



FATTY ACIDS OF BROWN ALGAE FROM THE RUSSIAN FAR EAST

SVETLANA V. KHOTIMCHENKO

Institute of Marine Biology, Far East Branch, Russian Academy of Sciences, Vladivostok 690041, Russia

(Received in revised form 9 March 1998)

Key Word Index—Phaeophyta; marine brown algae; fatty acids; chemotaxonomy.

Abstract—Twenty species of brown algae belonging to the Chordariales, Ralfsiales, Dictyosiphonales, Scytosiphonales, Desmarestiales, Laminariales and Fucales orders were investigated for their fatty acid composition by capillary gas chromatography. Polyunsaturated fatty acids (PUFAs) with 18 and 20 carbon atoms, palmitic and oleic acids were major components. No correlation between the taxonomic position of the brown algal species and the distribution of these acids was found. Peculiarities of fatty acid composition were shown for members of the Fucaceae only, which contained the highest percentages of 14:0 and 18:1(n-9), the lowest amounts of (n-3) PUFAs and had a C₂₀ non-methylene interrupted acid. *Agarum cribrosum* had fatty acid patterns unique for the Phaeophyta. The occurrence of the rare C₁₆ PUFAs, 18:2(n-4) and a high content of 22:5(n-3) were a characteristic feature of this alga. Fatty acid composition of brown algae depends on different factors and the extent of their influence is species-specific. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Fatty acids (FAs) found in marine algae have aroused considerable interest among researchers. This is because marine plants are recognized producers of C₁₈ and C₂₀ polyunsaturated fatty acids (PUFAs) [1,2]. These acids are essential for nutrition of many animals and humans [3–5], are used for the biosynthesis of eicosanoid hormones [6,7] and are of interest in biotechnology and, more recently, in cosmetics [8,9]. Although general trends of fatty acid distribution in various algal phyla have been elucidated [2,10,11], further experimental work has lost none of its importance. As has been shown earlier, certain red and green macrophytic algae, belonging to different taxonomic classes, orders or families and genera have distinguishing features of taxonomic value in their FA composition [12–15]. As to brown algae, similar information was obtained only for representatives of the genus *Dictyota* and the Dictyotaceae [16,17]. Studies of Phaeophyta fatty acids have not so far been systematic, although brown algae are the dominant flora of many temperate littoral zones. Relative to the large number of species within the Phaeophyta, which includes ca 265 orders and more than 1500 genera [18], limited numbers of algal

species have been examined for fatty acid composition, mainly those belonging to the Laminariales and Fucales [11,19–26]. Therefore, in the present paper, we report the fatty acid composition of 20 species of Phaeophyta belonging to seven orders to examine possible chemotaxonomic trends in the distribution of FAs among brown algae and potential suitability of algae as sources of PUFAs, possessing important physiological effects. A knowledge of the detailed fatty acid composition of the brown algae is necessary for further studies of their fatty acid metabolism or when assessing environmental effects. The majority of these algae were investigated for the first time. The significance of this study is supported by the fact that some brown algae are used as human food [27].

RESULTS AND DISCUSSION

Brown algal species belonging to the seven orders, Chordariales, Ralfsiales, Dictyosiphonales, Scytosiphonales, Desmarestiales, Laminariales and Fucales (Table 1) from the Russian Far East coast were examined for total fatty acid composition. Fatty acid analysis was performed by capillary GC and the data, shown in Tables 2 and 3, indicate the presence of ca 40 components. Particular attention

