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Fatty acid and sterol composition of gametophytes and sporophytes of *Chondrus crispus* (Gigartinaceae, Rhodophyta)*

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SUMMARY: Fatty acids and sterols of gametophytes and sporophytes of *Chondrus crispus* were isolated and identified. Although chemical variation in the proportions of other natural compounds (eg. different carrageenan type) between gametophytes and sporophytes has already been observed, this is the first comparative study of the lipid composition in both generations. The content of fatty acids and sterols in gametophytes (0.710 and 0.190 mg g^{-1} dry weight respectively) was higher than in sporophytes (0.622 and 0.113 mg g^{-1} dry weight). The main fatty acids were palmitic, palmitoleic, oleic, arachidonic and eicosapentaenoic acids. These five fatty acids represented more than 78% of the all fatty acid composition in both generations. In addition unsaturated fatty acids were present in a much greater quantity (>80%) than saturated fatty acids. However, differences in the distribution of fatty acids between the two generations were observed. Gametophytes demonstrated higher amounts of monounsaturated fatty acids while sporophytes had a relatively higher content of saturated and polyunsaturated fatty acids. The major sterol in both generations was cholesterol (>94%). Differences were also observed in the sterol distribution between gametophytes and sporophytes, the former containing smaller amounts of 7-dehydrocholesterol and stigmasterol, while the latter had smaller amounts of 22-dehydrocholesterol, campesterol and sitosterol. Gametophytes were lacking 24-methylenecholesterol. Fatty acids and sterol of algal class, families and sometimes even species are characteristic to those particular taxa, and could be useful as chemotaxonomic markers. The results of this study showed that intra-specific variation in algae can be occured.

Key words: *Chondrus crispus*, gametophyte, sporophyte, fatty acid, sterol.

INTRODUCTION

The determination of lipid composition in a given species is essential for further studies on lipid metabolism and on the effect of environmental factors. Moreover, some evidence suggests that the fatty acid and sterol composition may be useful for taxonomic purposes (Shameel, 1990; Dembitsky *et al*., 1991; Patterson, 1971, 1992; Herbreteau *et al*., 1997).

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Rhodophyta are characterized by a high content of C_{20} polyunsaturated fatty acids, mainly arachidonic (20:4 n-6) and eicosapentaenoic (20:5 n-3) acids (Ackman and McLachlan, 1977; Pohl and Zurheide, 1979; Fleurence *et al*., 1994). Other abundant fatty acids in this class are palmitic (16:0) and oleic (18:1) acids. The major sterol of red algae, with a few exceptions, belong to the type C_{27}. Cholesterol is the most commonly found and is usually present in the highest concentration (Doyle and Patterson, 1972; Goad and Goodwin, 1972; Patterson, 1992). Two other C_{27}-sterols frequently detected in

red algae are desmosterol and 22-dehydrocholesterol. The occurrence of C_{26}-, C_{28}- and C_{29}-sterols in varying quantities has been reported in a number of species of red algae (Patterson, 1971, 1992).

Many red algae have a complex life history with alternating haploid and diploid phases that are free living (Gabrielson and Garbary, 1986; Maggs, 1988; John, 1994). The morphology of these different phases can be identical (isomorphic) or different (heteromorphic). Seaweeds with life stages that differ in ploidy level might express recessive traits during haploid stages, or express other differences, that would allow these stages to vary in adaptability under different environmental conditions. Heteromorphic seaweeds are well know to have variable chemical traits, such as different carrageenan types and chlorophyll a concentrations, associated with their morphological differences (Dudgeon *et al*., 1995).

Most of the available information in the literature on the fatty acid and sterol composition of isomorphic red algae, as well as the majority of the other algae was obtained from plants not sorted according to their life history phase and no comparative studies has ever been done on *Chondrus crispus* Stackhouse. The present study was undertaken to determined the fatty acid and sterol composition of gametophytes and sporophytes of *C. crispus*, which has an isomorphic life history. This species was chosen because gametophytes and sporophytes can be easily separated by the resorcinol test (Yaphe and Arsenault, 1965).

