

Fatty Acids, α -tocopherol and Total Pigment Contents of *Cystoseira* spp., *Ulva* spp. and *Zostera* spp. from Sinop Bay (Turkey)

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Received: May 24, 2008

Accepted: July 30, 2008

Abstract

The present investigation was to study the nutritional value, fatty acids composition and α -tocopherol of *Cystoseira* spp., *Ulva* spp. and *Zostera* spp. in the Sinop Bay from Black Sea. The highest level of 20:5(n-3) was 10.96% of dry weight (DW) for *Cystoseira* spp. however, the lowest level was 4.40% DW for *Ulva* spp.. The α -tocopherol content in samples showed marked variation among samples and it was max; 17.10 \pm 0.10, and min; 9.10 \pm 0.50 μ g g⁻¹ DW. The highest level of total carotenoids was determined in the *Zostera* spp., contained as 1.09 \pm 0.14 mg g⁻¹ DW. Nevertheless, the highest level of chlorophyll *a* was observed in the *Ulva* spp. (0.71 \pm 0.07 mg g⁻¹). As a result, this present study results showed that *Cystoseira* spp., *Ulva* spp. and *Zostera* spp. in the Sinop Bay could be utilized as functional ingredients for the valuable nutritional properties for seafood industries.

Key words: marine plants, fatty acids, tocopherol, Black Sea, Sinop Bay

INTRODUCTION

Marine plants have been used since ancient times as animal and human food, fodder and fertilizer and as sources of medical drug [1], because they contain high amount of protein, fatty acids and minerals [2-4]. Currently, marine plants 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, α -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 [5]. It has also been reported that the fatty acids of certain marine plants have antiviral activity [6] and vitamin E is able to protect membrane lipids from oxidative damage [7]. It is commonly believed that some kinds of marine plants have 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 [8].

The nutrient contents of marine plants are vary greatly and demonstrate a dependence on such factors as geographical location [9], season and temperature [1,10]. Although the Black Sea region has many macroalgae species, there is scarce information on the fatty acids α -tocopherol, chlorophyll *a* and carotenoid for some marine plants 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 marine plants in the mentioned area. The

purpose of the present investigation was to contribute to a deep knowledge on the fatty acid composition, α -tocopherol and pigments of *Cystoseira* spp., *Ulva* spp. and *Zostera* spp. in Sinop Bay.

MATERIALS AND METHODS

Materials

The samples (*Cystoseira* spp. *Ulva* spp. and *Zostera* spp.) were collected by diving in October 2004 in Sinop Bay (Figure 1) in which belongs geographically to the West Black Sea Region of Turkey between latitude 42°02'06" N and longitude 35°09'36" E, when the temperature of seawater was about 18°C. The collected area of the samples was upper infralittoral zone of the Sinop Bay.

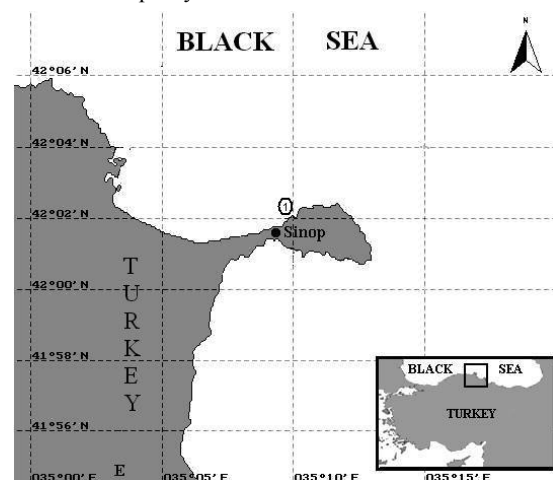


Figure 1. The map of Turkey where samples of *Cystoseira* spp. *Ulva* spp. and *Zostera* spp. collected from Sinop Bay.

Preparation of the samples

The samples, which were collected by hand were cut by lab knife manually after that all of them were completely washed in seawater. They were transported to the faculty laboratory by using the cooler container. The marine plants 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°C for 3 hours 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 to use for all analyses.