Table 1. List of algae investigated

No	Order	Family	Species	Total lipids*
1.	Chordariales	Corynophlaeaceae	<i>Leathesia difformis</i>	1.15 ± 0.25
2.		Chordariaceae	<i>Sphaerotreichia divaricata</i>	4.5 ± 0.6
3.	Ralfsiales	Ralfsiaceae	<i>Analipus japonicus</i>	14.5 ± 3.6
4.	Dictyosiphonales	Punctariaceae	<i>Punctaria plantaginea</i>	7.45 ± 3.7
5.		Dictyosiphonaceae	<i>Dictyosiphon foeniculaceus</i>	8.0 ± 1.3
6.	Scytosiphonales	Scytosiphonaceae	<i>Petalonia fascia</i>	10.4 ± 1.5
7.	Desmarestiales	Desmarestiaceae	<i>Desmarestia ligulata</i>	7.0 ± 0.7
8.	Laminariales	Chordaceae	<i>Chorda filum</i>	4.8 ± 1.0
9.		Laminariaceae	<i>Laminaria bongardiana</i>	6.8 ± 0.9
10.			<i>L. japonica</i>	5.75 ± 1.3
11.			<i>L. cichorioides</i>	6.3 ± 1.7
12.			<i>Costaria costata</i>	10.9 ± 2.6
13.			<i>Agarum cribrosum</i>	6.9 ± 1.8
14.		Alariaceae	<i>Arthrothamnus kurilensis</i>	5.2 ± 0.7
15.			<i>Alaria angustata</i>	6.6 ± 1.5
16.			<i>Undaria pinnatifida</i>	3.75 ± 0.25
17.	Fucales	Cystoseiraceae	<i>Cystoseira crassipes</i>	8.6 ± 2.0
18.		Sargassaceae	<i>Cocophora langsdorffi</i>	5.7 ± 2.8
19.		Fucaceae	<i>Fucus evanescens</i>	7.7 ± 1.2
20.			<i>Pelvetia wrightii</i>	8.0 ± 2.7

* mg per g⁻¹ fr. wt.Data are expressed as means ± s.d., where *n* = 3.

has been paid to the presence of positional isomers, unusual and rare fatty acids, and the ratio of major FAs.

Despite the marked morphological differences that exist between the brown algae examined they had similar fatty acid patterns. The major FAs encountered in the Phaeophyta were 14:0, 16:0,

18:1(*n*-9) and C₁₈ and C₂₀ PUFA: 18:2(*n*-6), 18:3(*n*-3), 18:4(*n*-3), 20:4(*n*-6) and 20:5(*n*-3), the same as has been reported for brown algae from other regions of the World Ocean [10, 16, 19–26, 28, 29]. Relationships between these acids show strong variations among different species. Polyenoic C₁₈ and C₂₀ fatty acids predomi-

Table 2. Fatty acid composition (% of total) of brown algae from the Chordariales, Ralfsiales, Dictyosiphonales, Scytosiphonales, Desmarestiales and Fucales

Fatty acid	Sample number*										
	1	2	3	4	5	6	7	17	18	19	20
14:0	5.4	5.0	6.9	4.1	5.2	5.2	7.2	3.4	4.0	11.0	9.6
14:1- <i>trans</i>	0.7	0.4	0.4	0.6	1.4	0.7	0.3	0.9	0.7	—	—
16:0	26.1	19.1	21.9	21.1	19.3	23.5	15.9	20.2	21.0	15.8	16.9
16:1(<i>n</i> -7)	2.8	0.6	2.0	0.6	1.5	2.6	0.9	1.3	1.4	1.2	1.6
16:1(<i>n</i> -5)	—	—	—	—	—	—	3.0	—	—	0.1	—
16:1- <i>trans</i>	tr	0.7	0.2	0.5	1.3	0.2	0.6	1.2	0.7	0.2	0.1
18:0	3.2	2.3	1.2	1.5	1.3	1.4	1.1	0.7	1.2	0.6	0.7
18:1(<i>n</i> -9)	17.5	10.5	16.1	16.2	9.6	17.7	7.3	9.5	7.7	38.1	38.0
18:2(<i>n</i> -6)	4.5	10.7	11.8	10.1	6.6	5.5	9.9	7.1	11.0	13.2	11.9
18:3(<i>n</i> -6)	0.5	2.3	0.6	0.9	0.9	1.0	0.6	0.4	0.9	0.6	0.9
18:3(<i>n</i> -3)	5.1	8.3	6.3	7.3	8.8	5.3	8.9	10.9	15.3	2.9	2.3
18:4(<i>n</i> -3)	5.6	12.4	6.4	8.0	15.6	8.6	13.7	12.2	10.9	1.2	1.4
20:0	1.0	0.7	0.8	0.8	0.6	1.3	0.3	0.2	0.3	0.2	0.3
20:3 NMI**	—	—	—	—	—	—	—	—	—	0.2	0.3
20:3(<i>n</i> -6)	0.4	0.8	0.6	0.4	0.8	0.5	0.6	0.8	0.6	0.7	0.9
20:4(<i>n</i> -6)	12.0	10.1	14.7	15.8	6.6	8.0	11.6	12.3	10.9	8.7	9.9
20:4(<i>n</i> -3)	0.5	0.6	0.4	0.2	1.0	0.5	0.5	0.6	1.3	0.2	0.2
20:5(<i>n</i> -3)	11.5	12.5	8.0	8.7	18.5	15.4	11.6	10.6	4.7	2.1	1.9
Other***	3.2	3.0	1.7	3.2	1.0	2.6	6.0	7.7	7.4	3.0	3.1
(<i>n</i> -3)PUFA	22.8	33.8	21.1	24.2	43.9	29.8	34.7	34.4	32.2	6.4	5.8
(<i>n</i> -6)PUFA	17.5	23.8	26.7	27.5	14.9	15.1	23.9	20.8	23.5	23.5	24.0
C ₁₈ PUFA	15.7	33.6	25.3	26.4	31.9	20.4	33.1	30.6	38.1	17.9	16.5
C ₂₀ PUFA	24.5	24.0	23.9	25.3	26.9	24.5	25.3	24.6	17.6	12.0	13.1

