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Lipids, Fatty Acids and Sterols of *Cystoseira abies-marina*

P. Moreno[a], G. Petkov[b,*], Z. Ramazanov[a] and G. Garsia[a]

[a] Institute of Applied Algology, E-35214 Muelle de Taliarte, Telde, Las Palmas, Spain
[b] Institute of Plant Physiology, Bulgarian Academy of Sciences bl. 21, Sofia 1113, Bulgaria

* Corresponding author

Lipid content, fatty acids and sterol composition of *Cystoseira abies-marina* and total fatty acids of *C. humilis* from Gran Canaria were studied. A high content of polyunsaturated fatty acids 16 : 3 and 16 : 4 was found. Their content in monogalactosyldiacylglycerols is over 90%. Triacylglycerols and phospholipids contain mainly arachidonic and eicosapentaenoic acids. Reasons for the differences in the composition of fatty acids and sterols of various species of *Cystoseira* are discussed. Some technological approaches are recommended.

Introduction

Brown algae of the genus *Cystoseira* are wide spread in the seas and represented by many species (Piattelli 1990, Amico 1995). Some species have been studied for their lipid composition. There are data on the total fatty acids of *Cystoseira barbata* (Good. *et* Wood.) Ag. (Piatek *et al.* 1978, Stefanov *et al.* 1988, 1990). A comparison of the fatty acid composition of *C. fibrosa* (Hudson) C. Ag. = *C. baccata* (S. Gmel.) Silva (Riguera *et al.* 1984) and *C. compressa* (Esper) Derb. *et* Sol. (Kanias *et al.* 1992) shows remarkable differences.

The sterol composition of several species of *Cystoseira* has been studied (Iatrides *et al.* 1983, Vilalta *et al.* 1984, Riguera *et al.* 1985, Milkova *et al.* 1997), including some seasonal variations (Francisco *et al.* 1977, Combaut *et al.* 1984, 1985). Fucosterol is the main sterol in all *Cystoseira* species studied. Its concentration varies between 60 and 96% of the total sterols. Al Easa *et al.* (1995) have found ergosterol in the sterol mixture of *C. trinodes* (Førssk.) C. Ag. but this result has not been confirmed by other authors.

There are no data about the composition of the lipophylic fraction of *C. abies-marina* (Turner) C. Ag., which is wide spread on the sublittoral rocks of the Canary Islands.

The aim of this paper was to study the lipid content, fatty acids and sterol composition of *Cystoseira abies-marina*. Moreover, there are substantial differences in the data published for the lipid, fatty acid and sterol composition of *Cystoseira* species and, in our opinion, these differences are worth a detailed discussion

Materials and Methods

The fresh plants of *Cystoseira abies-marina* (Turner) C. Ag. were collected from sublittoral rocks on the coast of Gran Canaria, washed and dried at 60 °C. The dry material (6.163 g) was extracted with ethanol 3 times for 0.5 h under reflux, the extract was evaporated, the residue was re-extracted with chloroform, and total lipids were estimated gravimetrically (0.591 g).

Further material was obtained after drying in the sun to constant dry weight. Water extracted material was obtained after extraction of sun-dried, milled materials. Lipids of fresh material (without drying) were extracted after the above mentioned method, converted to fatty acid methyl esters in methanol containing 6 wt% anhydrous HCl at 60 °C for 1.5 h, extracted with diethyl ether and purified by thin layer chromatography (TLC) on silica gel with hexan – diethyl ether (10 : 1 v/v).

Another part of the lipid extract from fresh material was saponified with 5% m/v KOH in 96% v/v ethanol for two hours under reflux. Unsaponifiable matter was extracted with diethyl ether. Sterols were separated on TLC and acetylated with a pyridine – acetic anhydride mixture (1 : 1 v/v) at 60 °C for 1 h. The reaction mixture was neutralized with 5% m/v aqueous $NaHCO_3$ Sterols were extracted with ether and purified on TLC.