Fatty acids

Fatty acid methyl esters were prepared according to Lepage and Roy [11] modified by Cohen et al. [12]. 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°C. Separation was done in a polyethylene glycol capillary column DB-WAX with 30m-length 0.25 mm i.d. and 0.25 µm film thickness from J&W Scientific (USA). Column was subjected to a temperature program starting at 180°C for 5 min, heating at 4°C/min for 10 min, and held up at 220°C for 25 min. The injector (split ratio 100:1) already mentioned temperature was kept constant at 250°C during the 40 min analysis.

α-tocopherol

The extraction was carried out following a method adapted from Chen et al. [13]. 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 ($\lambda_{exc} = 290$ nm and $\lambda_{em} = 300$ nm). The separation was carried out in a Lichrosorb Si 60-5 (250 mm x 3 mm i.d.) column from Chrompack (USA) protected by a silica pre-column S2-SS (10 mm x 2 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 (Tokyo, Japan) and eluted at a constant flow of 1ml/min. The data was recorded and analyzed using Borwin version 1.21 chromatographic software (JMBS Developpements, France).

Carotenoids

Analysis of pigments of samples was performed according to Gouveia et al. [14] and total carotenoid and chlorophyll a content in the samples was determined by using spectrophotometric quantification (Hitachi U-2010, Japon) after extraction with acetone.

RESULTS AND DISCUSSION

All the species studied in this work, *Cystoseira* spp. and *Ulva* spp. were macroalgae but *Zostera* spp. was sea grass. They were found indifferently both in the midlittoral and infralittoral 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 acids composition of *Cystoseira* spp. *Ulva* spp. and *Zostera* spp. was shown Table 1. The high level of monounsaturated fatty acids (MUFA) was seen in *Cystoseira* spp. as 32.37%. Additionally; the less level of MUFA was only obtained as 19.50% in *Ulva* spp.. The range of polyunsaturated fatty acid (PUFA) was determined from 26.33 to 33.76% of all species. The fatty acid 20:5(n-3) was dominant (10.96%) which was found in *Cystoseira* spp., while the lower level was found in *Ulva* spp. as 4.40%. The ratio 18:2(n-6)/ 20:4(n-6) was also around 50% lower in *Zostera* spp. indicating in this species a high enzymatic activity of Δ -6-desaturase, elongase and/or Δ -5-desaturase as was referred for other macroalgae species from de Black Sea [15]. Some studies on the fatty acid composition of marine plants have reported that algal fatty acid compositions have been affected by many factors such as temperature, salinity, nutrients and water depth. [16,17]. According to Plaza et al. [18], some brown algae (*Cystoseira* spp.) and green algae (*Ulva* spp.) 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 plants (*Enteromorpha* spp.) were higher as compared to soybeans and beans. So that, *Enteromorpha* spp was especially recommended for human consumption by Aguilera-Morales et al. [19]. Sanchez-Machado et al. [1] pointed out that while lipid contents of some seaweed were low; there were high levels of PUFA of the omega-3 and omega-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.

Table 1. Fatty acids composition (percentage of total fatty acids) of *Cystoseira* spp., *Ulva* spp. and *Zostera* spp. in the Sinop Bay (Turkey).

Fatty acids (%)	<i>Cystoseira</i> spp.	<i>Ulva</i> spp.	<i>Zostera</i> spp.
14:0	4.18	2.00	3.36
16:0	30.66	26.50	28.00
18:0	1.22	8.00	1.81
Other Saturated ^a	1.33	1.60	3.08
Σ Saturated	37.39	38.50	36.24
16:1(n-7)	20.51	12.10	17.52
18:1(n-9)	11.28	6.20	10.16
18:1(n-7)	0.06	-	1.17
Other			
Monounsaturated	0.52	1.20	1.42
Σ Monounsaturated	32.37	19.50	30.27
16:4(n-3)	-	5.41	-
18:2(n-6)	2.91	2.23	1.17
18:3(n-6)	0.83	1.74	3.76
18:3(n-3)	1.13	6.11	4.51
18:4(n-3)	1.75	7.31	3.14
20:4(n-6)	4.81	0.13	2.49
20:5(n-3)	10.96	4.40	9.54
22:2(n-6)	1.07	2.01	0.85
22:6(n-3)	2.31	1.12	3.60
Other Polyunsaturated	0.55	3.30	0.52
Σ Polyunsaturated	26.33	33.76	29.57
18:2n-6/20:4n-6	0.60	17.15	0.47

