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Separation and Identification of Lipids and Fatty Acids of the Marine Alga *Fucus vesiculosus* by TLC and GC—MS

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ABSTRACT. Ten fractions of polar and nonpolar lipids (phospholipids, glycolipids and neutral lipids) were isolated from the brown marine alga *Fucus vesiculosus* by TLC. In each fraction the quality and quantity of fatty acids were determined by GC—MS. *F. vesiculosus* is another type of marine alga suitable as a source of polyene fatty acids.

Abbreviations

PI — phosphatidylinositol, PC — phosphatidylcholine, PG — phosphatidylglycerol, PE — phosphatidylethanolamine, CL — cardiolipin (diphosphatidylglycerol), PA — phosphatidic acid, DGDG — digalactosyldiglyceride, MGDG — monogalactosyldiglyceride, TG — triacylglycerol.

In addition to marine algae of the genus *Porphyridium* producing polyene fatty acids (Ahern *et al.* 1983; Řezanka *et al.* 1987), these compounds, known as vitamin F, are synthesized by other genera. The content and composition of fatty acids in algae of the genus *Fucus* were studied by Ackman and McLachlan (1977), Jamieson and Reid (1972) and by Smith and Harwood (1984) who separated the lipids into MGDG, DGDG and polar lipids and analyzed fatty acids in the individual groups. The relationship between fatty acid production and exogenous factors (nitrogen content, water temperature and time of harvest) was described by Pohl and Zurheide (1982).

F. vesiculosus is widely distributed in nature and is described in the medical literature (Mac Nalty 1965). It is a component of medicinal teas supporting metabolism (*List of Czechoslovak Pharmaceutical Preparations 1986*) and individual components of lipid fractions and fatty acids bound in them and analyzed in the present work might also contribute to their possible dietetic properties.

MATERIAL AND METHODS

Ten grams of the marine alga *Fucus vesiculosus* (*Léčiva*, Prague) were homogenized and extracted according to Folch (Bergelson 1980) yielding a total of 270 mg lipids.

Two-dimensional TLC was done on 200 × 200 mm plates 0.5 mm thick (Kieselgel G Stahl, *Merck*, FRG) in $CHCl_3$—CH_3OH—H_2O (65 : 25 : 4) and in $CHCl_3$—CH_3OH—NH_4OH (65 : 28 : 5) in the first and second direction,

respectively. Ten mg samples of total lipids were applied per plate. Individual lipid classes were detected by Rhodamine 6G. Their relative occurrence was determined in the High Speed TLC Scanner CS-920 (*Shimadzu*, Japan). Individual compounds were identified according to identity of R_F values with those of standard compounds (Serum Lipid Mixture, *Supelco*, USA) and by specific colour reactions (Kates 1972; Bergelson 1980).

0.5—1 mg of each group of lipids and of total lipids was transesterified with a BF_3—CH_3OH mixture. Methyl esters were extracted with hexane, washed with water and dried.

Methyl esters of fatty acids of total lipids and of individual lipid classes were identified and quantified by means of the GC—MS method (total ion current) in the HP 5995 B apparatus (*Hewlett Packard*, USA) with a capillary column Carbowax 20 M (25 m × 0.25 mm), programmed temperature from 150 to 220 °C and at 3 L/min. Injector temperature was 260 °C, flow of helium as a carrier gas was 1.1 mL/min, split 1 : 50, ionization energy 11 aJ (70 eV).

Pyrrolidides were prepared according to Andersson (1978) and were chromatographed in the same apparatus with a capillary column (40 m × 0.25 mm) with a DC-510 phase (*Macherey-Nagel*, FRG) and programmed temperature from 200 to 280 °C at 3 K/min, injector temperature was 280 °C, helium flow was 1.7 mL/min.

RESULTS AND DISCUSSION

Differences in the occurrence of individual lipid classes are illustrated in Table I (from 1 to 28 %).

