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Screening of Red Algae Filaments As a Potential Alternative Source of Eicosapentaenoic Acid

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Abstract: Lipids were extracted by a supercritical fluid extraction method from 10 species of filamentous red algae obtained from culture collections and their fatty acid compositions were determined. The fatty acid profiles of the 10 species were similar. The major fatty acids were 16:0, 20:4ω6 and 20:5ω3 (eicosapentaenoic acid, EPA), which amounted to over 70% of the total fatty acids. The highest EPA content (29.8 mg/L), as a percentage of total fatty acids, was produced by *Liagora boergesenii* filaments, which has good potential for EPA mass production in pilot plants.

Key words: red algae, supercritical fluid extraction, eicosapentaenoic acid.

INTRODUCTION

The mass production of omega-3 (ω3) polyunsaturated fatty acids (PUFAs) is being studied because of their role in human health (Grima et al., 1995; Reis et al., 1996). The therapeutic value of PUFAs, especially eicosapentaenoic acid (EPA), has been shown in the reduction of blood cholesterol (Bonaa et al., 1990), in the prevention of blood-platelet aggregation, and as a protection against cardiovascular and coronary heart diseases (Simopoulos, 1986). Fish oil is generally the major source of PUFAs, but the product has a fishlike smell (Ackman et al., 1988). Several publications have pointed out the feasibility of PUFA production from microbial sources, with special emphasis on fungi (Kennedy et al., 1993) and microalgae (Bajpai and Bajpai, 1993). Although the microalgae have several advantages over fish oil (Reis et al., 1994), their production cost is high. Red algae contain high levels of PUFAs; the PUFA concentration is similar to that of fish oil and microalgae (Araki et al., 1986; Lu, 1992). Filaments of red algae in the conchocelis phase (2n) have the benefits of a faster growth rate, easy culture, and lower production cost (Hung, 1994). Furthermore, the PUFA content of the conchocelis is higher than that of the thalli (Lu, 1992), and our laboratory has set up an analytical-scale system to culture the filaments of this red alga.

In early extraction methods, removal of the organic solvent is difficult. More recently, supercritical fluid extraction (SFE) has been investigated as a good technique for the determination of fat in vegetable oil and meats (Walker et al., 1994; Taylor et al., 1997). That SFE has the advantages of savings in labor, operational costs, and laboratory space, waste minimization, nontoxicity, and increased selectivity over traditional extraction methods is generally true (Samyudia et al., 1996; Lehotay, 1997). However, there are no reports regarding the use of the SFE technique in algae. In

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this study, we determined the fatty acid composition of red algal filaments and evaluated their potential as EPA producers.

Materials and Methods

The algal species analyzed were *Bangia atropurpurea, Porphyra angusta, Porphyra dentata* (Bangiaceae); *Helminthocladia australis, Liagora orientalis, Liagora boergesenii* (Helminthocladiaceae); *Scinaia monoliformis, Galaxaura cylindrica* (Chaetangiaceae); *Grateloupia filicina, Halymenia ceylanica* (Grateloupiaceae). The filamentous plants (conchocelis) were originally germinated from a single carpospore of red algae using a method similar to that for *Porphyra* species (Chiang and Wang, 1980). Filaments of red algae were propagated by fragmentation and grown into colonies of filamentous clusters in SWM-III medium (Chen et al., 1969). The conchocelis was maintained at 20°C under fluorescent light (2000 lux) with a light-dark regimen (12:12 h). Mass cultures of the filaments were maintained in 20 L of polycarbonate (Nalgene Labware, Rochester, N.Y.) with continuous aeration by aquarium air pumps.

After 2 weeks of incubation, algae were harvested with a plankton net. Excess water was removed by filtration on a Buchner funnel using suction. Wet filamentous plants were lyophilized and then ground in a mortar. Filamentous fragments were extracted by high-pressure soxhlet extractor (J & W Scientific Co.). The SFE process was completed under 500 psi, 55°C, for 3 hours; the condenser temperature was 15°C; and the supercritical solvent was CO_2. The extract was dissolved in a small volume of chloroform and then methylated by diazald (Chen, 1993). Fatty acids were determinated as methyl esters using gas chromatography–mass spectroscopy. Pentadecanoic acid (Supelco) was used as a standard. Fatty acid methyl esters (FAMEs) were identified by comparison of retention times with known standards (FAME-1, Supelco). The extract was analyzed using HP 5890 series II GC and HP 5971 MSD. The GC-MS analysis conditions are summarized in Table 1.

