

Fatty acid composition of three species of *Codium* (Bryopsidales, Chlorophyta) in Chile

Composición de ácidos grasos en tres especies del género *Codium* (Bryopsidales, Chlorophyta) de Chile

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Resumen.- Las algas poseen patrones de composición lipídica particulares de acuerdo a su identidad específica. En el presente trabajo se identificaron y compararon los principales ácidos grasos de dos especies endémicas del género *Codium* presente en Chile: *C. dimorphum* y *C. fernandezianum*; y de la especie introducida *C. fragile*. El principal ácido graso en el género fue el ácido palmítico (28-54%), seguido del ácido oleico y del ácido linoléico. Se confirma por primera vez, la presencia de ácido hexadecatrienoico (C16:3), ácido graso propuesto como característico del género *Codium*, el cual puede aplicarse en estudios quimiotaxonómicos a nivel mundial.

Palabras clave: Algas, composición química, lípidos, ácido hexadecatrienoico

Abstract.- The fatty acid compositions of algae show characteristic patterns according to species. This paper is the first to identify and compare the main fatty acids of the two endemic *Codium* species: *C. dimorphum* and *C. fernandezianum* found in Chile. In addition the fatty acids of the introduced species *C. fragile* were investigated. Palmitic acid was shown to be the main fatty acid, comprising between 28 to 54%, followed by oleic and linolenic acids. We also confirm, for the first time, the presence of hexadecatrienoic acid (C16:3) in all three species, which is suggested to be a characteristic fatty acid for *Codium* genus, so this can be used in chemotaxonomy for these species around the world.

Key words: Algae, chemical composition, lipids, hexadecatrienoic acid

INTRODUCTION

There are about 100 species of *Codium* Stackhouse known around the world (Xu *et al.* 1998); three of them are native to Chile (Ramírez & Santelices 1991). The genus *Codium* belongs to the family Codiaceae (Chlorophyta), and is characteristically organized in branches that form spongy, dark green, coenocytic thalli of intertwined filaments (Hoffmann & Santelices 1997).

Lipid extracts obtained from *Codium* species have been shown to exhibit antibacterial, antiviral, antifungal and cytotoxic activities. A variety of sterols, fatty acids and terpenes are responsible for these activities (Caccamese *et al.* 1981). Due to their ability to produce polyunsaturated fatty acids C:18 and C:20 (PUFAs), marine algae have attracted considerable attention from researchers all over the world in the last two decades (Dembitsky *et al.* 2003). The nutritional value of fatty acids is indisputable, they are important components in the cellular structure, take part in the production of some hormones and bile salts, and have

important regulatory functions for cellular activity and gene expression. In addition they are related to the prevention and treatment of atherosclerosis, thrombosis, arthritis and cancer, mainly through their metabolic conversion into prostaglandins, thromboxanes and leucotriens (Ip 1997). They also have hypocholesterolemic, immunostimulant and antioxidant properties (Sanhueza *et al.* 2002).

The fatty acid composition of algae is complex and there is a greater variety in algae than in vascular plants (Dembitsky *et al.* 2003). The lipid composition of red, brown, and green algae, belonging to different classes, orders, families and genera presents distinguishing features that are taxonomically valuable. Such fatty acid patterns are characteristic for different algal divisions. In green algae C16 and C18 PUFAs have been shown to be generally the most abundant (Khotimchenko 2003, Graeve *et al.* 2002).

In a study considering six *Codium* species, Xu *et al.* (1998) detected 37 fatty acids. They determined palmitic acid (C16:0) (25%-45%) to be the most abundant, followed by oleic acid (C18:1), linolenic acid (C18:3), myristic acid (C14:0) and hexadecatrienoic acid (C16:3). They also reported on linolenic, arachidonic and eicosapentaenoic acids in concentrations of 1-10% in all taxa. Recently, in the northern Chile, Ortiz *et al.* (2009) detected 19 fatty acids in *Codium fragile*. They found linolenic acid to be the main fatty acid, making up 24.6% of the total fatty acid content, followed by palmitic (17.74%), stearic (17.38%) and oleic (12.25%) acids.