We have used the following mobile phases for the TLC separation of the lipid classes on silica gel G:

- monogalactosyldiacylglycerol (MGDG), $CHCl_3$ – MeOH – Me_2CO – AcOH (3.5 : 1.2 : 1.2 : 0.05 v/v)
- triacylglycerols (TG), n-hexan – Et_2O (3 : 1 v/v)
- Phospholipids (PL), $CHCl_3$ – MeOH – H_2O (65 : 25 : 4 v/v)
- Sterols, n-hexan – Et_2O (1 : 1 v/v)
- Sterol acetates, n-hexane – Et_2O (10 : 1 v/v)

The lipid spots were identified using reference compounds. The spots of reference compounds were stained with H_2SO_4 – EtOH (2 : 3 v/v) and heated at 110 °C.

Gas chromatography of fatty acid methyl esters was carried out on a 30 m long Supelcowax-10 capillary column at 195 °C. The methyl ester of pentadecanoic acid (15 : 0) was used as an internal standard for the quantification of the fatty acids. Sterols were separated on a 1.82 m, 2.7% OV-17 packed column at 280 °C.

Cystoseira humilis Kütz. was collected at the same location as *C. abies-marina* and the fatty acids were isolated according to the procedures described above.

Results and Discussion

Lipid, fatty acid and sterol contents of *C. abies-marina* are shown in Table I. The lipid content was much greater than that found in *C. barbata* (1.1%) by Alfimov (1963). The differences in the lipid content of fresh and dried material are due to the loss of volatile substances and to a more difficult penetration of solvents in dry material. Wetting the material prior to extraction procedures leads to more complete extraction of the lipids (Georgiev *et al.* 1992).

The lipid yield of milled, water extracted material significantly decreases. This is a result of the action of liberated hydrolases. For this reason, delipidisation of the material should be carried out before the water extraction, as previously described (Petkov and Dilov 1987).

The fatty acid composition of *C. abies-marina* differs substantially from that of other species studied. The comparison of our data and data obtained by other authors is valid because all of them have used the same pretreatment of algal material. Fatty acids 16 : 3 and 16 : 4 are most abundant in the total lipids (Table II). Our experience with microalgae, as well as data in the literature, indicate that 16 : 4 is localised predominantly in the thylacoid membranes as a component of MGDG, digalactosyldiacylglycerols (DGDG) and sulfoquinovosyldiacylglycerols (SQDG). Moreover, the high concentration of 16 : 4 in the total fatty acids is evidence for a good physiological state of photosynthesis. Some physiological stress conditions: nitrogen starvation, temperatures above optimum, heterotrophic nutrition, provoked a decrease of 16 : 4 and even its total disappearance in *Scenedesmus*, *Dunaliella* and *Coelastrum* (Ben-Amotz *et al.* 1985, Petkov *et al.* 1986, 1990, Furnadzhieva *et al.* 1987). The data published in these papers lead to the conclusion that a low content of 16 : 4 and the high one of 18 : 0, 18 : 2, 20 : 4, 20 : 5 is possibly connected to seasonal changes of heterotrophic nutrition, because of water pollution with organic substances. As shown in Table II, 16 : 3 and 16 : 4 constitute more than 90% of the composition of MGDG; phospholipids (PL) are built mainly of unsaturated 20 : 4 and 20 : 5 acids. Triacylglycerols (TG), which are storage substances, increase at nitrogen starvation or unfavourable conditions. In our case, the acid 20 : 5 prevails in TG. Our data confirm the results of Hulanicka *et al.* (1964) with *Euglena*, who found that autotrophic cultivation increased the percentage of

Table I. Lipid composition of *C. abies-marina*.

Substances	%
Lipids in oven dried material	9.6
Lipids in sun-dried material	8.5
Lipids in water-extracted material	2.0
Fatty acids in fresh material	4.3
Fatty acids in lipids	48.8
Unsaponifiable fraction of lipids	7.0
Sterols in unsaponifiable fraction	5.4

Table II. Fatty acid composition of *Cystoseira* species.