Table 1. Gas Chromatography–Mass Spectroscopy Analysis Conditions

Injection mode: Split (1:27)
Injection amount: 0.5 μl
Injection temperature: 250°C
Column: Supelco capillary column (#2330) (30 m × 0.25 id)
Column temperature: 50°C (1 min wait)→20°C/min→140°C (7 min wait)→3°C/min→180°C→5°C/min→220°C (4 min wait)→end
Carrier gas: He
Flow rate: 25 cm/s
Detector temperature: 280°C

Results

All of the 10 red algae species grew rapidly under laboratory conditions and reached maximum growth after 10 to 14 days, the biomass increased about 10 to 12 fold (10 ± 2 g wet wt/L and 2.12 ± 0.34 g dry wt/L). *Liagora boergesenii* only required a 10-day cultivation period and yielded the highest cell mass (12.0 g wet wt/L). The crude lipid contents of the species are presented in Table 2. *Liagora boergesenii* showed a significantly higher value (21.5% ± 0.5%, 2.58 g/L) (analysis of variance [ANOVA], $P < 0.05$) than the others. The value for *Galaxaura cylindrica* (19.8% ± 0.4%) was a little lower than that for *Liagora boergesenii,* but was higher than those for other red algae filaments. The extraction rate of crude lipid by the SFE method (11.2%–21.5%) was significantly higher than that obtained using organic solvents (i.e., MeOH-$CHCl_3$ [2:], 7.5–15.3%; ANOVA, $P < 0.05$) (Lu, 1992).

Table 2. Crude Lipid Contents from Selected Red Algae Filaments (mean ± SE, $n = 2$)

Sample name	Extractable crude lipid in dry mass base (% wt/wt)
Liagora boergesenii	21.5 ± 0.5
Grateloupia filicina	13.6 ± 0.2
Scinaia monoliformis	17.0 ± 0.4
Helminthocladia australis	19.7 ± 0.3
Galaxaura cylindrica	19.8 ± 0.4
Halymenia ceylanica	18.8 ± 0.3
Liagora orientalis	17.6 ± 0.4
Porphyra angusta	12.4 ± 0.1
Porphyra dentata	11.2 ± 0.1
Bangia atropurpurea	13.3 ± 0.1

The fatty acid compositions of 10 red algae filaments are shown in Table 3. The major fatty acids were 16:0, 20:4ω6, and 20:5ω3 (EPA), which amounted to over 70% of the total fatty acids. Furthermore, *Bangia atropurpurea* showed the highest content of PUFA (62.6%), and the Σω3/Σω6 value of *Liagora boergesenii* (2.40) was significantly

Table 3. Relative Percentages (% wt/wt) of the Fatty Acids from Ten Species of Filamentous Red Algae

