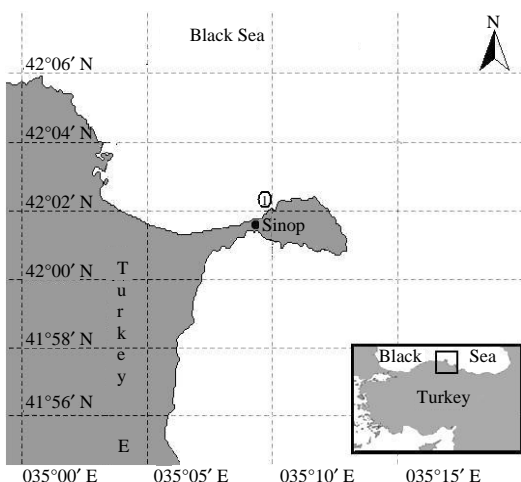


Fig. 1: The map of Turkey where samples of red macroalgae collected from Sinop Bay (Turkey)

the mid-littoral and infra-littoral zones, although they had more surface cover and thalli which were more slender and flexuous near the surface. The wide distributions in Black Sea of species are due to its capacity to colonize both transparent and very cloudy waters in both sheltered and wave-exposed areas.

The total fatty acid composition of four red macroalgae (Table 1) has a distribution characteristic of the phylum (Li *et al.*, 2002) with high relative levels of the acids 16:0, 18:1 (n-7), 18:1 (n-9), 20:5 (n-3) and 20:4 (n-6). The high level of Monounsaturated Fatty Acids (MUFA) was seen in *Ceramium* sp. as 34.64%. Additionally, the less level of MUFA was only obtained as 10.82% in *Antithamnion* sp. The range of Polyunsaturated Fatty Acid (PUFA) was determined from 25.13-54.35% in the all macroalgae species. The highest level of fatty acids 20:5 (n-3) and 22:2 (n-6) were found in *Antithamnion* sp. While the high level of 20:5 (n-3) was found as 27.18% in *Antithamnion* sp., the lower level was found in *Ceramium* sp. as 11.90%. The results demonstrated that the level of 18:3 (n-6) was similar in *Laurencia* and *Antithamnion* sp., as 1.88 and 1.78%, respectively while *Antithamnion* sp. has the lowest level of 18:3 (n-3) in each sample. The ratio 18:2 (n-6)/20:4 (n-6) was also around 30% lower in *Antithamnion* sp. indicating in this species a high enzymatic activity of  $\Delta$ -6-desaturase, elongase and/or  $\Delta$ -5-desaturase as was referred for other macroalgae species from de Black Sea (Yazici *et al.*, 2007). The two species (*Laurencia* sp., *Ceramium* sp.) presented the lowest level of 20:4 (n-6) fatty acids, probably

due to a high utilization in eicosanoid production (Yazici *et al.*, 2007). Li *et al.* (2002), 20:5 (n3) obtained 16-20, 23.4 and 31.5% in *Ceramium* sp., *Laurencia okumurai* and *Corallina pilulifera* in Bohai Sea, respectively and this results were higher than species of Sinop Bay. Comparative analysis of fatty acids of three species with same species from Bohai Sea (Li *et al.*, 2002) gave an interesting observation: The species from Sinop Bay are richer in 18:1 (n-9) and and lower in 20:5 (n-3), 20:4 (n-6). Some studies on the fatty acid composition of macroalgae have reported that algal fatty acid compositions have been affected by many factors, such as temperature, salinity, nutrient and water depth (Xu *et al.*, 1998; Graeve *et al.*, 2002). According to Plaza *et al.* (2008), some brown algae (*Cystoseira* sp.) and green algae (*Ulva* sp.) contain natural sources of protein, carbohydrates, minerals and vitamins with low levels of lipids. For this reason, some kind of algae could be used as functional ingredients. Both fatty acid and mineral content of marine algae (*Enteromorpha* sp.) were higher as compared to soybeans and beans. The content of 20:5 (n-3) and 22:6 (n-3) was higher than in the other type of seaweeds (Aguilera-Morales *et al.*, 2005). *Enteromorpha* sp. was especially recommended for human consumption by Aguilera-Morales *et al.* (2005). Sanchez-Machado *et al.* (2004) pointed out that while lipid contents of some seaweed were low; there were high levels of polyunsaturated fatty acids of the  $\Omega$ -3 and -6 families. Fatty acids are used for many chemical reactions in human and animal body such as in cell structure, hormones and energy activities. Moreover, they provide some beneficial effects on human metabolism for enzyme activities, muscle and tissues.