MATERIALS AND METHODS

The algal samples were collected from a population of Muros (A Coruña). In order to avoid differences in lipid composition related to the microgeographic environmental conditions and seasons, shoots were collected separated only 10 cm from the same rock, in the same position and orientation. Ten gametophytes and sporophytes of *C. crispus* were lyophilized. The dried material was homogenized by pulverization and divided into two parts. The first one was used to determine the fatty acid composition and the second the sterol composition. The analytical data are the means of ten analyses.

Fatty acid extraction and analysis

Dried samples (2.50 g) were extracted with trichloromethane-methanol (2:1 v/v) in a soxhlet apparatus for 48 h. The extract was evaporated under vacuum. Ethanol with 6% KOH was added to the residue and the reaction mixture was saponified by refluxing at 100ºC for 1 h with pyrogallol. The mixture was cooled, concentrated under reduced pressure and thereafter H_2O and ethanol were added and extraction with ether was repeated three times. The aqueous alkaline fraction was acidified with 6N HCl to pH 1 and then extracted several times with hexane. The organic fraction was dried over anhydrous Na_2SO_4 and evaporated under reduce pressure.

The fatty acid fraction was dissolved in methanol-H_2SO_4 (97.5:2.5 v/v) with pyrogallol methylated. The fatty acid methyl esters were extracted with hexane:ether (2:1 v/v). The organic fraction was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The fatty acid methyl esters were purified by silica thin layer chromatography (TLC) and analyzed on a Varian 3400 CX gas chromatograph equiped with a flame ionization detector and a OV-1 Interchin column (30 m x 0.32 mm). The column temperature was programmed from 180 to 200ºC at a rate of 1ºC min^{-1} and from 200 to 240ºC at a rate of 2ºC min^{-1}. The injection temperature was 270ºC and the detector temperature 300ºC. The gas carrier was helium at a pressure of 1 bar. Methyl esters were identified by comparison of the retention times with those of heptadecanoic acid and by comparison with authentic standards.

Sterol extraction and analysis

Dried samples (2.50 g) were extracted in 2:1 (v/v) dichloromethane-methanol using a Soxhlet apparatus for 24 h. The extract was concentrated under reduced pressure and fractionated, in order to obtain total sterols, by preparative TLC on Silica gel plates (0.25 mm) developed in the first dimension in 92:8 (v/v) hexane-ethyl acetate (18 cm length of run) to separate steryl esters (SE), and, in a second dimension in 90:10:0.5 (v/v) dichloromethane-methanol-water (12 cm length of run) to separated free sterols (FS), acylated steryl glycosides (AGS) and steryl glycosides (GS) (Hartmann and Benveniste, 1987). The SE, FS, AGS and GS bands were located according to Rf values of standards simultaneously chromatographed: cholesterol palmitate, cholesterol (Sigma), cholesterol-3-O-6-palmitoyl-glucopyranoside and cholesterol-3-O-glucopyranoside (Matreya, Pleasant Gap, USA). Spots of standars were visualized with Liebermann-Burchard reagent

(Rf = 0.95, 0.70, 0.55 and 0.35 respectively). The different bands were scrapped off and eluted with dichloromethane for SE and FS and with 2:1(v/v) dichloromethane-methanol for AGS and GS.

The SE were saponified by 1 h reflux with 6% (w/v) methanolic KOH with 0.5% (w/v) pyrogallol. The AGS and GS were separately hydrolized in a 1% (v/v) ethanolic H_2SO_4 by refluxing for 4 h. Sterols were recovered by partition into hexane and acylated by acetic anhydride in anhydrous pyridine at room temperature in the dark for 48 h. Acetyl derivates were dried in a stream of nitrogen gas and purified by silica gel TLC plates developed in dichloromethane with colesteryl acetate as the standard. Identification was determined as mentioned above.

