

## FAT SOLUBLE VITAMINS AND FATTY ACIDS COMPOSITION OF BLACK SEA CYSTOSEIRA BARBATA

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Brown alga *Cystoseira barbata* is the most widely distributed seaweed in the Black Sea. There is limited information about fat soluble vitamins content and fatty acids composition of this specie from Bulgarian Black Sea coast. The aim of this study was to determine fat soluble vitamins, pigments, total lipid and fatty acid composition of *Cystoseira barbata*. Fat soluble vitamins (vitamin E and D), pigments ( $\beta$ -carotene and astaxanthin) and total cholesterol were analyzed simultaneously using HPLC/UV/FL system equipped with RP analytical column. Sample preparation procedure includes alkaline saponification, followed by liquid-liquid extraction. Brown seaweed *Cystoseira barbata* contained high amounts of  $\alpha$ -tocopherol and  $\beta$ -carotene. Lipids were extracted by following the method of Bligh and Dyer. The residual lipid fraction was methylated using base-catalyzed transmethylation with methanolic potassium hydroxide. Fatty acid composition was analyzed by GC/MS. *Cystoseira barbata* was rich in linoleic (C18:2n6) and eicosapentaenoic acid (C20:5n3) although total lipid content was generally low. High levels of  $\alpha$ -tocopherol correlate with high levels of polyunsaturated fatty acids. As an antioxidant  $\alpha$ -tocopherol preserves tissue PUFA from oxidation.

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### Introduction

Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine. Nowadays seaweeds represent an inexhaustible source of the raw materials used in pharmaceutical, food industries, medicine and cosmetics. They are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety of prepared foods. In particular, seaweeds contain significant quantities of protein, lipids, minerals and vitamins (Manivannan et al., 2008).

Lipids represent only 1-5% of algal dry matter and exhibit an interesting polyunsaturated fatty acid (PUFA) composition particularly omega 3 and omega 6 acids which play an important role in the prevention of cardio vascular diseases, osteoarthritis and diabetes. Brown algae are rich in fatty acids with 20 carbons: eicosapentaenoic acid (EPA, C 20:5 n3) and arachidonic acid (AA, C 20:4 n6) (Banerjee et al., 2009). Marine algae are rich in PUFAs of the n-3 and n-6 series, which are considered essential fatty acids for humans and animals. Some of these FAs (20:3n-6, 20:4n-6, 20:5n-3) have high biological activity and are converted into eicosanoids. In addition, PUFAs are of interest in cosmetics as components of sun lotions and as regenerating and anti-wrinkle products. Because of the huge and renewable biomass and the fact that many of them could easily be cultivated in the sea on a large

scale, seaweeds are a potential source of fatty acids for biotechnology and a dietary source of essential fatty acids (Khotimchenko et al., 2002).

Seaweeds are a good source of some water- (B<sub>1</sub>, B<sub>2</sub>, B<sub>12</sub>, C) and fat-soluble ( $\beta$ -carotene with vitamin A activity, vitamin E) vitamins. Seaweed vitamins are important not only due to their biochemical functions and antioxidant activity but also due to other health benefits such as decreasing blood pressure (vitamin C), prevention of cardiovascular diseases ( $\beta$ -carotene), or reducing the risk of cancer (vitamins E and C, carotenoids) (Skrovankova, 2011).

Bulgarian Black Sea coast is rich in algae, regarding biomass and algal biodiversity. According to Guiry and Guiry (2013), taxonomically, the species *Cystoseira barbata* belongs to the division *Ochrophyta*, class *Phaeophyceae*, order *Fucales*, family *Sargassaceae*, genus *Cystoseira*. It represents a group of macroscopic, multicellular algae, widely distributed along the coastal cliffs and rocks, which often form underwater "meadows". Negreanu-Pirjol et al., (2012) reported that lipid soluble substances of *Cystoseira barbata* possess higher antioxidant activity, compared to other species from the Black Sea. Information available about the lipid composition of this macroalgae is scarce. The main objective of this investigation was to provide knowledge on chemical composition of *Cystoseira barbata* as an alternative source of fatty acids and fat soluble vitamins.