Vitamin E is a generic term applied to the tocopherols and tocotrienols which show similar nutritional properties to  $\alpha$ -tocopherol (Sanchez-Machado *et al.*, 2002). The variations in the amount of  $\alpha$ -tocopherol detected in red seaweeds are shown in Table 2. The  $\alpha$ -tocopherol content of the samples showed very marked variation among the four types of sample. The mean level determined in the present study on red seaweeds was 21.2 $\pm$ 0.2 at the

Table 1: Fatty acid composition (percentage of total fatty acids) of red macroalgae in the Sinop Bay (Turkey)

Fatty acid (%)	<i>Antithamnion</i> sp.	<i>Ceramium</i> sp.	<i>Corallina</i> sp.	<i>Laurencia</i> sp.
14:0	1.93	3.52	2.67	1.94
16:0	22.94	30.00	24.75	19.66
18:0	1.50	1.59	2.61	1.65
$\Sigma$ other SAT <sup>a</sup>	3.29	1.52	2.24	1.99
$\Sigma$ SAT	29.66	36.63	32.25	25.24
16:1n-7	2.31	18.12	12.44	8.62
18:1n-7	1.09	1.60	2.29	4.37
18:1n-9	6.78	13.38	12.49	8.57
$\Sigma$ other MUFA <sup>b</sup>	0.64	1.53	3.19	2.02
$\Sigma$ MUFA	10.82	34.64	30.41	23.59
18:2n-6	0.12	2.47	3.58	1.83
18:3n-6	1.78	0.61	0.97	1.88
18:3n-3	0.27	0.80	1.72	0.46
18:4n-3	4.79	1.41	1.96	4.54
20:4n-6	2.66	1.88	2.59	1.48
20:5n-3	27.18	11.90	12.84	13.96
22:2n-6	13.68	2.82	1.78	11.54
22:6n-3	1.47	2.49	6.33	3.87
$\Sigma$ other PUFA <sup>c</sup>	2.39	0.74	1.60	2.93
$\Sigma$ PUFA	54.35	25.13	33.38	42.49
18:2n-6/20:4n-6	0.05	1.31	1.38	1.24

<sup>a</sup>Other SAT are 12:0, 13:0, 14:0 isobr, 15:0, 16:0 iso, 16:0 anteiso, Phytanic Acid, 20:0 and 22:0; <sup>b</sup>Other MUFA are; 17:1, 20:1w9, 20:1w7, 22:1w11 and 22:1w9; <sup>c</sup>Other PUFA are 16:4w3, 20:4w3, 22:5w6 and 22:5w3

Table 2: Proximate composition (g/100 g dw) of red macroalgae in the Sinop Bay (Turkey)

Compositions	<i>Antithamnion</i> sp.	<i>Ceramium</i> sp.	<i>Corallina</i> sp.	<i>Laurencia</i> sp.
Protein (%)	28.1 $\pm$ 0.3 <sup>a</sup>	28.5 $\pm$ 0.5 <sup>a</sup>	4.0 $\pm$ 0.3 <sup>c</sup>	16.4 $\pm$ 0.3 <sup>b</sup>
Lipid (%)	12.4 $\pm$ 2.6 <sup>c</sup>	2.5 $\pm$ 0.2 <sup>c</sup>	1.9 $\pm$ 0.1 <sup>c</sup>	6.3 $\pm$ 2.7 <sup>b</sup>
Ash (%)	31.4 $\pm$ 1.1 <sup>b</sup>	20.0 $\pm$ 0.7 <sup>c</sup>	80.5 $\pm$ 0.7 <sup>a</sup>	30.5 $\pm$ 0.8 <sup>b</sup>
Fiber (%)	0.05 <sup>b</sup>	0.14 <sup>a</sup>	0.08 <sup>b</sup>	0.13 <sup>a</sup>
$\Sigma$ Carotenoids (mg g <sup>-1</sup> )	1.69 $\pm$ 0.46 <sup>c</sup>	0.39 $\pm$ 0.16 <sup>c</sup>	0.34 $\pm$ 0.05 <sup>c</sup>	0.84 $\pm$ 0.01 <sup>b</sup>
Chlorophyll a (mg g <sup>-1</sup> )	0.25 $\pm$ 0.01 <sup>a</sup>	0.10 $\pm$ 0.02 <sup>b</sup>	0.09 $\pm$ 0.03 <sup>b</sup>	0.26 $\pm$ 0.01 <sup>a</sup>
$\alpha$ -tocopherol ( $\mu$ g g <sup>-1</sup> )	21.2 $\pm$ 0.2 <sup>a</sup>	9.1 $\pm$ 0.5 <sup>b</sup>	8.5 $\pm$ 0.3 <sup>b</sup>	8.8 $\pm$ 0.8 <sup>b</sup>

