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Fatty acids composition of macroalgae from Bulgarian Black Sea coast

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Abstract Lipids and fatty acids (FA) composition of three Black Sea macroalgae *Cladophora vagabunda, Ceramium rubrum* and *Cystoseira barbata* were studied. Fatty acids composition was analyzed by GC/MS. Total lipids content varied widely among the species and ranged between 0.66 and 0.98 g per 100 g fresh weight. Generally, saturated fatty acids were major components (62–71%), with 16:0 as the most abundant saturate (41–57%). Total polyunsaturated FAs and monounsaturated FAs ranged from 28% to 38%. The green alga *Cladophora vagabunda* showed higher C18 PUFAs contents than did C20 PUFAs while for red alga *Ceramium rubrum* the trend was opposite. *Cystoseira barbata* belonging to the group of brown algae showed similar amounts of C18 and C20 PUFAs contents. *Cladophora vagabunda* was rich in linoleic acid and *Ceramium rubrum* in arachidonic acid (AA) while *Cystoseira barbata* was rich in both linoleic acid and eicosopentaenoic acid. All of the studied species had a nutritionally beneficial n6/n3 ratio (1.24–2.84:1).

Keywords: Black Sea algae, fatty acids, GC/MS

1. Introduction

Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine. Today seaweeds are the raw material for many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries. 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 [1].

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. The red and 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) [2]. 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, seaweeds are a potential source of FAs for biotechnology and a dietary source of essential fatty acids [3].

The n-3 PUFAs cannot be synthesized by humans and are thus obtained through diet. In view of their promising medical and nutritional applications, they have been extensively investigated. However, the studies on efficient exploitation of natural sources for these compounds are limited. At present, marine fishes and fish oils are the main commercial sources of PUFAs but their suitability for human consumption has been questioned from a biosafety perspective, raising the need to search for alternative sources of high quality PUFAs [4]. Consequently, marine macroalgae have been studied as alternative potential sources, as many of them could easily be cultivated in the sea on a large scale. Also, the PUFAs present in the fishes enter the food chain from different trophic levels as a result of consuming primary producers, such as phytoplankton and seaweeds, which synthesize and store them in good quantities [5].

Bulgarian Black Sea coast is rich in algae, regarding biomass and algal biodiversity. Three species of algae – *Cladophora vagabunda*, *Ceramium rubrum* and *Cystoseira barbata* belonging to the three phyla Chlorophyta, Rhodophyta and Phaeophyta, respectively, were chosen for the

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present study based on their wide distribution in the littoral zone of Bulgaria. There is limited information about the fatty acids contents of Bulgarian Black Sea macroalgae in literature.

The aim of this study is to determine lipids and fatty acids composition of three algal species *Cladophora vagabunda*, *Ceramium rubrum* and *Cystoseira barbata* from Bulgarian Black Sea coast.

2. Experimental

2.1. Algal samples

Cladophora vagabunda, *Ceramium rubrum* and *Cystoseira barbata* were collected in October 2011 from the region of Balchik, 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°C prior to analysis.

2.2. Lipids extraction and fatty acids methyl esters preparation

Lipids were extracted by following the method of Bligh and Dyer [6]. Algal tissue was extracted first with chloroform:methanol (1:2, v/v) and the residue was 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 lipids fraction was methylated by base-catalyzed transmethylation using 2M methanolic potassium hydroxide and n-hexane according to BDS EN 5509:2000 [7]. After 10 minutes centrifugation (3500 rps), the hexane layer was taken for GC analyses.

2.3 GC–MS analysis

Gas chromatography of fatty acid methyl esters (FAME) was performed according to BDS EN ISO 5508:2000 [8] 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 30 m length and 0.25 mm i.d, with a wide range of applications from 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°C for 4 min followed by 10°C per minute until 235°C and final temperature reach was 280°C for 5 min. The sample volume was 1 µl. The three parallel analyses were made from each methanolysed sample. The injector was a split/splitless injector operated in the split mode. Peaks were identified according to two parameters: Retention Time (RT) based on available FAME mix standard (SUPELCO F.A.M.E. Mix C4-C24) (**Fig. 1**) and mass spectra (ratio m/z) – compared to internal Data Base (Thermo Sciences Mass Library, USA). FAMEs were identified and quantified by comparison with the RT and peak areas of

