Pergamon

PII: S0031-9422(98)00115-0

Phytochemistry, Vol. 48, No. 8, pp. 1335–1339, 1998

Printed in Great Britain
0031-9422/98/$—see front matter

FATTY ACIDS OF SIX *CODIUM* SPECIES FROM SOUTHEAST AUSTRALIA

XIN-QING XU,*† VIET HUNG TRAN,† GERRY KRAFT‡ and JOHN BEARDALL§

†Food Science Australia, Melbourne Laboratory, Private Bag 16, Werribee, Victoria, 3030, Australia; ‡School of Botany, University of Melbourne, Parkville, Victoria, 3052, Australia; §Department of Ecology and Evolutionary Biology, Monash University, Clayton, Victoria, 3016, Australia

(*Received in revised form* 22 *December* 1997)

Key Word Index—*Codium species*; Chlorophyta; algae; fatty acid composition; lipid content.

Abstract—Fatty acid profiles, total lipid contents of 12 green macroalgae collected from south east Australia were analysed. These algae belong to six *Codium* species. The major fatty acids are 16:0, 18:1ω9, 18:3ω3, 16:3ω3, 18:2ω6 and 20:4ω6. Total polyunsaturated fatty acid contents ranged from 17.2 to 54.4%. Total lipid contents were from 7.3 to 21.1%. There were large variations in individual fatty acid contents according to species, season and location.

INTRODUCTION

Codium species belong to the family Codiaceae in the order Codiales, which is monogeneric and contains *ca* 100 species worldwide. Although our knowledge of the fatty acid composition of green macroalgae is still poor, there are about ten publications on the fatty acid composition of nine *Codium* species; these are somewhat contradictory. Some authors [1–5] reported that 16:3 is a characteristic fatty acid for *Codium* species. Others [6–9], however, could not detect the presence of this fatty acid, even for the species from the same area of the Sea of Japan. Some reports [6–8] could not detect polyunsaturated fatty acids with two or more double bonds in the samples. We present here the fatty acid composition of 12 samples of six *Codium* species collected from the States of Victoria, Tasmania and South Australia in Australia and provide more useful information about *Codium* fatty acids. Of the six *Codium* species investigated in this paper, fatty acid compositions of four of the species have not been reported previously.

RESULTS AND DISCUSSION

The fatty acid composition of total lipids determined by capillary gas chromatography, as well as total lipid content of the 12 samples belonging to six *Codium* species are shown in Table 1. There are 37 fatty acid compounds identified in the samples examined. The major fatty acids are 16:0, 18:1ω9, 18:3ω3, 16:3ω3, 18:2ω6 and 20:4ω6. Palmitic acid (16:0) was the most abundant fatty acid in all species, ranging from 25% to 45%, with the average of 33.2% in the 12 samples. Oleic acid (18:1ω9) and linolenic acid (18:3ω3) were the next most abundant fatty acids on average but showed large variations in their contents. There was a three-fold difference in the content of 18:1ω9 from 7 to 23% with an average of 14.1% and a 14-fold difference in the content of 18:3ω3 from 1.5 to 22%, with an average of 10.4%. Hexadecatrienoic acid (16:3ω3) was detected in all 12 samples, ranging from 0.7 to 13.9%, with an average of 6.7%. Linoleic acid (18:2ω6) and arachidonic acid (20:4ω6) were also abundant in these samples ranging from 2.9% to 15.6% (5.9% on average) and from 1.5% to 10.9% (4.2% on average), respectively.

Total lipid contents in the *Codium* samples ranged from 7.3% to 21.1%, with an average of 14.2%, which was higher than in most of the green seaweeds reported previously and investigated by us so far (unpublished data). Four *C. duthieae* samples collected in the same season but at different locations had a large variation from 12.2 to 20.7%, which may be due to the difference in the local growth environment.

There were eight saturated fatty acids present in the *Codium* species examined. Pentadecanoic acid (15:0), heptadecanoic acid (17:0), eicosanoic acid (20:0) and tetracosanoic acid (24:0) were detected in most of the samples but in small quantity (< 0.9%). Myristic acid (14:0) was relatively higher in *C. fragile* and *C. pomoides* with contents of 5.3% and 7.8%, respectively. In the other samples, it was less than 3.5%. It is interesting to note that the average percentage of 22:0

*Author to whom correspondence should be addressed.

Table 1. Fatty acid composition and total lipid content (in dry weight, mean of tow determinations) of twelve samples belong to six *Codium* sepcies. The date and location are also listed in the table (V: Victoria, S: South Australia, T: Tasmania).