In recent decades, these lipids have sparked the interest of chemotaxonomists. It has been proposed hexadecatetraenoic acid (C16:4) as taxonomically important for green algae, with only trace appearances in higher plants. But for years, hexadecatrienoic acid (C16:3) has been considered to be a characteristic fatty acid of the family Codiaceae, considered as the most important chemotaxonomic feature in the genus (Dembitsky *et al.* 2003, Khotimchenko 2003). However, C16:3 could not be detected in some *Codium* species by other researchers (Aliya *et al.* 1991, 1992, Hainaux *et al.* 1998, Ortiz *et al.* 2009). In this current investigation we describe the fatty acid composition of the two endemic *Codium* species in Chile (*C. fernandezianum* Setchell, *C. dimorphum* Svedelius) and one other recently introduced species (*C. fragile* (Suringar) Hariot).

MATERIAL AND METHODS

COLLECTION OF BIOLOGICAL MATERIAL

Codium fernandezianum was collected from the Robinson Crusoe Island in the Juan Fernández Archipelago (33°37'S, 78°49'W), *C. dimorphum* was collected from Horcón Cove (33°40'S, 79°00'W) and *Codium fragile* was obtained from Caldera Bay (27°03'S, 70°51'W). The macroalgae were manually collected by SCUBA diving. The postrate algae *C. dimorphum* was collected with a trowel. Epiphytes were removed carefully with tweezers. The cleaned algae were stored at -20°C until further processing in the laboratory.

Part of the macroalgae was fixed in 96% ethanol for its taxonomic identification. Algae were identified by examination of the utricles and fronds, dichotomies, presence of filaments, and reproductive structures (Hoffmann & Santelices 1997, Campbell 1999).

Fresh samples (3-5 kg) of each of the three algae were cut into pieces separately. The pieces were extracted with methanol for 3 days in a 5 L extraction flask, protected from light and shaken every day by hand (modified from Dembitsky *et al.* 2003). Fresh algae contain a great amount of water, so extraction of mebolites can be better achieved by methanol, which is a polar solvent. The extracts were then filtered and concentrated under low pressure with a

Table 1. Mean values of the percentage of total fatty acid content in the *Codium* species studied herein. Numbers refer to peaks in figure 1 / Valores promedio del porcentaje del contenido de ácidos grasos total en las especies de *Codium* estudiadas. Los números corresponden a los picos en figura 1

Fatty acid	<i>C. fernandezianum</i> %	<i>C. dimorphum</i> %	<i>C. fragile</i> %
Myristic acid (C14:0)	0.49	0.34	0.82
Palmitic acid (C16:0)	29.27	54.49	28.22
(C16:2)	1.74	1.68	0.74
Hexadecatrienoic acid (16:3)	9.57	7.25	7.50
Stearic acid (C18:0)	0.50	0.24	0.58
Oleic acid (C18:1)	15.14	20.39	5.20
Linoleic acid (C18:2)	8.25	2.17	0.74
Linolenic acid (C18:3)	16.93	9.83	6.74
Arachidic acid (C20:0)	0.55	0.34	4.22
Arachidonic acid (C20:4)	6.41	0.80	3.10
Behenic acid (C22:0)	2.65	1.68	2.26
Total SAFA	30.81	55.41	33.84
Total MUFA	15.14	20.39	5.20
Total PUFA	42.90	21.73	18.82