* Number designates algal species in Table 1: 1. *L. difformis*, 2. *S. divaricata*, 3. *A. japonicus*, 4. *P. plantaginea*, 5. *D. foeniculaceus*, 6. *P. fascia*, 7. *D. ligulata*, 17. *C. crassipes*, 18. *C. langsdorffi*, 19. *F. evanescens*, 20. *P. wrightii*.

** NMI, non-methylene interrupted.

*** Other: 12:0, 14:1, br-15:0, 15:0, br-16:0, 16:1(*n*-9), 16:2(*n*-6), 17:0, 17:1, 18:1(*n*-7), 18:1(*n*-5), 20:1, 20:2(*n*-6), 20:3(*n*-3), 22:0, 22:5(*n*-3).

Average values of three replicates are shown.

nated in all species and their content varied from 29.6 to 69.8% of the total FAs. This is typical of all brown algae [1, 2, 11, 30] and distinguishes representatives of the Phaeophyta from members of the Rhodophyta and Chlorophyta.

Comparative analysis of major PUFA distribution in brown algae showed that representatives of the Phaeophyta may tentatively be divided into three groups. Half of the algal species had C_{18} PUFAs as the most abundant polyenoic FAs, the content varying from 16.5% of total FAs in *Pelvetia wrightii* to 41.1% in *Alaria angustata*. These algae belong to the first group (spp. 2, 5, 7, 8, 10, 15, 17, 18, 19, 20 in Tables 2 and 3). Five other algal species contained C_{20} polyenoic FAs at a greater proportion than C_{18} PUFAs (spp. 1, 6, 9, 11, 13) and their content varied from 17.0 (*Agarum cribrosum*) to 35.3% (*Laminaria bongardiana*). The remaining five algal species were placed in the third group; they had nearly the same C_{18} and C_{20} PUFA content, for example, *Punctaria plantaginea* (spp. 3, 4, 12, 14, 16). Polyunsaturated FAs belong to two main series (n-3) and (n-6). PUFAs of the (n-3) series were abundant in most of the Phaeophyta, the highest content being found in *Alaria angustata* (50.2%). There are some algal species with ca equal (n-3) and (n-6) PUFA contents, e.g., *Laminaria cichorioides*, *Undaria pinnatifida*, and other species in which (n-6) PUFAs dominated (spp. 3, 4, 12, 19, 20). Representatives of the Fucaceae differed from other algal species by the lowest level of (n-3) PUFAs, 5.8–6.4% of the total FAs as compared with 21.1–50.2% in other species (Tables 2 and 3). Our results show that, in general, there is no correlation between the systematic position of the brown algal species and the distribution of the main C_{18} and C_{20} polyenoic FAs of (n-3) and (n-6) series among them, except for the Fucaceae.

Oleic acid belongs to a group of main FAs in brown algae examined in this work. It is a major unsaturated FA in a few algal species from different orders, because its content exceeds that of any PUFA. Two members of the Fucaceae, *Fucus evanescens* and *Pelvetia wrightii* were richest in 18:1(n-9), which made up 38% of the total FAs. Previously, the same high content of oleic acid had been reported in *Pelvetia wrightii* from the Sea of Japan [31] and in *P. canaliculata* from Scotland [10], suggesting that high 18:1(n-9) content is typical of algae from this genus. Literature data on oleic acid proportion in *Fucus* species are very contradictory. *Fucus spiralis* from Scotland had 38.2% of 18:1(n-9) in April but in November the content of this acid was served [10]. The proportion of oleic acid in *Fucus serratus* varied by season. From August to October the amount of 18:1 in total lipids was more abundant, reaching the maximum level in September (34.1%) and it decreased in

December down to 11.4% [32]. This acid in *F. vesiculosus* and *F. serratus* ranged from 6.9 to 27.4% of total FAs [10, 21, 26]. Hence, the content of 18:1(n-9) in *Fucus* algae varied widely with season.