^a: Other saturated fatty acids are 12:0, 13:0, 14:0 isobr, 15:0, 16:0 iso, 16:0 anteiso, Phytanic Acid, 20:0 and 22:0. ^b: Other monounsaturated fatty acids are 17:1, 20:1(n-9), 20:1(n-7), 22:1(n-11) and 22:1(n-9). ^c: Other polyunsaturated fatty acids are 20:4(n-3), 22:5(n-6) and 22:5(n-3).

Vitamin E is a generic term applied to the tocopherols and tocotrienols, which show similar nutritional properties to α -tocopherol [20]. The variations in the amount of α -tocopherol detected in *Cystoseira* spp., *Ulva* spp. and *Zostera* spp were shown in Table 2. The α -tocopherol content of the samples showed very marked variation among the four types of sample. The mean level determined in the present study on marine plants was max; 17.10 \pm 0.10, and min; 9.10 \pm 0.50 $\mu\text{g g}^{-1}$ DW and the highest level of α -tocopherol was observed in the *Cystoseira* spp.. Sanchez-Machado et al. [20] obtained that the α -tocopherol level of macroalgae *Himantalia elongate* was dehydrate; 33.20 \pm 4.20 $\mu\text{g g}^{-1}$ DW and canned; 12.00 \pm 2.00 $\mu\text{g g}^{-1}$ DW. These authors referred that the levels recorded in these marine plants were superior to that registered in foods known as rich in α -tocopherol. This study results also showed that *Cystoseira* spp., *Ulva* spp. and *Zostera* spp could be used as a supplement of vitamin E.

Table 2. Carotenoids, chlorophyll *a* and α -tocopherol of *Cystoseira* spp., *Ulva* spp. and *Zostera* spp. in the Sinop Bay (Turkey).

	<i>Cystoseira</i> spp.	<i>Ulva</i> spp.	<i>Zostera</i> spp.
Σ Carotenoids (mg g ⁻¹)	0.81 \pm 0.15	0.31 \pm 0.03	1.09 \pm 0.14
Chlorophyll <i>a</i> (mg g ⁻¹)	0.36 \pm 0.06	0.71 \pm 0.07	0.44 \pm 0.07
α -tocopherol ($\mu\text{g g}^{-1}$)	17.10 \pm 0.10	9.10 \pm 0.50	9.90 \pm 0.10

Mean values, $n=3$, dry weight basis

The highest level of total carotenoids was observed in the *Zostera* spp., contained as 1.09 \pm 0.14 mg g⁻¹ DW (Table 2). However, the highest level of chlorophyll *a* was observed in the *Ulva* spp. (0.71 \pm 0.07 mg g⁻¹ DW). They were particularly rich in pigmentation and 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 [21].

The results justified that the studied samples can be used in human nutrition and in balanced diets for animal nutrition and the results obtained in the present study can lead to new research areas. The chemical compositions of marine plants have been evaluated for human foods and there have been several reports about use of healthy food [9, 22-24]. In general, the chemical composition of these *Cystoseira* spp., *Ulva* spp. and *Zostera* spp. species from the Black Sea showed that, *Cystoseira* spp. was a good source of lipids with a good level of 20:5(n-3) and 20:4(n-6), and α -tocopherol. *Ulva* spp. and *Zostera* spp. presented the highest chlorophyll *a* and carotenoids, respectively. In Europe, the development of novel foods such as functional foods could be a new possibility for the use of marine plants, especially for fatty acids and α -tocopherol-rich species. Therefore, the result of the study has demonstrated that marine plants in the Sinop Bay (Turkey) could be used as ingredient in functional foods for human consumption.

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