Resulting total steryl acetates were analyzed on a Carlo Erba 2900 gas chomatograph coupled with a Jeol JMS-D300 mass spectrometer. Gas chromatography was carried on 30 m x 0.32 mm fused silica capillary column with a 0.1 mm film of DB5 (J&W Scientific, Folsom, USA). The column was operated at a pressure of 0.5 bars of helium. Temperature was increased from 260 to 278ºC at a rate of 2ºC min^{-1}, then to 300ºC at a rate of 5ºC min^{-1} and held at 300º C for 10 min. The injector temperature was held at 275ºC and the detector temperature 300ºC.

Electronic impact mass spectra was measured at 70 eV and an ionization temperature of 150ºC. Identifications were based on the retention times of the steryl acetates relative to cholesterol acetate and their mass spectra. For quantification, 5 α-cholestane was added to each sample as internal standard. Relative abundances of sterols were calculated by measurements of spectra which were proportional to content.

RESULTS

The quantitative estimation of the fatty acid and sterol content of *C. crispus* revealed that gametophytes had larger amounts of fatty acids and sterols than sporophytes (Table 1).

The relative percentages of the identified fatty acids are presented in Table 2. From the ten fatty acids identified, palmitic (16:0), palmitoleic (16:1 n-7), oleic (18:1 n-9), arachidonic (20:4 n-6) and eicosapentaenoic (20:5 n-3) acids were the main fatty acids. These five fatty acids represented more than 78% of all the fatty acid composition in both generations. Sporophytes had smaller proportions of palmitoleic and oleic acids than gametophytes, but larger amounts of stearic, palmitic and linolenic acids. Unsaturated fatty acids were present in much greater proportions than saturated fatty acids, with slightly higher levels in gametophytes than in sporophytes. The content of monounsaturated fatty acids in gametophytes was more than twice than in sporophytes. Polyunsaturated fatty acids were less abundant in gametophytes than in sporophytes.

The sterol profiles of gametophytes and sporophytes are summarized in Table 3. The major sterol

TABLE 1. – Fatty acid and sterol content of gametophytes and sporophytse of *Chondrus crispus* (mg g^{-1} dry weight). Values are means ±s.d. (n=10).

Type	Gametophytes	Sporophytes
Fatty acids	0.710 ± 0.010	0.622 ± 0.004
Sterols	0.190 ± 0.015	0.113 ± 0.018

TABLE 2. – Fatty acid composition of gametophytes and tetrasporophytes of *Chondrus crispus* (% of total fatty acids). Values are means ± s.d. (n=10).

Type	Common name	Gametophytes	Sporophytes
14:0	Myristic acid	0.88 ± 0.03	1.18 ± 0.01
16:0	Palmitic acid	10.85 ± 0.91	13.26 ± 0.04
18:0	Stearic acid	1.81 ± 0.33	5.25 ± 0.01
16:1 (n-7)	Palmitoleic acid	8.98 ± 0.15	3.15 ± 0.01
18:1 (n-9)	Oleic acid	13.22 ± 0.51	5.51 ± 0.02
18:2 (n-6)	Linoleic acid	1.42 ± 0.05	1.85 ± 0.10
18:3 (n-3)	Linolenic acid	2.85 ± 0.26	5.77 ± 0.02
18:4 (n-3)	Octadecatetraenoic acid	5.82 ± 0.98	7.30 ± 0.02
20:4 (n-6)	Arachidonic acid	21.84 ± 0.40	19.03 ± 0.50
20:5 (n-3)	Eicosapentaenoic acid	32.33 ± 1.08	37.70 ± 0.35
SFA[1]		13.54 ± 0.84	19.69 ± 0.34
UFA[2]		86.46 ± 0.81	80.31 ± 0.34
MUFA[3]		22.20 ± 0.71	8.66 ± 0.34
PUFA[4]		64.26 ± 0.40	71.65 ± 1.05
UFA/SFA[5]		6.44 ± 0.44	4.07 ± 0.09

[1]Saturated fatty acids. [2]Unsaturated fatty acids.
[3]Monounsaturated fatty acids. [4]Polyunsaturated fatty acids.
[5]Unsaturated: polyunsaturated fatty acid ratios

TABLE 3. – Sterol composition of gametophytes and sporophytes of *Chondrus crispus* (% of total sterols). Values are means ±s.d. (n=10).