Among the 20 algal species analysed, *Agarum cribrosum* was unique in having fatty acid patterns that were not found in other members of the Phaeophyta. It had C_{16} PUFAs, which are rare in algae viz: 16:2(n-7), 16:2(n-4), 16:3(n-4), 16:3(n-1), 16:4(n-1) and 18:2(n-4). This alga had the highest content (10.9%) of 22:5(n-3). Usually C_{22} PUFAs are not obligatory components of brown algae and present in concentration from traces to 1.9% of the total FAs in a few algae [10, 16, 17, 24]. A similar fatty acid profile has been reported for this species from Nova Scotia [21, 22], confirming that this unusual fatty acid composition is typical of *A. cribrosum* and, hence, this alga is very interesting from the point of view of pathways for FA synthesis.

Desmarestia ligulata had an unusually high content of 16:1(n-5), where identity was further confirmed by comparison of its equivalent chain lengths (ECL) and retention times (R_t) with those reported for 16:1(n-5) acid from *Dictyota dichotoma* [17]. Its content reached 3% of all FAs in *D. ligulata*. Another *Desmarestia* species, *D. aculeata* from Scotland, was reported to have approximately the same quantity of *trans*-16:1 [10]. Because of difficulties in GC separation of 16:1(n-5) and *trans*-16:1, it is possible that 16:1(n-5) rather than *trans*-16:1 was also present in *D. aculeata*.

Comparison of fatty acid composition of algal species belonging to the orders Chordariales, Dictyosiphonales and Laminariales, revealed no specific features in FA composition characteristic of algae from these orders that would have a taxonomic value. Nine algal species examined in this work belonged to the order Laminariales. They differed in fatty acid composition significantly (Table 3). Among them there are species rich in (n-3) PUFAs (*Alaria angustata*), those with approximately equal amount of (n-3) and (n-6) PUFAs (*L. cichorioides*) and species with uncommon fatty acid patterns (*A. cribrosum*).

Only *Pelvetia wrightii* and *Fucus evanescens* from the Fucaceae had similar features in fatty acid composition, having highest contents of 14:0 and especially of 18:1(n-9), the low concentrations of (n-3) PUFAs (18:3, 18:4, 20:5). Similar peculiarities in FA composition have been found in *Pelvetia canaliculata* [10] and several *Fucus* species from other regions [10, 21, 26]. In addition to the C_{20} PUFAs of the (n-3) and (n-6) series, a 20:3 non-methylene interrupted (NMI) fatty acid was found in *P. wrightii* and *F. evanescens*. NMI fatty acids have been detected in several brown algae [10, 21, 22, 24], but their amount was very low