## Data and methodology

*Cystoseira barbata* was collected in July, 2012 from the region of Varna Bay, Bulgaria. All of the samples were harvested manually from their respective sites and then transported to the laboratory in wet tissue towels in an ice box. They were thoroughly cleaned to remove epiphytes and detritus attached to the fronds. Cleaned samples were frozen and stored at  $-20^{\circ}\text{C}$  prior to analysis. The moisture content was determined by oven method at  $105^{\circ}\text{C}$  until constant weight (AOAC, 2000).

## GC-MS analysis

Lipids were extracted by following the method of Bligh and Dyer (1959). Algal tissue was extracted first with chloroform: methanol (1:2, v/v) and the residue were extracted thrice with small portions of chloroform: methanol (1:1, v/v). All the extracts were pooled together, filtered and mixed with an equal volume of chloroform and water (1:1, v/v) for phase separation. The lower organic phase was collected and evaporated to dryness in a vacuum, and the total lipids were determined gravimetrically. The residual lipid fraction was methylated by base-catalyzed transmethylation using 2M methanolic potassium hydroxide and n-hexane. After 10 minutes centrifugation (3500 rps), the hexane layer was taken for GC analyses. Gas chromatography was performed by a model FOCUS Gas Chromatograph with autosampler A 2000, equipped with Polaris Q MS detector (Thermo Scientific, USA). The capillary column used was a TR-5 MS (Thermo Scientific, USA) universal column 30m length and 0.25mm i.d., with a wide range of applications for food analysis. Helium was used as a carrier gas at flow rate 1 ml/min. Chromatographic separation was achieved by temperature range: initial temperature  $-40^{\circ}\text{C}$  for 4 min followed by  $10^{\circ}\text{C}$  per minute until  $235^{\circ}\text{C}$  and final temperature reach was  $280^{\circ}\text{C}$  for 5 min. The injection volume was 1 $\mu\text{l}$ . Peaks were identified according to two parameters: Retention Time (RT) based on available FAME mix standard (SUPELCO F.A.M.E. Mix C4-C24) and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA). All analytical determinations were performed in triplicate. Values were expressed as percentage of total fatty acid mass as a mean value and  $\pm$  standard deviation (SD). All of the chemicals used in the experiments were analytical grade and GC grade (Sharlau).

## HPLC analysis

Astaxanthin,  $\beta$ -carotene,  $\alpha$ -tocopherol, ergocalciferol and total cholesterol were determined simultaneously using HPLC/UV/FL system equipped with RP analytical column. Sample preparation was performed following the method described by Dobрева, Galunska and Stancheva (2011) with some modifications. Generally, an aliquot of the homogenized tissue ( $1.500 \pm 0.005$ g) was weighed into a glass tube with a screw cap and 1% of methanolic L-ascorbic acid and 0.3M methanolic potassium hydroxide were added. Six parallel samples of algae tissue were prepared and saponified at 40°C for 30 min. After cooling the lipids were extracted with n-hexane: dichloromethane. The extracts were pooled together and evaporated under nitrogen. The dry residue was dissolved in methanol: dichloromethane and injected into the HPLC system (Thermo Scientific Spectra SYSTEM). Chromatography was run as a gradient at 1.1 mL/min with a Synergi 4 $\mu$  Hydro-RP 80A pore 250x4,6 mm reversed-phase column. Solvent A contained methanol-water (93:7), solvent B contained acetonitrile and solvent C – 2-propanol. The gradient changed as follows: 0-16.0 min, 100 % solvent A, 20.0-30.0 min, 60% solvent B and 40 % solvent C, 30.0-40.0 min, 50 % of solvent B and 50% solvent C. The gradient was then returned to 100 % of solvent A. Detection of astaxanthin,  $\beta$ -carotene, ergocalciferol and cholesterol was performed by UV detector (for astaxanthin  $\lambda=474$ nm, for  $\beta$ -carotene  $\lambda=450$ nm, for ergocalciferol  $\lambda=265$  nm, for cholesterol  $\lambda=208$  nm). Concentration of  $\alpha$ -tocopherol was measured by fluorescence at  $\lambda_{ex} = 288$  nm and  $\lambda_{em} = 332$  nm. The quantitation was performed by the method of the external calibration, comparing the chromatographic peak areas of the samples with those of the corresponding standards.

