Liver Lipids and Fatty Acids of the Sting Ray *Dasyatis bleekeri* (Blyth)

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ABSTRACT: The sting ray, *Dasyatis bleekeri* (Blyth), has been studied for lipids and fatty acids of its liver. The neutral lipids identified were hydrocarbons, wax esters, steryl esters, 1-*O*-alkyl-2,3-diacylglycerols, triacylglycerols, and sterols. Neutral lipids were predominant (91.8%), major components being triacylglycerols (92.7%). Polyenoic fatty acids of n-3 series, *viz*. eicosapentaenoic acid and docosahexaenoic acid, were high in the phospholipid and neutral lipid fractions. Cholesterol was the major component (67.9%) in the steryl ester fraction. Glyceryl ethers, with chainlengths up to 30 carbons, were recorded with unsaturated, anteiso, iso, and normal chains. In wax ester alcohols, up to 32-carbon chains were recorded. Hydrocarbons were up to 36-carbon chains with anteiso, iso, and normal chains. Among branched chain hydrocarbons, pristane was the major component (6.7%) and squalene was present at the level of 3.5%. Chimyl and batyl alcohol backbones were the major components found in 1-*O*-alkyl-diacylglycerols.
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KEY WORDS: *Dasyatis bleekeri,* docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), fatty acid, lipid.

The sting ray, *Dasyatis bleekeri* (Blyth), belonging to the family Dasyatidae of the class Chondrichthyes, occupies the lowest group of all living vertebrates with movable jaws and paired appendages. *Dasyatis bleekeri* is a dominant cartilaginous fish of the lower zone of the Hooghly-Matlah estuarine complex of West Bengal, India. Because of its abundant availability, this fish is regularly caught and marketed for human consumption. The liver of *D. bleekeri* is the largest organ in the body and may constitute one-seventh to one-sixth of the weight of a ray.

Nutritionists and food scientists need lipid and fatty acid composition data to aid them in dietary formulation, nutrient labeling, processing, and product development (1). Currently, the biochemical effects of fish oils in human health and nutrition (2,3) have placed renewed emphasis on the apparent difference in the compositions of fish oils (4). Lipid and fatty acid data for different marine and fresh water fish species are available in the literature (5–8). No such data is available on *D. bleekeri*, which is an important edible seafood in India.

In the present investigation, the lipids and fatty acids of the liver of *D. bleekeri* were studied with a view to exploring the possibilities of commercial exploitation of the highly available species as a source of marine oils and polyunsaturated fatty acids of n-3 series.

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EXPERIMENTAL PROCEDURES

Sample collection. Sunderbans, the major part of the coastal region of West Bengal, India, is situated between the latitude 21°30′ N to 22°31′ N and longitude 88°08′ E to 89°51′ E. *Dasyatis bleekeri* inhabits the coastal water of West Bengal and is generally captured within 2–3 km of the coast line. A mature *D. bleekeri* was collected from this area during the month of January, 1994. The fish studied in this investigation was 25 inches long and 22 inches wide, with a 49-inch long tail, and weighed 12.50 kg. Weight of the liver, which was yellowish in color, was 1.85 kg. It consisted of two lobes, connected anteriorly. The ventral portion of the fish was dissected and the liver was separated from the body, immediately frozen, and stored at –20°C until analyzed.

Extraction of lipid. The total lipid from the liver was extracted by the method of Bligh and Dyer (9). BHT (butylated hydroxy toluene) was added at a level of 100 mg/L to the solvent as antioxidant. The isolated lipids were stored under nitrogen in distilled hexane and kept at –20°C for future use.

Column chromatography. A portion of the total lipid was subjected to column chromatography using a silicic acid (Mallinckrodt, 300 mesh) column as described by Rouser *et al.* (10). The neutral-, glyco-, and phospholipids were eluted by 10, 40, and 10 column vol of chloroform, acetone, and methanol, respectively. Solvents were evaporated and fractions were kept in distilled hexane at –20°C. Each class of lipids was estimated by weighing, in a micro balance.