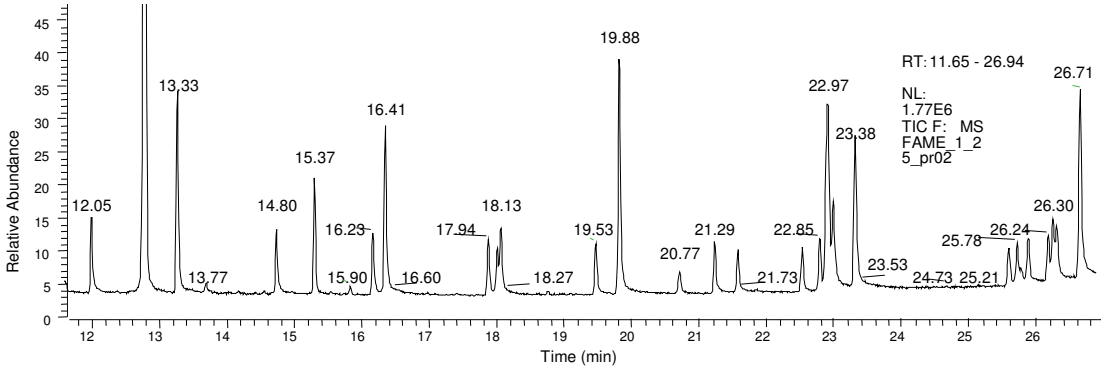

Fig. 1. Chromatogram of FAME mix standard solution (SUPELCO F.A.M.E. Mix C4-C24)

SUPELCO standards. Three replicate GC analyses were performed and the values of FA were expressed as percentage of total FA mass as a mean value and ± standard deviation (SD). All of the chemicals used in the experiments were analytical grade and GC grade (Sharlau).

2.4. Statistical analysis

All analytical determinations were performed in triplicate and the mean values were recorded. Student's t-test was employed to estimate the significance of values.

3. Results and Discussions

3.1. Total lipids

The total lipids contents of various algal species analysed are presented in **Table 1.** The lipids varied within the investigated algal species and ranged from 0.66 ± 0.22 to 0.98 ± 0.13 g per 100 g on a fresh weight basis.

Table 1. List of investigated algae

Algae	Total lipids (g per 100 g fresh weight)
Chlorophyta	
Cladophorales	
Cladophoraceae	
Cladophora vagabunda	0.66±0.22
Rhodophyta	
Ceramiales	
Ceramiaceae	
Ceramium rubrum	0.98±0.13
Phaeophyta	
Fucales	
Sargassaceae	
Cystoseira barbata	0.84±0.21

Data are expressed as means ± SD, where n=3

There was no significant differences (p=0.05) within the species investigated. *Ceramium rubrum* registered the highest lipids content 0.98 ± 0.13 g per 100 g, followed by *Cystoseira barbata* with 0.84 ± 0.21 g per 100 g and *Cladophora vagabunda* – 0.66 ± 0.22 g per 100 g.

3.2. Fatty acids composition

The fatty acids compositions of investigated algae are listed in **Table 2.** Saturated fatty acids (SFA) were major components accounting from 62.21% ± 5.37% for *Cladophora vagabunda* and 62.59% ± 0.93% for *Cystoseira barbata* to 71.61% ± 3.08% for *Ceramium rubrum*. The total sum monounsaturated fatty acids (MUFAs) ranged from 8.05% to 12.12%, whereas total sum of PUFAs were 16.28 – 29.37%.

Palmitic acid was the major fatty acid in all species tested. It accounted more than a half of the total acids content for *Ceramium rubrum* (57.90%) and *Cystoseira barbata* (54.26%). For *Cladophora vagabunda* palimic acid content was 41.97%. Fatty acids composition of algal lipids varies widely with species, habitat, light, salinity, pollution and environmental conditions [9, 10] but in most studies palmitic (C 16:0) acid is predominant [11, 12, 13].

The second major fatty acid varied in the three species. For *Cladophora vagabunda* it was linoleic acid (C18:2 n-6) accounting 14.14% of total fatty acids composition. In *Ceramium rubrum*, after palmitic acid – arachidonic (AA C20:4 n-6) and oleic (C18:1 n-9) acids were most abundant with 4.52% and 3.87%, respectively. In previous study similar pattern was established for other *Ceramium sp.* from Bohai Sea [12].