## Results and discussion

The total lipids content of *Cystoseira barbata* accounted for  $3.35 \pm 0.58$ g per 100 g on a dry weight basis. The fatty acid composition is presented in Table 1. Saturated fatty acids (SFA) were most abundant components with 70.63% of total fatty acids (TFA). The total sum of monounsaturated fatty acids (MUFAs) was 13.08%, whereas total PUFAs were 16.29%.

Algae are a source of vitamins (liposoluble and hydrosoluble) available all along the Black Sea coast, yet not usually consumed fresh or dried. Results obtained from HPLC analysis for various liposoluble constituents in *Cystoseira barbata* are shown in Table 2. The value of  $\beta$ -carotene in algae in this study exceeds those reported by Gayathri et al., (2004) for fresh carrot (88.33  $\mu$ g/g) and other vegetables of mass consumption like spinach, cabbage, Swiss chard, averaging 25 $\mu$ g/g. Another interesting carotenoid was detected in *Cystoseira barbata* algal samples. Astaxanthin content accounted for  $1.49 \pm 0.04$  mg per 100g dry weight. Many studies have associated high dietary intake of carotenoids with lower risks of certain pathologies (Tapiero et al., 2004).

The major fatty acids encountered were palmitic (C16:0), palmitoleic (C16:1), oleic (C18:1, n-9), linoleic (C18:2, n-6; LA) and eicosapentaenoic (C20:5, n-3; EPA) that collectively contributed to 84.76% of TFAs. Fatty acids composition of algal lipids varies widely with species, habitat, light, salinity, pollution and environmental conditions (Kim et al., 1996; Ratana-Arporn & Chirapart, 2006) but in most studies palmitic (C 16:0) acid is predominant (Khotimchenko et al., 2002; Li et al., 2002; Gressleret et al., 2010). The main proportion of PUFA consisted of n-3 FA. The amount of n-3 FA was 9.76% of total FAME.

According to Sies and Stahl (2004), carotenoids directly provide photoprotection against UV light photooxidation in the skin. The ketocarotenoid astaxanthin is believed to play a key role in the

prevention of several human pathological processes, such as skin UV-mediated photooxidation, inflammation, prostate and mammary carcinogenesis, ulcers and age-related diseases (Guerrin et al., 2003).

Table 1: Fatty acid composition of *Cystoseira barbata* given as means  $\pm$  SD (% of total FAME), n=3

SFA	<i>Cystoseira barbata</i>	MUFA	<i>Cystoseira barbata</i>	PUFA	<i>Cystoseira barbata</i>
C 6:0	0.12 $\pm$ 0.02	C 14:1	0.59 $\pm$ 0.09	C 18:3 n6	n.d.
C 8:0	n.d.	C 15:1	n.d.	C 18:2 n6	4.87 $\pm$ 0.34
C 10:0	0.52 $\pm$ 0.08	C 16:1	5.50 $\pm$ 0.28	C 18:3 n3	1.55 $\pm$ 0.67
C 11:0	n.d.	C 17:1	0.61 $\pm$ 0.09	C 20:5 n3	7.56 $\pm$ 0.43
C 12:0	0.82 $\pm$ 0.12	C 18:1 n9	6.38 $\pm$ 0.17	C 20:4 n6	n.d.
C 13:0	n.d.	C 20:1	n.d.	C 20:3 n6	n.d.
C 14:0	1.76 $\pm$ 0.13	C 22:1 n9	n.d.	C 20:2	1.02 $\pm$ 0.11
C 15:0	n.d.	C 24:1	n.d.	C 20:3 n3	n.d.
C 16:0	60.45 $\pm$ 2.86			C 22:6 n3	0.65 $\pm$ 0.08
C 17:0	0.62 $\pm$ 0.09			C 22:2	n.d.
C 18:0	1.40 $\pm$ 0.17				
C 19:0	n.d.				
C 20:0	1.10 $\pm$ 0.16				
C 21:0	0.59 $\pm$ 0.09				
C 22:0	1.28 $\pm$ 0.19				
C 23:0	0.67 $\pm$ 0.12				
C 24:0	1.30 $\pm$ 0.20				
$\Sigma$ SFA	70.63 $\pm$ 0.58	$\Sigma$ MUFA	13.08 $\pm$ 0.24	$\Sigma$ PUFA	16.29 $\pm$ 0.59