Fatty Acids	*Codium duthiae* 7/95 V	*duthiae* 7/95 V	*duthiae* 7/95 V	*duthiae* 8/95 S	*Codium fragile* 7/94 V	*Codium galeatum* 7/94 V	*galeatum* 2/95 V	*Codium harveyi* 7/95 V	*Codium harveyi* 8/95 S	*muelleri* 8/95 S	*Codium muelleri* 10/95 T	*pomoides* 7/95 V
14:0	2.8	2.1	2.7	1.4	5.3	3.1	3.1	3.5	2.8	2.9	1.0	7.8
15:0	0.1	0.1	0.1	0.1	0.2	0.3		0.1	0.1	0.1	0.1	
16:0	28.6	32.4	26.6	36.9	40.5	31.4	41.2	26.8	44.8	38.8	25.6	25.0
16:1*trans*	0.2	0.3	0.3	0.2	0.8	0.3	0.1	0.3	0.1	0.3	0.1	0.2
16:1ω7	1.5	1.3	1.9	2.5	2.3	2.8	2.3	2.6	2.3	4.0	0.5	0.5
16:1ω5	0.6		0.1			0.9	0.2	0.1			0.5	0.4
16:2ω9	0.1	0.5	1.8	0.5	1.2	0.6	0.3	1.7	0.2	0.6	1.4	3.5
16:2ω6									0.1	0.1	0.1	
17:0	0.1	0.1	0.1	0.1	0.3	0.3	0.4					0.2
16:3ω3	12.3	9.5	10.2	5.3	3.0	2.1	0.7	11.5	2.2	5.7	13.9	3.8
16:4ω3						1.3	0.3					
18:0	0.7	0.7	1.1	1.2	0.7	3.8	2.5	0.9	1.7	1.1	0.4	1.0
18:1ω9	6.9	9.6	13.6	9.5	23.3	11.4	12.7	13.1	15.8	19.6	18.3	15.4
18:1ω7	1.0	1.0	1.7	1.2	2.1	3.4	2.8	2.7	2.8	2.4		1.4
18:1ω5			0.1		0.2			0.1				
18:2ω6	5.8	5.2	7.4	5.9	4.0	4.8	3.8	7.0	2.9	3.6	5.1	15.6
18:3ω6	3.9	5.0	5.8	3.1	0.9	2.4	2.7	4.9	2.4	2.1	1.9	3.5
18:3ω3	22.1	16.9	12.7	12.2	5.6	4.7	1.5	12.3	4.3	8.0	20.8	3.6
18:4ω6				4.4						1.8		
18:4ω3	3.0	3.3	1.4	3.4	0.3	0.7	1.9	1.3	3.3	1.2	1.3	0.3

20:0	0.1	0.1	0.2	0.2	0.2	0.9	0.3	0.3	0.3	0.2	0.1	0.4
20:1ω9	0.1		0.2		0.9	0.7	0.1				0.3	0.5
20:1ω7			0.1	0.2	0.7	1.4	0.3	0.1	0.2	0.2	0.1	0.3
20:3ω6	0.4	0.3	0.5	0.3	0.2	0.3	0.3	0.4	0.3	0.1	0.2	0.2
20:3ω3	0.1											
20:4ω6	4.2	4.8	5.0	4.2	1.5	2.5	10.9	4.0	2.9	1.8	3.6	4.5
20:4ω3	0.3	0.2	0.1	0.2	0.1	0.2		0.1			0.1	0.2
20:5ω3	2.4	2.4	1.2	1.9	0.5	1.2	2.0	1.1	0.6	0.6	1.5	4.5
22:0	1.7	1.7	0.9	2.3	1.1	3.3	2.4	0.8	2.2	1.8	0.9	
22:1ω9						1.0						
22:3ω6						0.4	0.4					
22:3ω3			0.1									
22:4ω6	0.1	0.4	0.3	0.1		0.9		0.1	0.2	0.1	0.0	0.1
22:5ω3		0.8	0.5			3.8	3.2					
22:6ω3			0.1			0.2	0.5					
24:0	0.2	0.2	0.4	0.2	0.5		0.8	0.3	0.8	0.2	0.3	0.3
24:1ω9							0.4					
unknown	1.2	1.3	2.8	2.6	3.8	9.0	2.0	3.8	6.5	3.1	1.9	6.8
SAFA	34.2	37.4	32.1	42.4	48.7	43.0	50.7	32.7	52.8	45.1	28.5	34.7
MUFA	10.2	12.2	17.9	13.5	30.3	22.0	18.9	19.0	21.2	26.4	19.7	18.7
PUFA	54.4	49.2	47.2	41.4	17.2	26.1	28.5	44.4	19.4	25.5	50.0	39.8
ω3PUFA	40.0	33.1	26.4	23.0	9.4	14.2	10.1	26.2	10.5	15.4	37.7	12.4
Total lipids	20.7	15.4	12.2	21.0	21.1	7.8	9.6	12.1	8.8	7.3	20.8	13.7