	Bangiaceae			Helminthocladiaceae			Chaetangiaceae		Grateloupiaceae	
Fatty acids (%)	*Bangia atropurpurea*	*Porphyra angusta*	*Porphyra dentata*	*Helminthocladia australis*	*Liagora orientalis*	*Liagora boergesenii*	*Scinaia monoliformis*	*Galaxaura cylindrica*	*Grateloupia filicina*	*Halymenia ceylanica*
14:0	1.50 ± 0.01	2.25 ± 0.18	1.49 ± 0.34	4.66 ± 0.34	2.92 ± 0.02	3.53 ± 0.28	4.00 ± 0.71	1.61 ± 0.21	2.13 ± 0.24	2.45 ± 0.01
16:0	20.03 ± 1.15	29.47 ± 0.68	22.20 ± 1.96	36.76 ± 0.89	37.01 ± 2.63	32.21 ± 1.47	25.05 ± 1.80	41.34 ± 1.87	31.62 ± 2.09	34.09 ± 0.21
16:1ω9	0.90 ± 0.02	0.93 ± 0.01	trace	0.87 ± 0.00	1.16 ± 0.15	0.65 ± 0.01	1.30 ± 0.05	trace	0.88 ± 0.06	trace
16:1ω7	0.90 ± 0.03	0.93 ± 0.07	trace	trace	3.13 ± 0.29	0.99 ± 0.04	trace	3.56 ± 0.09	0.86 ± 0.00	1.14 ± 0.02
18:0	1.07 ± 0.05	1.20 ± 0.01	1.60 ± 0.02	1.14 ± 0 ± 0.03	1.23 ± 0.01	0.77 ± 0.03	2.78 ± 0.09	0.87 ± 0.06	0.62 ± 0.01	1.02 ± 0.00
18:1ω9	9.08 ± 0.01	7.52 ± 0.02	6.82 ± 0.10	11.28 ± 0.52	11.17 ± 0.22	7.20 ± 0.11	8.02 ± 0.15	4.06 ± 0.24	7.83 ± 0.11	16.27 ± 0.23
18:1ω7	2.29 ± 0.00	2.32 ± 0.04	1.76 ± 0.01	0.61 ± 0.02	2.25 ± 0.07	1.64 ± 0.05	trace	3.14 ± 0.19	4.80 ± 0.12	4.88 ± 0.07
18:2ω6	2.17 ± 0.01	3.34 ± 0.04	4.20 ± 0.06	1.32 ± 0.05	0.81 ± 0.00	1.07 ± 0.03	1.13 ± 0.12	0.79 ± 0.07	1.93 ± 0.09	0.81 ± 0.01
20:1ω9	1.57 ± 0.15	1.14 ± 0.02	2.76 ± 0.12	0	2.41 ± 0.10	0	0	2.34 ± 0.17	0	0
18:4ω3	0	0	0	0	0.64 ± 0.01	0	0	0	0	0
20:2ω6	0.85 ± 0.04	0.75 ± 0.02	2.58 ± 0.08	0	0	0	0	0	0	0
20:3ω6	0.73 ± 0.02	1.20 ± 0.00	1.08 ± 0.08	1.03 ± 0.04	0	0.78 ± 0.04	4.34 ± 0.25	1.80 ± 0.14	2.19 ± 0.19	0
20:4ω6	39.60 ± 0.49	43.10 ± 0.65	27.97 ± 0.99	20.00 ± 0.20	18.49 ± 1.31	13.60 ± 0.37	44.20 ± 1.40	15.18 ± 0.64	28.32 ± 1.62	32.56 ± 0.08
22:1ω9	trace	0.76 ± 0.01	1.00 ± 0.13	0	0	0.60 ± 0.34	0	0	0	0
20:5ω3	19.25 ± 0.51	5.19 ± 0.02	26.52 ± 1.07	22.42 ± 0.71	18.65 ± 1.68	37.06 ± 0.75	9.37 ± 0.36	25.05 ± 0.71	18.74 ± 0.66	6.79 ± 0.04
∑ PUFA	62.60	53.58	62.35	44.77	38.59	52.51	59.04	42.82	51.18	40.16
∑ MUFA	14.74	13.60	12.34	12.76	20.12	11.08	9.32	13.10	14.37	22.29
∑ SFA	22.60	32.92	25.29	42.56	41.16	36.51	31.83	43.82	34.37	37.56
∑ ω3/∑ ω6	0.45	0.11	0.80	1.00	1.00	2.40	0.19	1.41	0.58	0.20

*Values of less than 0.05% are presented as "trace" (mean ± SE, $n = 2$). PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid

higher than that of other algal samples (ANOVA, $P < 0.05$). *Liagora boergesenii* filaments also contained the highest percentage of EPA (37.06%) relative to the other red algae samples (ANOVA, $P < 0.05$). However, 22:6ω3 (docosahexaenoic acid, DHA) was not found in all red algae samples. Because DHA is considered to have some different nutritional and pharmacologic effects from EPA, the use of EPA-producing cultures that essentially lack other long-chain PUFAs has the potential to reduce the substantial problems of EPA recovery in downstream processing. *Bangia atropurpurea* showed the highest total fatty acid content among the 10 samples. *Liagora boergesenii* had the highest concentration of EPA (6.78 mg/100 mg crude lipid); that is, the EPA content was 29.8 mg/L. This EPA content is higher than that of fish oil and microalgae (Ackman et al., 1988; Grima et al., 1995).

In conclusion, *Liagora boergesenii* could be used for EPA mass production in a pilot plant, since it shows rapid growth rate and high lipid and EPA content. Furthermore, filamentous red algae will increase EPA content under higher light intensity (Lu, 1992). We are now optimizing the culture conditions for increased EPA production and also the SFE control strategies for highly efficient extraction.

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