Table 3. Fatty acid composition (% of total) of brown algae of the Laminariales

Fatty acid	Sample number*								
	8	9	10	11	12	13	14	15	16
14:0	4.7	5.3	5.3	5.4	5.9	3.0	5.2	4.3	4.4
14:1- <i>trans</i>	0.1	1.4	1.7	1.2	tr	0.9	0.5	1.3	0.5
16:0	22.6	16.5	12.3	19.7	24.7	17.5	18.1	10.1	26.8
ECL 16:17	—	—	2.3	—	—	—	—	2.4	—
16:1(n-7)	2.5	2.7	3.9	3.7	1.0	8.0	2.7	2.1	0.1
16:1- <i>trans</i>	0.6	1.0	0.3	0.2	0.2	0.3	0.5	0.8	tr
16:2(n-4)	—	—	0.3	0.3	0.4	1.8	0.3	0.1	0.2
16:3(n-4)	—	—	—	—	—	2.9	—	—	—
16:3(n-1)	—	—	—	—	—	0.5	—	—	—
16:4(n-1)	—	—	—	—	—	5.4	—	—	—
18:0	0.7	0.6	1.0	2.9	2.5	1.5	1.8	0.6	2.9
18:1(n-9)	10.6	7.9	8.4	13.9	21.4	9.2	15.3	6.2	17.9
18:2(n-6)	10.6	5.1	8.4	7.2	9.1	6.1	8.1	5.3	6.2
18:2(n-4)	—	0.2	0.4	0.3	0.1	1.1	—	—	—
18:3(n-6)	0.5	1.0	4.2	3.2	1.2	0.5	0.3	1.4	1.2
18:3(n-3)	8.0	5.7	6.1	3.9	3.8	4.1	6.9	9.4	5.8
18:4(n-3)	11.4	14.4	13.9	5.9	4.0	2.2	10.4	25.0	8.7
20:0	0.2	0.2	0.3	0.7	0.9	0.4	0.5	0.3	0.9
20:4(n-6)	9.4	14.6	14.0	10.9	13.7	6.8	10.1	12.4	12.7
20:5(n-3)	13.2	19.4	14.0	13.0	5.6	9.2	15.6	15.2	7.5
22:5(n-3)	—	0.6	—	—	—	10.9	0.4	—	—
Other**	4.9	3.4	3.2	7.6	5.5	7.7	3.0	3.1	4.2
(n-3)PUFA	33.3	34.4	34.7	23.4	13.6	15.8	33.5	50.2	22.5
(n-6)PUFA	21.3	21.4	28.0	22.2	25.5	14.5	20.2	19.6	21.1
C ₁₈ PUFA	30.5	26.4	32.8	20.2	19.0	12.6	26.6	41.1	22.0
C ₂₀ PUFA	24.1	35.3	29.3	25.4	20.3	17.0	26.9	28.7	21.6

* Number designates algal species in Table 1: 8. *C. filum*, 9. *L. bongardiana*, 10. *L. japonica*, 11. *L. cichorioides*, 12. *C. costata*, 13. *A. cribrosum*, 14. *A. kurilensis*, 15. *A. angustata*, 16. *U. pinnatifida*.

** Other: 12:0, 14:1, br-15:0, 15:0, br-16:0, 16:1(n-9), 16:2(n-6), 17:0, 17:1, 18:1(n-7), 18:3(n-1), 20:1, 20:2(n-6), 20:3(n-6), 20:3(n-3), 20:4(n-3).

Average values of three replicates are shown.

(less than 0.1% of all FAs). Appreciable amounts of 20:3 NMI acid (about 0.8%) were found in *Pelvetia canaliculata*, *Fucus vesiculosus*, *F. spiralis* and *F. serratus* [10, 21]. It is possible that 20:3 NMI and perhaps other C₂₀ NMI fatty acids, are typical components of the Fucaceae. Thus, further study of the distribution of NMI fatty acids in brown algae is needed, especially in representatives of the Fucaceae and the Fucales.

Three species from the genus *Laminaria*, *L. japonica*, *L. cichorioides* and *L. bongardiana* had similar fatty acid patterns but differed in the ratio of main FAs. C₂₀ PUFAs dominated among polyenoic FAs in *L. bongardiana* and this alga had a high content of 20:5(n-3). In *L. japonica*, C₁₈ PUFAs were predominant FAs and 18:4(n-3), 20:4(n-6) and 20:5(n-3) acids were present in *ca* equal amounts. In these two *Laminaria* species, (n-3) PUFA content was higher than that of (n-6) PUFAs, while in *L. cichorioides* polyenoic fatty acids of the (n-3) and (n-6) series were found in comparable quantities. A survey of published data on fatty acids of *Laminaria* shows significant differences between studies [10, 20–24]. Lipids of *L. japonica* have been studied by many researchers and the information obtained on the ratio between the main FAs is inconsistent, although all authors found C₁₈ PUFAs as dominant components [20, 21, 24]. We have previously studied

the fatty acids of *L. japonica* from the Yellow Sea collected in winter (water temperature *ca* 4°C) [30]. It significantly differed in (n-3)/(n-6) PUFAs from the same species taken in summer (water temperature *ca* 22°C) examined in this work. The Chinese *L. japonica* was lower in 18:4(n-3) and 20:5(n-3) than alga from the Sea of Japan. This may not be an effect of water temperature, because it has been shown that low water temperature is favourable to biosynthesis and accumulation of PUFAs [33]. Possibly other environmental factors, such as light, the concentration of nitrogen and other compounds in the water, affect the fatty acid content in algae [2, 33]. Recently, Honya *et al.* [34] have shown the influence of factors, such as growth stage, on the (n-3)/(n-6) PUFA ratio in cultured *L. japonica*, in addition to seawater temperature. Thus, the main FA ratio in *L. japonica* is affected by many factors. Hence, this algal species may provide a good model for the study of the influence of different factors on the fatty acid composition and the culturing of algae with desirable a fatty acid ratio.