(1.6% on average) is slightly higher than those of 18:0 (1.3% on average) and 20:0 (0.3% on average). Total saturated fatty acids (SAFA) accounted for 28.5% to 52.8% of the total fatty acids, values similar to those published results using fresh samples to extract lipids for fatty acid analysis [1–4].

Among the monounsaturated fatty acids (MUFA), 16:1*trans*, 16:1ω5, 18:1ω5, 20:1ω9, 20:1ω7 and 22:1ω9 were present in small amounts in the algae. Oleic acid was the second most abundant fatty acid in these *Codium* species. Total MUFAs accounted for 10.2% to 30.3% (with an average of 19.2%) among these samples.

Total polyunsaturated fatty acids (PUFA) with C_{16}, C_{18}, C_{20}, and C_{22} accounted for 17.2 to 54.4% (average 36.9%) and shared a three-fold difference in quantity. Total ω3 PUFAs accounted for 9.4 to 40% (average 21.5%) and, thus, had a four-fold difference in relative content. There were also large variations in PUFAs within one species. Four *C. duthieae* samples collected in the same season but at different locations had large differences (*ca* 10% and 7% variations, respectively) in 18:3ω3 and 16:3ω3 content. Two *C. galeatum* samples collected at different seasons and locations in Victoria had a major difference in 20:4ω6 content with one 8.4% higher than the other. Two pairs of *C. harveyi* and *C. muelleri* samples collected in the same season, but, at different locations had differences in their 18:3ω3 and 16:3ω3 contents ranging from 8 to 10% and 8 to 13%, respectively. These suggest that fatty acid composition is not a good indicator for chemotaxonomy at the species level when algae are grown under different conditions, at least for *Codium* species.

Hexadecatrienoic acid was the fourth most abundant fatty acid in the 12 samples with an average content of 6.7% of all fatty acids. Khotimchenko [1], Aknin *et al.* [2], Al-Hasan *et al* [4], and Sato [5] all detected a high content of 16:3ω3 in three *Codium* species, including *C. fragile*, and Sato [5] suggested that 16:3ω3 was a characteristic fatty acid of the Codiaceae. The content of 16:3ω3 was 3% or lower in four of the *Codium* samples examined, but only 0.7% in one of the *C. galeatum*. There were also large variations among different samples from a species. *Codium duthiae* had a 16:3ω3 content varying from 5.3 to 12.3%, while two *C. harveyi* had 16:3ω3 contents of 11.5% and 2.2%, respectively. Therefore, our data does not fully support the concept that 16:3ω3 is a characteristic fatty acid of the Codiaceae. More analysis needs to be done in the future.

Aknin *et al* [2], reported the presence of 16:4ω3 in *C. dichotomum* and *C. elongatum*. We only detected 16:4ω3 in one *Codium* species, namely *C. galeatum*. This fatty acid was detected only in this alga probably due to some tiny red algae grown on *C. galeatum*. These above *Codium* species are the only three species in which 16:4ω3 has been reported to date. Linolenic acid showed large variations among species, with the lowest of 1.5% in *C. galeatum* and the highest of 22.1% in *C. duthieae*. Even the same species of *C. duthieae* collected at the same season but at different locations still showed variations in 18:3ω3 content from 12.2 to 22.1%. Arachidonic acid (20:4ω6) ranged from 1.5 to 10.9% and showed a seven-fold difference in percentage among these species and a four-fold difference in the same species of *C. galeatum*. Eicosapentaenoic acid (20:5ω3) was detected in all the samples from 0.5–4.5% with an average of 1.7%. These two fatty acids are of particular interest due to their biological function and possible benefit to human health. As in most green seaweeds, their contents were lower compared with those in brown and red algae (unpublished data).