Source: Authors

Note: n.d. – not detected

Table 2: Various constituents of *Cystoseira barbata* given as means  $\pm$  SD, n=6

Constituent	mg per 100g dry weight
$\beta$ -carotene	27.20 $\pm$ 1.32
Retinol equivalent	4.53 $\pm$ 0.22
$\alpha$ -tocopherol	15.77 $\pm$ 0.21
Ergocalciferol	0.62 $\pm$ 0.06
Astaxanthin	1.49 $\pm$ 0.04
Total cholesterol	4.67 $\pm$ 1.11

Source: Authors

The concentrations of  $\alpha$ -tocopherol in the present study (15.77 $\pm$ 0.21 mg per 100g d.w.) were found higher than those reported by Durmaz et al., (2008) for *Cystoseira* spp. from the Black Sea. The

authors concluded that in comparison to *Ulva spp.* and *Zostera spp.*, brown algae from *Cystoseira spp.* are better source of lipids with a good level of 20:5 (n-3) and 20:4 (n-6), and  $\alpha$ -tocopherol.

Total cholesterol content was  $4.67 \pm 1.11$  mg per 100g d.w. Although, further analysis of the individual sterol composition is needed, Milkova et al., (1997) reported that *Cystoseira barbata* from the Black Sea contained in the sterol fraction almost pure fucosterol.

## Conclusion

Lipids, fatty acids composition,  $\alpha$ -tocopherol, ergocalciferol,  $\beta$ -caroten, astaxanthin and total cholesterol in brown algae *Cystoseira barbata* were analyzed in the present study. Palmitic acid (C16:0) was the most abundant fatty acid, followed by EPA (20:5, n-3). The high concentrations of  $\alpha$ -tocopherol,  $\beta$ -carotene, polyunsaturated fatty acids and the presence of the powerful antioxidant astaxanthin demonstrate possible application of this macroalga as a supplement for use in food and pharmaceutical industry and aquaculture. Further studies concerning proximate analysis, amino acids, carbohydrates, biogenic and toxic elements, as well as individual sterol composition are necessary in order to provide more information for safer and more versatile utilization of these seaweeds.

## References

- AOAC. (2000). *Official methods of analysis*. (17th edition). Arlington, VA: Association of Official Analytical Chemists.
- Banerjee, K., Ghosh, R., Homechaudhuri, S. & Mitra, A. (2009). Biochemical Composition of Marine Macroalgae from Gangetic Delta at the Apex of Bay of Bengal. *African Journal of Basic & Applied Sciences*, 1(5-6), 96-104.
- Bligh, E. & Dyer, W. (1959). A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911–915. <http://dx.doi.org/10.1139/o59-099>  
PMid:13671378
- Dobрева, D., Galunska, B. & Stancheva, M. (2011). Liquid chromatography method for the simultaneous quantification of fat soluble vitamins in fish tissue. *Scripta Scientifica Medica*, 43(1), 276-279.
- Durmaz, Y., Duyar, H., Gokpinar, S., Taskaya, L., Ogretmen, Y., Bandarra, N. & Nunes, M. (2008). Fatty Acids,  $\alpha$ -tocopherol and Total Pigment Contents of *Cystoseira spp.*, *Ulva spp.* and *Zostera spp.* from Sinop Bay (Turkey). *International Journal of Natural and Engineering Sciences*, 2(3), 111-114.
- Gayathri, G., Platel, K., Prakash, J. & Srinivasan, K. (2004). Influence of antioxidant spices on the retention of  $\beta$ -carotene in vegetables during domestic cooking processes. *Food Chemistry*, 84(1), 35–43. [http://dx.doi.org/10.1016/S0308-8146\(03\)00164-X](http://dx.doi.org/10.1016/S0308-8146(03)00164-X)
- Gressler, V., Yokoya, N., Fujii, M., Colepicolo, P., Filho, J., Torres, R. & Pinto, E. (2010). Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. *Food Chemistry*, 120, 585–590. <http://dx.doi.org/10.1016/j.foodchem.2009.10.028>