Second major fatty acids in *Cystoseira barbata* were linoleic (LA C18:2 n-6) and eicosapentaenoic (EPA C20:5 n-3) accounting 13.33% and 9.24% of total fatty acids, respectively. This is inconsistant with a previous study [14] where *Cystoseira barbata* from Black Sea showed oleic acid (C18:1 n-9) as most abundant and higher contents of α-linolenic (C18:3 n-3) acid.

The amounts of monounsaturated fatty asids (MUFA's) varied significantly among the species. The total MUFA content for *Cladophora vagabunda* was 9.81%, for *Ceramium rubrum* and

Table 2. Fatty acids composition of different algal species given in means ± SD (% of total FAME)

Fatty acid, % of total FA	*Cladophora vagabunda*	*Ceramium rubrum*	*Cystoseira barbata*
C8:0	0.67±0.38	n.d.	n.d.
C10:0	1.14±0.72	1.03±0.20	0.51±0.14
C12:0	1.68±0.96	1.60±0.31	0.79±0.21
C13:0	1.08±0.63	n.d.	n.d.
C14:0	3.06±0.78	2.39±0.36	1.51±0.24
C16:0	41.97±15.74	57.90±5.58	54.26±2.67
C17:0	1.24±0.71	1.20±0.23	0.60±0.15
C18:0	2.51±1.51	2.51±0.47	1.32±0.27
C20:0	2.24±1.30	n.d.	1.08±0.28
C22:0	2.59±0.92	2.47±0.47	1.25±0.32
C23:0	1.34±0.78	n.d.	n.d.
C24:0	2.69±1.47	2.50±0.48	1.26±0.33
ΣSFA	**62.21±5.37^{a}**	**71.61±3.08^{b}**	**62.59±0.93^{a,c}**
C14:1	n.d.	1.15±0.22	n.d.
C16:1	2.22±0.53	2.40±0.09	2.26±0.28
C17:1	1.22±0.71	1.18±0.22	0.59±0.15
C18:1n9	4.07±1.73	3.87±0.59	4.11±0.22
C20:1	1.11±0.65	1.06±0.20	0.53±0.14
C22:1n9	n.d.	1.31±0.25	n.d.
C24:1	1.19±0.69	1.15 ±0.21	0.56±0.15
ΣMUFA	**9.81±3.89^{a,c}**	**12.12±1.75^{a}**	**8.05±0.44^{b,c}**
C18:3n6	1.24±0.68	1.14±0.22	0.70±0.14
C18:2n6	14.14±6.52	0.93±0.04	13.33±0.53
C18:3n3	2.20 ±1.07	1.95±0.36	0.97±0.28
C20:5n3	1.50±0.58	1.92±0.10	9.24±0.65
C20:4n6	1.43±0.73	4.52±0.40	1.50±0.08
C20:3n6	n.d.	1.43±0.21	1.26±0.15
C20:2	1.71±0.87	1.67±0.32	0.99±0.18
C20:3n3	2.84±1.63	2.72±0.51	1.38±0.38
C22:6n3	1.34±0.78	1.43±0.21	n.d.
C22:2	1.57±0.92	n.d.	n.d.
ΣPUFA	**27.97±2.42^{a,c}**	**16.28±1.34^{b}**	**29.37±0.69^{c}**

n.d. – not detected;
a–c Values in a row without a common superscript are significantly different at P = 0.05

Cystoseira barbata – 12.12% and 8.05% respectively. Oleic acid (C 18:1 n-9) was the most abundant MUFA in all species analyzed, followed by palmitoleic acid (C 16:1). C14:1 and C22:1n-9 were detected only in *Ceramium rubrum*.

Important long–chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA, C20:5 n-3), linoleic acid (LA, C18:2 n-6) and arahidonic acid (AA, C20:4 n-6) were found in significant levels. Linoleic acid was found to be the most dominant fatty acid in all PUFA's groups. The obtained value of (C18:2, n-6) was 14.14% for *Cladophora vagabunda* and 13.33% for *Cystoseira barbata*.