Lipids of *Undaria pinnatifida* have been studied by several groups [19, 20, 23, 24, 26, 35] and data on its FA composition are contradictory. The discrepancies concern mainly the quantity of 18:4(n-3) and 18:1(n-9) and, to a lesser degree, the content of other unsaturated FAs. Comparison between available data shows that the C₁₈ PUFA percentage was

higher than that of C₂₀ PUFAs in all *U. pinnatifida* samples examined and the amount of (n-3) PUFAs exceeded that of (n-6). Young and adult *U. pinnatifida* did not show any differences in fatty acid composition; hence, algal age did not affect FA ratio [30]. Chinese *U. pinnatifida*, collected in winter, was rich in octadecatetraenoic acid (25.3%) [30], while the same alga from the Sea of Japan, taken in summer, contained only 8.7% of this acid (Table 3). In winter, *U. pinnatifida* was richer in PUFAs than in summer. This agrees with the view that algae accumulate PUFAs when there is a decrease in environmental temperature [33, 36].

Discrepancies between the FA ratio in algal species obtained in this work and literature data were also found for *Punctaria plantaginea*. This algal species from Scotland was richer in 18:4(n-3), 20:5(n-3) and therefore in total (n-3) PUFAs [10] than that from the Sea of Japan. The fatty acid composition of *Leathesia difformis*, *Dictyosiphon foeniculaceus* and *Chorda filum* from the Russian Far East coast did not differ significantly from those of the same algal species from the Scotland [10] and had similar trends in FA ratio. Probably, the extent of FA ratio modification in brown algae by different factors (environmental, growth stage, age, etc.) depends on the algal species.

Our results show that the specific features of brown algae fatty acid compositions are high concentration of C₁₈ and C₂₀ PUFAs and oleic acid. There is no correlation between the systematic position of an algal species and the distribution of C₁₈ and C₂₀ PUFAs of (n-3) and (n-6) series among them. The ratio of these acids varies in algal species belonging to the same order, family or even genus. Among brown algae, there are species rich in (n-3) PUFAs (majority of algae in Tables 2 and 3) or in (n-6) PUFAs (spp. 3, 4, 12, 19, 20) and those with approximately equal amounts of (n-3) and (n-6) PUFAs (spp. 11, 13, 16). Peculiarities of fatty acid composition that may have a chemotaxonomic value were shown for members of the Fucaceae. The algae belonging to this family typically have a high content of 14:0, small amounts of (n-3) PUFAs and contain 20:3 NMI acid. The highest contents of 18:1(n-9) are characteristic of *Pelvetia* species. The occurrence of rare C₁₆ polyenoic fatty acids and 18:2(n-4) in appreciable amounts and highest content of 22:5(n-3) are a characteristic feature of *A. cribrosum*. The fatty acid composition of brown algae depends on different factors and the extent of influence of these factors is species-specific.

A possibly significant practical conclusion of the present study is that brown algae may be suitable as potential sources of PUFAs of the (n-3) and (n-6) series. In most marine animals, 18:2(n-6), 18:3(n-3) and also 20:4(n-6) and 20:5(n-3) are

obtained directly from the diet. Amounts of C₂₀ PUFAs are converted into anti-inflammatory leucotrienes [37] and prostaglandins necessary for growth and reproduction [38]. Hence, marine brown algae may provide valuable sources of essential PUFAs for marine animals. In addition, PUFAs have been shown to play a protective role against free radicals known to have an aging effect on skin [8] and, hence, PUFAs are being frequently used in cosmetics, especially in sun lotions and regenerating and anti-wrinkle products [9].

The high quantities and a large diversity of fatty acids found in brown algae, the huge and renewable biomass of these plants and varied species composition of the Phaeophyta make them possible sources of specific FAs for biotechnological uses and as a diet enriched in essential fatty acids.