Type	Gametophytes	Sporophytes
Cholesterol	94.42 ± 0.44	94.49 ± 0.41
22-Dehydrocholesterol	1.47 ± 0.23	0.85 ± 0.17
7-Dehydrocholesterol	0.87 ± 0.09	1.42 ± 0.20
Brassicasterol	0.77 ± 0.21	0.94 ± 0.18
Campesterol	1.24 ± 0.13	0.62 ± 0.11
24-Methylenecholesterol	-	0.48 ± 0.09
Sitosterol	0.85 ± 0.02	0.62 ± 0.11
Stigmasterol	0.38 ± 0.02	0.58 ± 0.08
C_{27}-Sterols	96.76 ± 0.13	96.76 ± 0.42
C_{28}-Sterols	2.01 ± 0.10	2.04 ± 0.40
C_{29}-Sterols	1.23 ± 0.01	1.20 ± 0.19

in both generations was cholesterol. Some differences were observed in the proportions of the minor sterols between gametophytes and sporophytes. The former containing lower proportions of 7-dehydrocholesterol and stigmasterol, while the latter had lower proportions of 22-dehydrocholesterol, campesterol and sitosterol. Moreover, no 24-methylenecholesterol was detected in gametophytes.

DISCUSSION

Variability in the total amounts of fatty acids and sterols between gametophytes and sporophytes of *C. crispus* was noted. Variations in the proportions of another natural compound, carrageenans, between gametophytes and sporophytes has already been observed in *C. crispus* (Percival and McDowell, 1967; McCandless *et al*., 1973, 1983; Rivera-Carro *et al*., 1990). In *Gracilaria verrucosa* (Huds.) Papenfuss, the different generations differed slightly in the amounts of lipids (Khotimchenko and Levchenko, 1997). Pettit *et al*. (1989b) and Pettit and Harwood (1989) suggested that the lipid composition of *C. crispus* may change in response to seasonal environmental conditions. The differences noted in this study are not due to different seasonal conditions as gametophytes and sporophytes were collected from the same population and at the same time.

Differences were also observed in the fatty acid and sterol distributions between the two generations. The main fatty acids were palmitic, palmitoleic, oleic, arachidonic and eicosapentaenoic acids as previously reported (Ackman and McLachlan, 1977; Pohl and Zurheide, 1979; Harwood *et al*., 1988; Pettit *et al*., 1989a). Contrary to this study Fleurence *et al*. (1994) reported higher amounts of arachidonic acid in this species than eicosapentaenoic acid. It seems that the proportion of these two fatty acids in a given species is negative. These fatty acids are the end products of byosynthetic pathways, and one common precursor (linoleic acid) was suggested in the synthesis of both fatty acids (Cohen and Heimer, 1990). If there is a fixed amount of linoleic acid one can understand the negative correlations between these fatty acids.

Major differences with the data in the literature are related to the different values of the ratio of unsaturated to saturated fatty acids. These differences may be due to the different locations and seasons where the plants were collected. Changes in major environmental conditions (light regime and temperature) are know to cause changes in fatty acid composition and metabolism (Pettit *et al*., 1989b; Pettit and Harwood, 1989). These authors suggested that illumination and low temperatures increased synthesis and desaturation of fatty acids. In this study, differences in unsaturated:saturated fatty acid ratios between gametophytes and sporophytes of *C. crispus* were observed. Levy *et al*. (1992) suggested that there are positive correlations between the growth periods of a given species and the environmental conditions on the one hand and the ratio unsaturated:saturated fatty acids on the other hand. In Galicia, differences in growth phase related to the culture conditions and requirements (light, temperature and nutrient levels) between gametophytes and sporophytes obtained from natural populations of the Galician coasts were observed (Tasende and Fraga, 1997, 1999).