The fatty acid composition of total cellular lipids provides potentially interesting information for marine algal taxonomy. Caution should, however, be taken when interpreting the available information about fatty acid composition from algae. From the results presented here, it is apparent that fatty acid composition not only varies considerably among different species within the same genus, but also varies in the same species collected in the same season but at different locations. There are many factors affecting algal fatty acid composition, such as growth stage, irradiance, temperature, nutrients, salinity and depth under water. In some previous investigations on fatty acid composition of *Codium* species (as well as other algal species) [6–8], fatty acids with two or more double bonds were not detected, an effect ascribed to the use of air-, solar- or oven-dried samples for fatty acid analyses. Dried algal samples always give less PUFAs since these compounds are very susceptible to oxidation. The degree of PUFA loss depends on the species, texture, time exposed to sun, high temperature or air, and the storage time and conditions (unpublished data). Results obtained by extracting fatty acids from freeze-dried samples, kept in a dark, dry and cold place for months and from those fresh samples stored in a freezer with temperature less than $-70°$ for months are still acceptable, but not as good as that from freshly extracted algae (unpublished data). The true fatty acid profile can only be obtained from freshly collected and then immediately extracted and analysed algal samples.

EXPERIMENTAL

Sample origin

Algae were collected at different locations and times from the Australian south east coast. All samples were identified by Dr. G Kraft. The six *Codium* species are *C. duthiae* Silver, *C. fragile* (Suringar) Hariot, *C. galeatum* J. Agardh, *C. muelleri* Kuetzing, *C. harveyi* Silver and *C. pomoides* J. Agardh. The collecting locations and times are listed in Table 1.

Extraction of total lipids

Total lipids were extracted in a Soxhlet for 3 hr using 2×2.5 g of freeze-dried sample with 100 ml of

MeOH-$CHCl_3$ (1:1). After evapn of the solvents, the lipids were dried at 100° for 30 min, then desiccated before weighing.

Extraction of lipids for fatty acid analysis

Lipids for fatty acid analysis were extracted using a modified method [10]. The fr. algal sample (10 g) was ground and then extracted with a mixt. of 20 ml MeOH and 10 ml $CHCl_3$ in a capped flask and shaken overnight at 2°. Before centrifugation, 10 ml $CHCl_3$ and 10 ml H_2O were added to the extract soln and shaken for 1 hr. The lower layer of $CHCl_3$ was transferred and evapd. under red. pres.

Fatty acid methylation

Fatty acids were converted to Me esters (FAME) prior to analysis by capillary GC. Total lipids were treated with 4 ml of 1 M MeOH-OH for 1 hr at room temp. FAME were extracted with hexane and injected into the GC immediately.

Fatty acid analysis by GC

Analysis of FAME was carried out using a fused silica capillary column (J&W Scientific, DB-23, 0.25 μm film thickness, 30 m × 0.25 mm I.D.) and a FID. N_2 was the carrier gas at a rate of 30 ± 1 ml min^{-1}. Initial column temp. was set at 145° and held for 2 min, then programmed to 240° at the rate of 2.5° min^{-1} and held for 10 min at 240°. Injector and FID detector temps were 250° and 280°, respectively. Sample of FAME (1 μl) was injected through a JADE valve. The results were computed and FAMEs identified by chromatography by comparison with authentic standards (Sigma).

REFERENCES

1. Khotimchenko, S. V., *Phytochemistry*, 1993, **32**, 1203.
2. Aknin, M., Moellet-Nzaou, R., Cisse, E., Knornprobst, J. M., Gaydou, E. M., Samb, A. and Miralles, J., *Phytochemistry*, 1992, **31**, 2739.
3. Banaimoon, S. A., *Botanica Marina*, 1992, **35**, 165.
4. Al-Hasan, R., Hantash, F. M. and Radwan, S. S., *Applied Microbiology and Biotechnology*, 1991, **35**, 530.
5. Sato, S., *Nippon Suisan Gakkaishi*, 1975, **41**, 1117.
6. Qasim, R., *Journal of Chemistry Society Pakistan*, 1986, **8**, 223.
7. Shameel, M. and Khan, R., *Botanica Marina*, 1991, **34**, 501.
8. Shameel, M., *Botanica Marina*, 1990, **33**, 429.
9. Pollesello, P., Toffanin, R., Murano, E., Paoletti, S., Rizzo, R. and Kvam, B. J., *Journal of Applied Phycology*, 1992, **4**, 315.
10. Bligh, E. G. and Dyer, W. J., *Canadian Journal of Biochemistry Physiology*, 1959, **37**, 911.