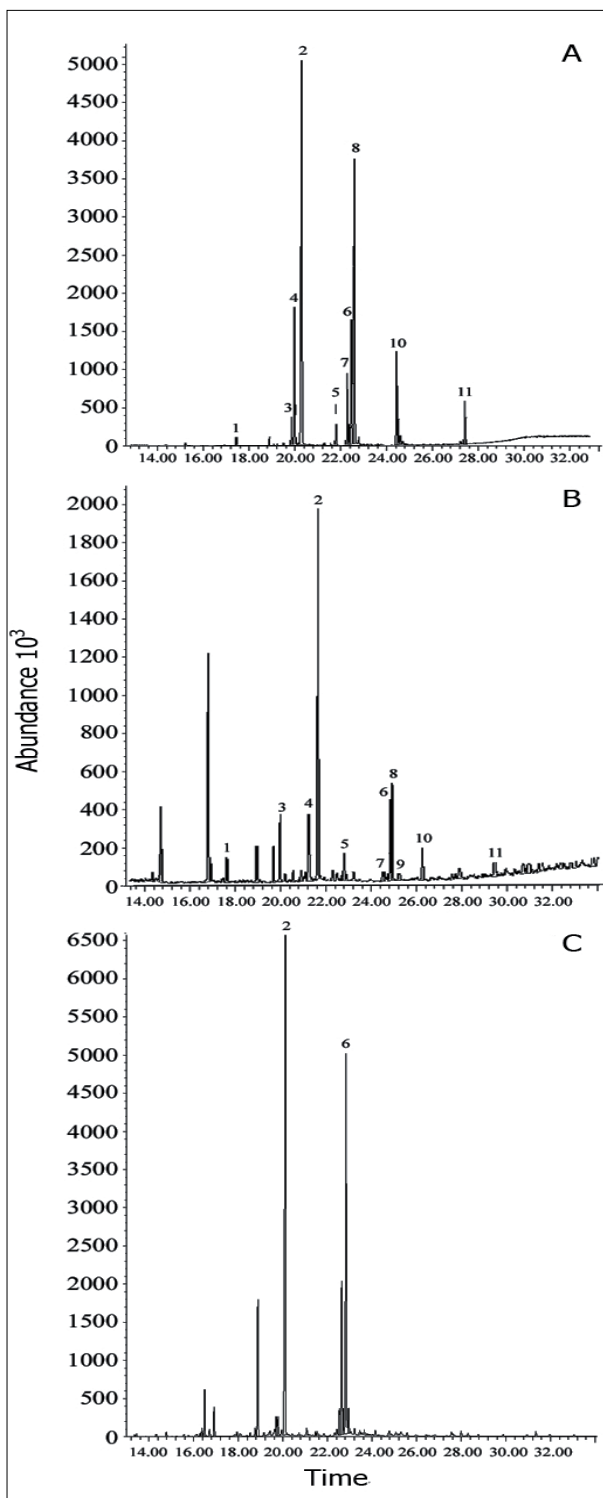


Figure 1. Fatty acids amounts of the green macroalgae, A) *Codium fernandezianum*, B) *Codium fragile* and C) *Codium dimorphum* and retention times (min) / Cantidad de ácidos grasos de las macroalgas clorofíceas, A) *Codium fernandezianum*, B) *Codium fragile* y C) *Codium dimorphum* obtenidos en el presente estudio y tiempos de retención (min)

rotary evaporator Heidolph® Laborota. Eighty grams of total extract were obtained.

The total extracts were fractionated with solvents of increasing polarity (hexane, dichloromethane, ethyl acetate, distilled water) in a liquid-liquid separation device. The fractions obtained were analyzed using thin layer chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS).

Lipids were extracted according to Khotimchenko (2003). Two milligrams of the samples (hexanic and dichloromethanic fractions) were combined before methylation. The fatty acids present were derivatized, converting them into methyl esters by using 1% KCl in MeOH and 5% HCl in MeOH (Dembitsky *et al.* 2003). The fatty acid methyl esters (F.A.M.E.s) were then identified through GC-MS. The analyses were done in duplicates.

The identification of the obtained compounds was done with gas chromatography with flame ionization detection (GC-FID) and mass detection (GC-MS). Mass analysis was carried out in a Chromatograph Hewlett Packard 5890 Series II (California, USA) with a mass detector Hewlett Packard model 5972, Series. Gas carrier was helium electronic grade, with a flux of 1 ml seg^{-1} , injector was 275°C, detector temperature 300°C, and the program for oven temperature was 100°C for the first 5 min, and increased 10°C per minute until reaching 275°C, with a final time of 15 min. Chromatographic fused silica column was a HP-5MS (J&W Scientific) of 30 m, internal diameter of 0.25 mm and a phase thickness of 0.25 μm . Standards (F.A.M.E. Mix C14-C22, C20:1-C20:5) were purchased from SUPELCO (Bellefonte, PA, USA).