The result of this study showed that the sterol composition of *C. crispus* was at least as complex, if not more so, than the one found in other red algae. the major sterol in gametophytes and sporophytes was cholesterol. Although slight differences in the proportion of the individual sterols were observed, the combined content of C_{27}-, C_{28}- and C_{29}-sterols was similar in both generations. In addition, although in gametophytes were lacking 24-methylenecholesterol, the combined content of campesterol and its precusor, 24-methylenecholesterol in the sporophytes was similar to the content of campesterol in gametophytes.

Large variations in the sterol composition in this species has been reported. Thus, although in earlier studies sitosterol was cited as the major sterol in *C. crispus* (Heilbron, 1942; Saito and Idler, 1966), now it is know that the major sterol is cholesterol as was observed in this study (Alcaide *et al*., 1968, 1969; Freire and Ribas, 1975; Goldberg *et al*., 1982). Moreover, Iatrides *et al*. (1978) cited cholesterol as the only sterol occurring in *C. crispus*. In addition, in other studies no C_{28}- and C_{29} sterols were detected. Patterson (1992) suggested that there are two probable reasons for the confusions of the sterol composition in a given species. First, when the early studies were done, the analytical techniques were not able to separate and identify closely-related sterols that were know to occur. Secondly, considerable variation may be due to seasonal, geographic and environmental changes.

Another frequently found C_{27} sterol in Gigartinaceae, desmosterol, was not determined in this study. Fattorusso *et al*. (1975) believed that desmos-

terol seemed to have some taxonomic significance since it was found in some species of Gigartinaceae (Goldberg *et al*., 1982; Patterson, 1992). However, Goad and Goodwin (1972) observed that the proportion of cholesterol and desmosterol can vary from sample to sample in a given species, with some samples containing only cholesterol or both C_{27} sterols.

Debates on the extent to which the morphologically identical phases of a species are physiologically and ecologically equivalent have been taking place for many years. However, morphological similarity does not imply physiological or ecological similarity, as rapid growth, reproduction, environmental tolerance, resistance to predation and competition have been observed to differ between isomorphic life stages of macroalgae (Dixon, 1973; Hannach and Santelices, 1985; Juanes and Puente, 1993). Life history traits may be determined at the cellular level by differential allocation of metabolic energy among physiological processes (Dudgeon *et al*., 1995)

Several studies demonstrated variability in ploidy ratios in natural populations of *C. crispus*, with dominance of haploid or diploid phases and a wide range of intermediate situations, due to physical environment or a differential reproductive and growth capacities (Bhattacharya, 1985; Chopin *et al*., 1988; Lazo *et al*., 1989; Mathieson, 1989; Fernández and Menéndez, 1991a, b; Chopin and Floc'h, 1992; Scrosati *et al*., 1994; Dudgeon *et al*., 1995; Lindgren and Aberg, 1996), which could reflect different physiological adaptations between the two generations. In addition, in culture, slight differences in growth between both generations were also observed (Chen and Taylor, 1980; Guiry, 1979; McLachlan *et al*., 1988; Tasende and Fraga, 1999).

In conclusion, although previous studies pointed out the fact that changes in the environmental conditions, such as temperature, salinity, light regime and nutrient availability, have an impact on the synthesis of fatty acid and sterols in marine macroalgae (Araki *et al*., 1990), this study showed variations in *C. crispus* related to the phases of the life history. Further research is needed to determine if changes in the fatty acid and sterol composition in the two generations are influenced by geographical locations and seasons.

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