- Guerrin, M., Huntley, M.E. & Olaizola, M. (2003). Haematococcus astaxanthin: applications for human health and nutrition. *Trends in Biotechnologies*, 21, 210–216. [http://dx.doi.org/10.1016/S0167-7799\(03\)00078-7](http://dx.doi.org/10.1016/S0167-7799(03)00078-7)
- Guiry, M. & Guiry, G. (2013). AlgaeBase. *World-wide electronic publication*. Galway, Ireland: National University of Ireland.
- Khotimchenko, S., Vaskovsky, V. & Titlyanova, T. (2002). Fatty acids of marine algae from the Pacific coast of North California. *Botanica Marina*, 45, 17-22. <http://dx.doi.org/10.1515/BOT.2002.003>
- Kim, M., Dubacq, J., Thomas, J. & Giraud, G. (1996). Seasonal variations of triacylglycerols and fatty acids in *Fucus serratus*. *Phytochemistry*, 43, 49-55. [http://dx.doi.org/10.1016/0031-9422\(96\)00243-9](http://dx.doi.org/10.1016/0031-9422(96)00243-9)
- Li, X., Fan, X., Han, L. & Lou, Q. (2002). Fatty acids of some algae from the Bohai Sea. *Phytochemistry*, 59, 157-161. [http://dx.doi.org/10.1016/S0031-9422\(01\)00437-X](http://dx.doi.org/10.1016/S0031-9422(01)00437-X)
- Manivannan, K., Thirumaran, G., Karthikai Devi, G., Hemalatha, A. & Anantharaman, P. (2008). Biochemical Composition of Seaweeds from Mandapam Coastal Regions along Southeast Coast of India. *American-Eurasian Journal of Botany*, 1(2), 32-37.
- Milkova, T., Talev, G., Christov, R., Dimitrova-Konaklieva, S. & Popov, S. (1997). Sterols and Volatiles in *Cystoseira Barbata* and *Cystoseira Crinita* from the Black Sea. *Phytochemistry*, 45(1), 93-95. [http://dx.doi.org/10.1016/S0031-9422\(96\)00588-2](http://dx.doi.org/10.1016/S0031-9422(96)00588-2)
- Negreanu-Pirjol, T., Negreanu-Pirjol, B., Sirbu, R., Paraschiv, G. & Meghea, A. (2012). Comparative Studies Regarding the Antioxidative Activity of Some Therapeutic Marine Algae Species along the Romanian Black Sea Coast. *Journal of Environmental Protection and Ecology*, 13(3A), 1744–1750.
- Ratana-Arporn, P. & Chirapart, A. (2006). Nutritional Evaluation of Tropical Green Seaweeds *Caulerpa lentillifera* and *Ulva reticulata*. *Kasetsart Journal: Natural Sciences*, 40(6), 75-83.
- Sies, H. & Stahl, W. (2004). Nutritional protection against skin damage from sunlight. *Annual Review of Nutrition*, 24, 173–200. <http://dx.doi.org/10.1146/annurev.nutr.24.012003.132320> PMID:15189118
- Skrovankova, S. (2011). Seaweed Vitamins as Nutraceuticals. In: Taylor, S. (Ed.). *Marine Medicinal Foods: Implications and Applications, Macro and Microalgae. Advances in Food and Nutrition Research Series*, 64, 357-369. Waltham, MA: Academic Press Inc.
- Tapiero, H., Townsend, D. & Tew, K. (2004). The role of carotenoids in the prevention of human pathologies. *Biomedicine & Pharmacotherapy*, 58, 100–110. <http://dx.doi.org/10.1016/j.biopha.2003.12.006> PMID:14992791