RESULTS

A total of 11 fatty acids were identified in all samples of *Codium dimorphum*, *C. fernandezianum*, and *C. fragile* (Table 1). There were similar fatty acid compositions among the three species. The main fatty acids in descending order were palmitic (C16:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and hexadecatrienoic (C16:3) acids.

Total saturated fatty acids (SAFA) in the studied macroalgae accounted for 30% to 55% of all fatty acids. Total monounsaturated fatty acids (MUFA) accounted for 5% to 20%. Oleic acid was the second most abundant fatty acid in *Codium dimorphum* and also important in the other two species.

Total polyunsaturated fatty acids (PUFA) with C16, C18, and C20 accounted for 18% to 42% in the three species studied. *Codium fernandezianum* was the species with the higher proportion of PUFA content. The genus contains linolenic acid as the dominant C18 PUFA and relatively high percentages of linoleic acid. Hexadecatrienoic acid, a possible chemotaxonomic character of the species of *Codium*, was found in relatively high amounts (7.2% to 9.5%) in all species while the hexadecatetraenoic acid (C16:4) was not detected.

Codium dimorphum, *C. fernandezianum* and *C. fragile* were found to be slightly distinguishable from one another in terms of their fatty acid amount (Table 1, Fig. 1).

DISCUSSION

Representatives of the genus *Codium* collected from a variety of places around the world have been investigated due to their fatty acid contents (Hainaux *et al.* 1998, Xu *et al.* 1998, Dembitsky *et al.* 2003, Khotimchenko 2003). However, there is just one published research on the species living in Chile. And the fatty acid composition of the two endemic species, *Codium dimorphum* and *C. fernandezianum* has not been reported previously.

As reported worldwide for green macroalgae all three *Codium* species had high C16 and C18 contents (Aliya *et al.* 1992, Hainaux *et al.* 1998, Graeve *et al.* 2002, Li *et al.* 2002, Khotimchenko 2003, Ortiz *et al.* 2009) (Table 1). Several studies have shown palmitic acid (C16:0) to be the main saturated fatty acid of green macroalgae. The same pattern has been observed globally and in the present study for *Codium* spp. (Al-Hasan *et al.* 1991, Aliya *et al.* 1992, Khotimchenko 1993, Xu *et al.* 1998).

Thus, macroalgae of the genus *Codium* have palmitic, oleic, linolenic, linoleic and hexadecatrienoic acids as important components totalling more than 50% of all fatty acids. The total content of saturated fatty acids (SAFA) and polyunsaturated fatty acids (PUFA) was higher than the monounsaturated fatty acids (MUFA). *Codium fernandezianum* was the species with the higher proportion of PUFA content (Table 1). A similar proportion in the levels of saturation of the fatty acids was previously reported in other 16 different species of *Codium* from different geographical locations (see Dembitsky *et al.* 2003, Khotimchenko 2003). And it seems that the specific features of the fatty acid composition typical of the genus remain constant.

In accordance to other studies of fatty acid configuration on Chlorophyta, the genus contains linolenic acid as the dominant C18 PUFA, relatively high percentages of linoleic

acid and minute amounts of 18:4 (Khotimchenko 2003), the latter fatty acid was not detected in this study. Between the members of Bryopsidales the monounsaturated fatty acid oleic acid (C18:1) has also a chemotaxonomic importance, it is described to be present in high amounts in the genus *Codium* (Khotimchenko 2003), as could also be shown in the present study. The presence of arachidonic acid (C20:4, Table 1) shows that the three studied *Codium* species are capable of synthesizing larger PUFAs.

A strong relationship has been found between biogeographical conditions and fatty acid contents. Environmental effects have to be taken in account. Salinity, temperature, solar radiation, nutrient availability, and environmental pollution affect the physiological state of the algae and thereby affect the fatty acid composition (Teoh *et al.* 2004). Al-Hasan *et al.* (1991) reported variations in macroalgae fatty acid concentrations, but not in the composition pattern, when the temperature varied. Also an increased desaturation of fatty acids was detected in various species of brown macroalgae during the winter season (Pohl & Zurheide 1979).