TABLE I. Representation of individual lipid types in *F. vesiculosus*

Lipid	%	Lipid	%
PI	3	PA	1
PC	19	DGDG	7
PG	5	MGDG	9
PE	12	TG	28
CL	1	Unidentified	15

Liem and Laur (1974) described higher values of PA (up to 5 %) in *F. serratus* and *F. vesiculosus*. Smith and Harwood (1984), working with *F. serratus*, described differences in the formation of MGDG and DGDG between the vegetative and reproductive parts of the alga — 1 % MGDG and 4 % DGDG in the vegetative part, 16 % MGDG and 29 % DGDG in the reproductive part. Our values detected in unspecified algal parts range from 7 to 9 %. The content of PC, PI, PG, CL and PE is comparable with the data of Liem and Laur (1974) and of Smith and Harwood (1984) obtained in *F. serratus* and *F. vesiculosus*. Similarly to Smith and Harwood in *F. serratus* (1984), we detected no PS. However, Liem and Laur (1984) described 6—19 % PS in these organisms. TG content was determined to be 1 % by Smith and Harwood (1984). The quantity of TG varies in individual seasons of the year (Pohl and Zurheide 1979). When studying the content of fatty acids in individual lipid groups it was found (Table II) that all acids have an even

TABLE II. Content of fatty acids in individual lipid classes after TLC (relative %).

TLC fraction	Fatty acid													
	14:0	16:0	16:1[a]	18:1[a]	18:2[a]	18:3[b]	18:3[c]	18:4	20:2	20:3	20:4[d]	20:4[e]	22:5[a]	Minority
PI	5.8	18.1	3.2	12.8	10.1	9.7	6.4	1.4	1.2	0.7	18.3	3.1	—	9.2
PC	11.2	7.4	4.8	25.3	10.6	7.7	2.4	2.1	0.8	1.1	8.4	2.7	—	15.5
PG	9.3	11.7	5.0	21.6	9.5	11.0	2.3	2.0	2.1	3.0	9.8	2.7	—	20.0
PE	10.9	17.3	3.2	12.3	8.3	8.3	0.7	0.3	1.9	2.1	19.6	7.1	—	8.3
CL	8.3	4.2	3.1	7.4	6.5	25.2	8.4	2.2	4.3	7.3	7.2	3.2	—	9.6
PA	9.2	10.1	3.7	22.3	8.6	10.1	2.8	0.7	1.4	0.8	9.6	2.3	—	18.4
DGDG	5.4	4.8	3.6	16.2	10.2	8.2	0.5	23.4	0.7	1.2	6.2	2.1	3.2	14.3
MGDG	3.4	6.3	3.5	14.7	7.8	4.3	1.3	29.1	0.6	0.9	4.6	1.7	2.0	19.8
TG	27.3	22.5	12.3	23.4	4.7	1.6	0.7	—	0.2	—	—	—	—	7.3
Unident.	11.8	7.3	5.0	23.1	8.6	10.4	2.8	1.7	2.1	1.1	8.6	0.9	—	16.6

[a]Sum of position isomers.
[b]6,9,12-18:3
[c]9,12,15-18:3.
[d]5,8,11,14-20:4.
[e]8,11,14,17-20:4.

number of C atoms and that the 18:0 acid is always absent; of saturated acids, only 14:0 (found primarily in PC, PE and mainly in TG) and 16:0 (primarily in PI, PG, PE, PA and TG) were detected. Liem and Laur (1974) demonstrated the dominance of the 16:0 acid in PE, PC, PI and PA fractions of three representatives of the genus *Fucus*. Unsaturated fatty acids within the range of 16:1—22:5 were detected with a pronounced variability in individual lipid fractions. The monoenoic acid 16:1 is highly represented in TG, 18:1 was detected in all studied lipid fractions, except for CL. The dienoic acid 20:2 was detected as a minor component in all fractions, whereas 18:2 was found only in CL and FG. Of trienoic acids, 6,9,12-18:3 and 9,12,15-18:3 were detected in *F. vesiculosus*. The former was highly represented in PI, PG and CL. Ackman and McLachlan (1977) described 6,9,12-18:3 and *trans*-3-hexadecenoic acids as major acids in the genus *Fucus*. A high content of the 18:4 acid is characteristic for DGDG and MGDG, in agreement with Jamieson and Reid (1972) and Smith and Harwood (1984) who also described 5,8,11,14-20:4 in PI and PE. The 22 : 5 acid was clearly a minor component in the strain used in this work and was only found in DGDG and MGDG.