EXPERIMENTAL

Algal species studied are listed in Table 1. *Leathesia difformis*, *Punctaria plantaginea*, *Petalonia fascia*, *Laminaria japonica*, *L. cichorioides*, *Costaria costata*, *Undaria pinnatifida* were collected in July, *Sphaerotreichia divaricata* and *Anelipes japonicus* in August, *Desmarestia ligulata* and *Agarum cribrosum* in May, *Dictyosiphon foeniculaceus*, *Chorda filum*, *Cystoseira crassipes*, *Cocophora langsdorffii* and *Pelvetia wrightii* were taken in June in the Sea of Japan in the southern region of the Russian Far East, (water temp. was 15–22°C). *Laminaria bongardiana*, *Alaria angustata*, *Arthrothamnus kurilensis* and *Fucus evanescens* were sampled in July on the coast of the Kurile Islands (Sea of Okhotsk) at 0–2 m depth. Freshly collected adult algae were thoroughly cleaned to remove epiphytes, small invertebrates and sand particles. Lipids were extracted by homogenization with CHCl₃-MeOH (1:2) [39]. The residue was reextracted 2–5 times with small portions of CHCl₃-MeOH (1:1). Combined extracts were filtered and then mixed with CHCl₃ and H₂O for phase separation. The CHCl₃ layer was collected, evapd under red. pres. and the total lipid content determined gravimetrically.

Fatty acid methyl esters (FAMES) were prepared by transmethylation of lipid samples by adding 1% Na in MeOH, followed by heating for 15 min at 55° and then adding 5% HCl in MeOH, followed by heating for 15 min at 55° [40]. FAMES were purified by TLC using benzene and analyzed by FID-GC using silica capillary columns (30 m × 0.25 mm) coated with Supelcowax 10 M and SPB-5 at operating temps of 210° and 220°, respectively; the carrier gas was He.

Individual peaks of FAMES were identified by comparing *R*_fs with those for authentic standards and using Equivalent Chain Length (ECL) analysis [41, 42]. In addition FAMES were separated by prep. AgNO₃-TLC [43]. Silica gel covered plates

(6 × 6 cm) were developed in MeOH saturated with AgNO₃ and then plates were dried. FAMEs were applied to the plates and developed with hexane–Et₂O–HOAc (94:4:3). FAME-containing zones were made visible by spraying a narrow edge of plate with 10% H₂SO₄ in MeOH and then charring. Frs recovered from TLC were then analysed by GC.

Acknowledgements—The author is grateful to Tamara Titlyanova for identification of algal species, Dr V. I. Kharlamenko for collection of algae from the Sea of Okhotsk and Dr V. E. Vaskovsky for constant support and fruitful help. This work was partly supported by grant No. RJEOOO from the International Science Foundation.