The function of fatty acids in algae is related to cell membranes, energy storage and metabolic processes (Orhan *et al.* 2003). The function of polyunsaturated C20 fatty acids is not completely clear, although they are important components of the lipids of chloroplastidial thylakoids (Al-Hasan *et al.* 1991). Fatty acids may play important roles in the ecology of aquatic ecosystems: Research on the biological activity of fatty acids has shown antibacterial activity of oleic acid, lauric acid, palmitic acid, linoleic acid, and linolenic acid, among others (Kabara *et al.* 1972). Also, they can play a role in defense (Jüttner 2001), and are capable to control microalgal growth (DellaGreca *et al.* 2001). A mixture of saturated, monosaturated or polyunsaturated fatty acids in microalgae has shown to be active against green microalgae and phytoplanktonic organisms (Ikawa 2004).

In regards to chemotaxonomy, hexadecatrienoic acid (C16:3) was found in the three species studied, although hexadecatetraenoic acid (C16:4) was not. This apparently important chemotaxonomic marker of *Codium* spp. was present in a relatively high amount (7-9%) in the three studied species as demonstrated for other studies around the world (Khotimchenko 2003). This agrees Al-Hassan *et al.* (1991), Khotimchenko (1993) and Xu *et al.* (1998). However, Ortiz *et al.* (2009) could not detect these two fatty acids in *Codium fragile* from Chile in an earlier study. Extensive analyses on variability of fatty acids in *Codium* carried out in several species around the world (Dembitsky *et al.* 2003) have not been included for

Southamerican species. Because chemical composition vary with biogeographical conditions, it is interesting to study the chemical composition of both endemic and introduced species growing in this part of the world.

Recently, molecular studies have helped to clarify a number of long standing issues related to the phylogeny of Bryopsidales (Lam & Zechman 2006). Diverse information like chemical composition, morphology, life cycle and other molecular studies will allow us to understand the evolution in these diverse green algae (Khotimchenko 2003). Morphology in macroalgae can be quite plastic, and may be dependent upon environmental factors including light, wave action and position in the water column, among others. This often leads to difficulties in taxonomical identifications when basing only in morphology. Chemotaxonomic approaches can be complements for taxonomy, as it has been a successful technique applied on brown algae (Laird *et al.* 2010). Thus, chemotaxonomy of certain metabolites, including pigments, terpenes, sterols and fatty acids, should also be taken into account for identification purposes (Vallim *et al.* 2005)

The three *Codium* species analyzed in this study (*C. fernandezianum*, *C. fragile* and *C. dimorphum*) come from environments with different physico-chemical characteristics (Santelices & Meneses 2000). These algae presented a similar pattern between them and between configurations described elsewhere for this genus, though the particular amounts could differ. The observed result is therefore, interesting from both a biochemical and chemotaxonomic point of view. The present work contributes to the global information on the chemical composition of the genus.

Fatty acids appear to be a promising tool to study trophic relationships in the marine environment (Silva *et al.* 1984), as the lipids composition of macroalgae is largely made up by characteristic PUFAs (Graeve *et al.* 2002). According to Trowbridge (1998), the first step is to integrate chemical and ecological information, in order to get a better understanding of the ecological interactions among algae and consumers, competitors and symbionts. Furthermore, compounds such as vegetable lipids, fatty acids and also volatile compounds offer valuable information for designating taxonomic positions (Dembitsky *et al.* 2003).

Another important branch that is newly arisen is the use of these compounds for example in cosmetics and for synthesizing eicosanoid hormones (Khotimchenko 1998). Lipids determination in algae is critical for the possible industrial exploitation of species rich in specific,

commercially valuable steroids and/or carotenoids (Pollesello *et al.* 1992). Exhaustive studies are imperative to understand the natures of these compounds and to obtain new biologically active molecular prototypes that can be useful to humans (Silva *et al.* 1984). Algae are an important resource to consider.

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