TABLE III. Total content of fatty acids in *F. vesiculosus*

Acid[a]	Relative %	Acid[a]	Relative %
8:0	0.16	6,9,12-18:3	8.26
9:0	0.12	9,12,15-18:3	2.65
10:0	0.21	6,9,12,15-18:4	2.25
12:0	0.24	UU	0.21
13:0	0.09	20:0	0.40
i-14:0	0.14	11-20:1	0.72
14:0	10.83	11,14-20:2	1.25
U	0.17	5,8,11-20:3	1.21
9-14:1	0.93	11,14,17-20:3	0.53
i-15:0	0.33	5,8,11,14-20:4	7.12
ai-15:0	0.35	20:3	0.17
br-15:0[b]	0.07	20:3	0.51
15:0	0.55	20:4	0.39
16:0	8.19	U	0.12
9-16:1	4.89	8,11,14,17-20:4	3.02
3*t*-16:1[c]	0.30	22:0	0.83
16:1	0.16	13-22:1	0.86
7,10-16:2	0.23	U	0.09
17:0	0.08	7,10,13,16-22:4[d]	0.14
17:1	0.49	4,7,10,13,16-22:5[d]	0.13
U	0.05	U	0.10
18:0	0.07	U	0.09
9-18:1	26.80	7,10,13,16,19-22:5[d]	0.11
11-18:1	0.49	24:0	0.08
9,12-18:2	12.01	15-24:1	0.81

[a] i — iso acid, ai — anteiso acid; numbers before the hyphen indicate the double-bond position(s); U — unidentified acid.

[b] Methyltetradecanoic acid (branch position undetermined).

[c] *trans*-3-Hexadecenoic acid.

[d] Double-bond position derived on the basis of identical retention times with respect to those of standards.

Table III presents the total contents of fatty acids. Saturated fatty acids C_8—C_{24} (predominantly even-numbered) with myristic and palmitic acids as the major ones, and branched-chain fatty acids were detected. As compared with Jamieson and Reid (1972), we identified separately saturated and monoenoic acids among the C_{20} and C_{24} acids. Among monoenoic fatty acids, even-numbered C_{14}—C_{24} and odd-numbered C_{17} acids, with oleic acid as the major one, were detected (see also Ackman and McLachlan 1977). We determined the double bond position of the 24:1 acid to be at $C_{(15)}$.

Polyenoic acids have the most extensive qualitative variation range. One C_{16}, four C_{18}, eight C_{20} and three C_{22} acids are present; in this connection it should be mentioned here that Iatrides *et al.* (1978) did not detect any polyene C_{20}—C_{22} acids.

One of the 20:3 acids with undetermined double bond position is probably 5,11,14-20:3 according to its chromatographic behavior, and similarly one of the 20:4 acids is the unusual isomer 5,11,14,17-20:4. On the basis of retention behavior, double bond positions were determined in two minority 22:4 and 22:5 acids, *viz.* 7,10,13,16-22:4 and 4,7,10,13,16-22:5, respectively.

It follows from the experimental and literature data that differences exist in the content of individual classes of lipids and fatty acids, depending apparently on the studied species or genus of *Fucus* and, to a certain extent, on the time of harvest and way of storage. Nevertheless, it could be demonstrated that marine algae of the genus *Fucus* may serve as another suitable source of unsaturated fatty acids or, occasionally, of some lipid classes. Among fatty acids, the production of 18:1, 6,9,12-18:3, 18:4 and 5,8,11,14-20:4 is of interest, among lipidic fractions PC, PE, MGDG, DGDG and TG are of significance.

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