REFERENCES

- Wood, B. J. B., in *Microbial Lipids*, ed. C. Ratledge and S. G. Wilkinson. Academic Press, London, 1988, p. 807.
- Kayama, M., Araki, S. and Sato, S., in *Marine Biogenic Lipids, Fats and Oils*, ed. R. G. Ackman, Vol. 2. CRC Press, Florida, 1989, p. 3.
- Duerberg, J., *Nutrition Reviews*, 1986, **44**, 125.
- Uki, N., Sugiuza, M. and Watanabe, T., *Nippon Suisan Gaikkashi*, 1986, **52**, 1013.
- Sardesai, V. M., *Journal of Natural Biochemistry*, 1992, **3**, 154.
- Gerwick, W. H. and Bernart, M. W., in *Marine Biotechnology*, vol. 1: Pharmaceutical and Bioactive Natural Products, ed. D. H. Attaway and O. R. Zaborsky. Plenum Press, New York, 1993, p. 101.
- Radwan, S. S., *Applied Microbiology and Biotechnology*, 1991, **35**, 421.
- Raildi, G., *Skin. Appl. Sci. Vevy Eur.*, 1992, **9**, 92.
- Servel, M.-O., Claire, C., Derrien, A., Coiffard, L. and De Roeck-Holtzhauer, Y., *Phytochemistry*, 1994, **36**, 691.
- Jamieson, G. R. and Reid, E. H., *Phytochemistry*, 1972, **11**, 1423.
- Khotimchenko, S. V. and Svetashev, V. I., *Biologiya Morya (Vladivostok)*, 1987, **6**, 3.
- Miralles, J., Akinin, M., Micouin, E. M., Gaydou, E. M. and Kornprobst, J. M., *Phytochemistry*, 1990, **29**, 2161.
- Akinin, M., Moellet-Nzaou, R., Cisse, E., Kornprobst, J. M., Gaydou, E. M., Samb, A. and Miralles, J., *Phytochemistry*, 1992, **31**, 2739.
- Khotimchenko, S. V., *Phytochemistry*, 1993, **32**, 1203.
- Khotimchenko, S. V., *Botanica Marina*, 1996, **38**, 509.
- Akinin, M., Dogbevi, K., Samb, A., Kornprobst, J. M., Gaydou, E. M. and Miralles, J., *Comparative Biochemistry and Physiology*, 1992, **102B**, 841.
- Khotimchenko, S. V., *Phytochemistry*, 1995, **38**, 1411.
- South, G. R. and Whittick, A., *Introduction to Phycology*. Blackwell Scientific Publication, 1987, p. 497.
- Pohl, P., Wagner, H. and Passig, T., *Phytochemistry*, 1968, **7**, 1565.
- Hayashi, K., Kida, S., Kato, K. and Yamada, M., *Nippon Suisan Gaikkashi*, 1974, **40**, 609.
- Ackman, R. G. and McLachlan, J., *Proc. N. Scot. Inst. Sci.*, 1977, **28**, 47.
- Ackman, R. G., in *New Sources of Fats and Oils*, ed. E. H. Pryde, L. H. Princen and K. D. Mukherjee. American Oil Chemists Society, Champaign, Illinois, 1981, p. 189.
- Takagi, T., Asahi, M. and Itabashi, Y., *Yukagaku*, 1985, **34**, 1008.
- Kaneniwa, M., Itabashi, Y. and Takagi, T., *Nippon Suisan Gaikkashi*, 1987, **53**, 861.
- Dembitsky, V. M., Rozentsvet, O. A. and Pechenkina, E. E., *Phytochemistry*, 1990, **29**, 3417.
- Fleurence, J., Gutbier, G., Maubeau, S. and Leray, C., *Journal of Applied Phycology*, 1994, **6**, 527.
- Chapman, V. J. and Chapman, D. J., *Seaweeds and Their Uses*. Chapman and Hall, London and New York, 1980.
- Johns, R. B., Nichols, P. D. and Perry, G. J., *Phytochemistry*, 1979, **18**, 799.
- Stefanov, K., Konaklieva, M., Brechany, E. Y. and Christie, W. W., *Phytochemistry*, 1988, **27**, 3495.
- Vaskovsky, V. E., Khotimchenko, S. V., Xia, Bangmei and Hefang, Li, *Phytochemistry*, 1996, **42**, 1347.
- Khotimchenko, S. V. and Svetashev, V. I., *Biologiya Morya (Vladivostok)*, 1983, **5**, 45.
- Kim, M. K., Dubacq, J. P., Thomas, J. C. and Giraud, G., *Phytochemistry*, 1996, **43**, 49.
- Pohl, P. and Zurheide, F., in *Marine Algae in Pharmaceutical Science*, Vol. 2, ed. H. Hoppe and T. Levring, 1979, p. 65.
- Honya, M., Kinoshita, T., Ishikawa, M., Mori, H. and Nisizawa, K., *Journal of Applied Phycology*, 1994, **6**, 25.
- Sato, S., *Nippon Suisan Gaikkashi*, 1975, **41**, 1177.
- Kayama, M., Iijima, N., Kumahara, M., Sado, T., Araki, S. and Sakurai, T., *Nippon Suisan Gaikkashi*, 1985, **51**, 687.
- Tocher, D. R. and Sargent, J. R., *Comparative Biochemistry and Physiology*, 1987, **87B**, 733.
- Ruggeri, B. and Thoroughgood, C. A., *Marine Ecology Progress Ser.*, 1985, **23**, 301.
- Bligh, E. G. and Dyer, W. J., *Canadian Journal of Biochemistry and Physiology*, 1959, **37**, 911.

40. Carreau, J. P. and Dubacq, J. P., *Journal of Chromatography*, 1978, **151**, 384.
41. Kramer, J. K. G., Fouchard, R. C. and Jenkins, K. J., *Journal of Chromatographic Science*, 1985, **23**, 54.
42. Napolitano, G. E., Ratnayake, W. N. and Ackman, R. G., *Phytochemistry*, 1988, **27**, 1751.
43. Dudley, P. A. and Anderson, R. E., *Lipids*, 1975, **10**, 113.