Fatty acids	*Cystoseira abies-marina*					*C. humilis*
	Total	TG	MGDG	PL	Sun dried	Total
14 : 0	0.5	2.7	0.5	5.7	24.9	3.1
14 : 1	–	–	–	–	5.7	–
16 : 0	1.2	18.0	1.2	25.6	25.1	8.1
16 : 1	0.1	7.9	0.3	5.8	2.4	6.2
16 : 1	0.1	–	–	–	–	–
16 : 2	7.4	3.8	3.7	2.2	–	0.6
16 : 3	40.7	–	30.7	1.2	–	0.8
16 : 4	47.0	4.6	60.3	1.4	–	1.2
18 : 0	0.1	3.9	0.1	3.7	1.5	6.2
18 : 1	0.5	9.2	0.6	11.8	14.4	9.1
18 : 2	0.4	8.5	0.5	4.2	10.4	7.8
18 : 3	tr.	–	tr.	–	–	1.9
18 : 3	0.2	1.5	0.2		3.5	19.3
18 : 4	0.1	–	tr.	3.7	–	13.4
20 : 4	0.9	4.9	0.7	9.8	11.5	11.4
20 : 5	0.5	34.9	1.4	24.8	–	10.9

16 : 4 whereas heterotrophic cultivation increased the percentage of 20 : 4 and 20 : 5. The data are in a good agreement with the results of Stefanov *et al.* (1988, 1990) for *Cystoseira barbata* from the Black Sea.

Neither seasonal changes nor heterotrophic nutrition completely explain the simultaneous absence of 16 : 3, 16 : 4 and 20 : 4, 20 : 5 reported in some of the publications on *Cystoseira* species (Piatek *et al.* 1978, Riguera *et al.* 1984, Kanias *et al.* 1992). For a comparison we studied the fatty acid composition of another Canarian alga *Cystoseira humilis* and found 16 : 3 and 16 : 4 acids, even if at a low percentage. In this case, the concentration of 18 : 4, 20 : 4, 20 : 5 was rather high (Table II). It is important to mention that *C. abies-marina* was collected at the beginning of October and the *C. humilis* in March.

The sterol composition of *C. abies-marina* is typical for an alga of the genus *Cystoseira* (Table III). The UV-spectrum of the sterols from fresh material and quick analytical procedures showed an absence of sterols with conjugated $\Delta^{5,7}$-double bonds. So, the sterol composition of *C. trinodes* studied by El Easa *et al.* (1995) appears to be an exception in the genus *Cystoseira*.

Table III. Percentage of sterols of *C. abiea-marina*

Sterol	%
22-Dehydrocholesterol	tr.
Cholesterol	0.7
Brassicasterol	1.2
24-methylenecholesterol*	1.1
Fucosterol	96.9

* together with campesterol

Kanias *et al.* (1992) have reported campesterol from *C. compressa*. Unfortunately, under the GC-conditions they used, there is an almost full overlapping of the peaks of 24-methylenecholesterol and campesterol. Studies of Milkova *et al.* (1997) confirm this. Al Easa *et al.* (1995), using a 30 m DB 1 column, have separated 24-methylenecholesterol and campesterol but they showed that campesterol was absent in *C. trinodes*. It has to be accepted that in *Cystoseira* there is no campesterol, or if there is any, it is in trace amounts. Authors who did not separate these two sterols, gave a small value for their sum (Kanias *et al.* 1992). We may conclude that all the species of *Cystoseira* studied, have one and the same qualitative sterol composition and show quantitative diferences only.

Sterols, except $\Delta^{5,7}$, are more stable to peroxidation compared to polyunsaturated fatty acids. We consider, that this is one of the reasons why the data on fatty acid composition differ significantly in different publications, while the data on sterol composition are similar. If sterols, which are secondary metabolites, coincide in the species of the same genus, it is very unlikely that primary metabolites such as fatty acids, will differ so much. For instance, *Chlorella* was subdivided by Kolattukudy (1976) into Δ^5, Δ^7, $\Delta^{5,7}$-genotypes according to its sterol composition but all species of the genera had the same fatty acid composition.

We have to conclude that all species of *Cystoseira* have the same fatty acids but with different percentages, depending on the physiological and ecological conditions.

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