Linoleic acid (C18:2, n-6) and α-linolenic acid (C18:3, n-3) are two PUFAs which cannot be synthesized by humans and other vertebrates. The PUFAs include two metabolic series of compounds: the n-6 and the n-3 FAs. Linoleic acid belongs to the n-6 series while linolenic acid refers to both α-linolenic (C18:3, n-3) and γ-linolenic acid (C18:3, n-6). Within the body both can be converted to other PUFAs such as arachidonic acid (C20:4, n-6), eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) [15]. The major PUFA in *Ceramium rubrum* was C20:4, n-6 with 4.52%. In *Cystoseira barbata* C20:5n3 accounted 9.24% of total fatty acids.

Sum of C18 and C20 PUFAs, n-3 and n-6 contents in algae investigated, as well as their ratios are given in **Table 3.**

Table 3. Groups and ratios of fatty acids in *Cladophora vagabunda, Ceramium rubrum* and *Cystoseira barbata* given in means ± SD (% of total FAME)

Group and ratios of fatty acids	*Cladophora vagabunda*	*Ceramium rubrum*	*Cystoseira barbata*
C18 PUFA	17.58±5.00^{a}	4.02±0.55^{b}	15.00±0.48^{a,c}
C20 PUFA	7.49±3.80^{a}	12.26±0.79^{b}	14.36±0.33^{c}
PUFA/SFA	0.45±0.07^{a}	0.23±0.03^{b}	0.47±0.02^{a,c}
n-6	16.81±5.19^{a}	8.02±0.20^{b}	16.79±0.48^{a,c}
n-3	7.88±4.04^{a}	6.59±0.97^{a,b}	11.59±0.28^{c}
n6/n3	2.84±1.62^{a}	1.24±0.18^{b}	1.45±0.04^{c}

a–c Values in a row without a common superscript are significantly different at P = 0.05

Despite the variations among the fatty acids composition, the three macroalgae showed typical profiles corresponding to their respective phyla, i.e., *Cladophora vagabunda* being a green alga was rich in C18 PUFAs while *Ceramium rubrum* being a red alga was rich with C20 PUFAs, and *Cystoseira barbata* being brown alga was rich in both. Such trends have already been established earlier in several studies [5, 11, 12, 16].

Simopolous et al. and Erkkila et al. reported that several studies have found inverse correlation between the PUFA/SFA ratios and cardiovascular diseases and suggested that replacement of SFA with PUFA in the human diet will decrease similar health problems [17, 18]. In this study the PUFA/SFA ratio was found lower than one in all analyzed species.

Major source of n-3 and n-6 long-chain PUFAs, such as arachidonic acid, EPA and DHA, is fish oil. However, the original source of these long-chain PUFAs is not the fish itself, but marine algae and phytoplankton which form their major dietary source [15]. Among the species analyzed *Cystoseira barbata* was found to be the richest source of n-3 fatty acids, especially eicosapentaenoic acid.

The n-6: n-3 ratio for the species analyzed was found to be 2.84 for Cladophora vagabunda, 1.24 for Ceramium rubrum and 1.45 for Cystoseira barbata. The n-6/n-3 ratio, which is currently recommended by the WHO [19] to be lower than 10 in the diet, can possibly be improved by addition of certain edible seaweeds, because of their high n-3 content. Seaweeds are also reported to contain much lower concentrations of trans fatty acids than today's diet [20, 21].

4. Conclusions

Lipids and fatty acids (FA) composition of three Black Sea macroalgae *Cladophora vagabunda, Ceramium rubrum* and *Cystoseira barbata* were studied. Total lipids varied within the investigated algal species and ranged from 0.66 ± 0.22 to 0.98 ± 0.13 g per 100 g on a fresh weight basis.

Fatty acids composition was analyzed by GC/MS. Saturated fatty acids (SFA) were major components. Palmitic acid was the major acid in all species tested. Eicosapentaenoic acid (C20:5 n-3) was found in significant quantities in *Cystoseira barbata* and accounted for 9.24% of total fatty acids in this brown alga.

All of the studied species had a nutritionally beneficial n6/n3 ratio (1.24–2.84:1), which is within the recommendations by the World Health Organization to be lower than 10.

5. References

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