Mean values, n = 3, dry weight basis; Different letters between the columns indicate significant difference at 5% by Duncan multiple range test

maximum and  $8.5 \pm 0.3$  at the minimum  $\mu\text{g g}^{-1}$  DW and the highest level of  $\alpha$ -tocopherol was observed in the *Antithamnion* sp., from red macroalgae. Sanchez-Machado *et al.* (2002) obtained that the  $\alpha$ -tocopherol level of macroalgae *Himanthalia elongate* was dehydrate;  $33.2 \pm 4.2 \mu\text{g g}^{-1}$  DW and canned;  $12.0 \pm 2.0 \mu\text{g g}^{-1}$  DW. These researchers referred that the levels recorded in the *Antithamnion* sp. were superior to that registered in foods known as rich in  $\alpha$ -tocopherol. This study also shows that these macroalgae can be used as a supplement of vitamin E.

The highest level of total carotenoids was observed in the *Antithamnion* sp. as  $1.69 \pm 0.46 \text{ mg g}^{-1}$  DW (Table 2). However, the highest level of chlorophyll a was observed in the *Laurencia* sp. ( $0.26 \pm 0.01 \text{ mg g}^{-1}$  DW). Red seaweeds are particularly rich in pigments and they can be powerful antioxidants. Recent studies have shown the correlation between a diet rich in carotenoids and a diminishing risk of cardiovascular disease, cancers as well as ophthalmological diseases (Burtin, 2003).

The variations in the amount of protein, lipid, ash and fiber detected in red seaweeds are shown in Table 2. Protein content of the samples ranged from  $4.0 \pm 0.3$  to  $28.5 \pm 0.5\%$ . The highest values for protein content were found in *Antithamnion* sp. ( $28.1 \pm 0.3\%$ ) and *Ceramium* sp. ( $28.5 \pm 0.5\%$ ). The greatest range in ash values occurred in *Corallina* sp. attaining a level of  $80.5 \pm 0.5\%$ . The minimum ash percentage was obtained in *Ceramium* sp. as  $20.0 \pm 0.7\%$ . It could be said that the ash contents of some vegetables are inferior to that recorded in seaweeds. In addition, the lower fiber percentage ( $0.05$ ) was found in *Antithamnion* sp. The highest fiber content result was almost three times higher than  $0.05\%$ . The protein content of some other brown algae species *Laminaria japonica*, *Hizikia fusiforme* and *Undaria pinnatifida* has been estimated 7.5, 11.6 and  $19.8/\text{g } 100 \text{ g DW}$ , respectively. However, protein content for both *Porphyra* and *Porphyra* sp., which are also red algae has been found higher than  $30.0 \text{ g } 100 \text{ g}^{-1}$  DW (Dawczynski *et al.*, 2007). Other studies showed that the values of the crude protein as  $18.4/\text{g } 100 \text{ g DW}$  of *Hypnea charoides* and  $19.0/\text{g } 100 \text{ g DW}$  of *H. japonica* (Wong and Cheung, 2000). It has been pointed out that the protein value of *Ulva lactuca* was measured as  $27.2/\text{g } 100 \text{ g DW}$  whereas those of *Durvillaea antarctica* was  $10.4/\text{g } 100 \text{ g DW}$  (Ortiz *et al.*, 2006).

The chemical compositions of red macroalgae have been evaluated for human foods and there have been several reports about the use as ingredient in healthy food (Li *et al.*, 2002; Orhan *et al.*, 2003; Kuda *et al.*, 2005; Dawczynski *et al.*, 2007).

Studies of red macroalgae from the Sinop Bay indicated that these macroalgae have typical chemical composition patterns of red algae from other regions. In general, the chemical composition of these four species macroalgae species from the Black Sea showed that *Antithamnion* sp., is a good source of lipids with a high level of EPA, total carotenoids and  $\alpha$ -tocopherol. *Ceramium* and *Laurencia* sp. presented the highest fiber content and *Ceramium* sp. evidenced to be an excellent protein source. In addition, high ash content was a common feature in all algae studied and based on the results these seaweeds may serve as a food supplement to reach the recommended daily dose of minerals in human. In Europe, the development of novel foods, such as functional foods could be a new possibility for the use of these macroalgae in human nutrition, especially.

## CONCLUSION

The results obtained in the present study demonstrated that red macroalgae in the Sinop Bay could be used as ingredient in functional foods for human consumption.

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