Thin-layer chromatography (TLC). Thin-layer chromatography was performed on 20 × 20 and 14 × 20 cm chromatoplates coated with silica gel G (0.25–0.50 mm thickness). The neutral lipid (NL) was fractionated by preparative TLC using light petroleum ether (40–60°C)/diethyl ether/acetic acid (80:20:1, vol/vol/vol) according to Mangold (11). While 1-*O*-alkyldiacylglycerols (ADAG), triacylglycerols (TG), and sterols were clearly separated, hydrocarbon, steryl ester (SE), and wax ester (WE) bands overlapped and were rechromatographed using a solvent system of light petroleum ether (40–60°C)/diethylether (98:2, vol/vol) according to Misra and

Ghosh (12). Spots of separated lipid components were detected by iodine vapor and by specific spray reagents. All the lipids were identified by comparing the R_f with those of authentic standards. NL components were estimated by direct weighing.

Saponification of various lipids. Aliquots of NL, glycolipids (GL), and phospholipids (PL) and separated components of NL, *viz.* WE, SE, ADAG, and TG were saponified according to Christie (13). The lipid sample (100 mg) was hydrolyzed by refluxing it with 1.5 M solution of potassium hydroxide in methanol (5 mL) for 1 h in an atmosphere of nitrogen gas. After saponification, methanol was removed under vacuum. The soap thus obtained was dissolved in water, cooled, transferred to a separatory funnel, and extracted three times with diethyl ether (3 × 5 mL) to remove nonsaponifiable materials. Whenever necessary centrifugation was done to break any emulsion that was formed. The solvent extracts were washed several times with water and dried over anhydrous sodium sulfate. The non-saponifiable material was recovered after removal of solvent in a rotary evaporator. The aqueous layer was acidified with 4 N H_2SO_4 and extracted with peroxide-free diethyl ether (3 × 5 mL). The free fatty acids were recovered after washing the extracts with water, drying over anhydrous sodium sulfate, and removing the solvent in a rotary evaporator.

Methylation of fatty acids. Fatty acids of each fraction obtained by the above process were methylated using an ethereal solution of diazomethane according to Schlenk and Gallerman (14).

*Acetylation of alcohols, sterols, and 1-*O*-alkylglycerols.* The alcohols, sterols, and 1-*O*-alkylglycerols obtained from alkaline hydrolysis of WE, SE, and ADAG, respectively, were acetylated from the non-saponifiables along with the free sterols according to Privett and Nutter (15).

Estimation of sterols. Sterols obtained by preparative TLC were estimated colorimetrically using ferric chloride reagent, as described by Kates (16).

Gas-liquid chromatography (GLC). For determination of the various lipid components, the samples were analyzed by GLC in a Hewlett-Packard instrument, model 5890, series II (Hewlett-Packard Company, Avondale, PA), equipped with a glass column (1.8 m × 2 mm i.d.) and fitted with a flame ionization detector (FID). Quantitation was done by an integrator (Hewlett-Packard model 3394A) attached to the gas chromatograph. Columns used were packed with nonpolar 3% OV-17 (polymer of methyl siloxane) and polar 10% DEGS (diethylene glycol succinate polyester); both liquid phases were supported on Chromosorb-W (HP) 80–100 mesh and packed into the glass column. The standards for GLC were obtained from Sigma Chemical Company, St. Louis, MO.

Analysis of hydrocarbons. Gas chromatography of the hydrocarbons obtained from the liver of *D. bleekeri* was carried out without derivatization on a nonpolar 3% OV-17 column. Oven temperature was kept isothermal at 200°C for 2 min at the start and then programmed at the rate of 4°C/min up to 320°C and kept isothermal for 15 min. The detector and injection port temperatures were 380 and 350°C, respectively. Carrier gas used was nitrogen with a flow rate of 30 mL/min. An authentic mixture of *n*-alkanes was also analyzed under the same conditions of temperature and other operational parameters. Identification was done primarily by comparing the retention times of the authentic compounds with those of the samples according to Misra and Ghosh (12).

Analysis of alcohol acetates from wax ester. GLC of alcohol acetates obtained from the nonsaponifiables of WE was done on a 3% OV-17 column. Oven temperature was programmed from 210 to 330°C at the rate of 6°C/min. Flow rate of nitrogen was 30 mL/min. Identification was done by comparing the retention times of the authentic compounds with those of the samples, as well as by semilogarithimic plots at two different isothermal temperatures (12).

Analysis of steryl acetates. GLC of steryl acetates was done on a 3% OV-17 and SE-30 column. Oven temperature was kept isothermal at 280°C. Detector and injection port temperatures were 350 and 330°C, respectively. Nitrogen was used as carrier gas with a flow rate of 30 mL/min. Identification was done following the methods of Patterson (17), by comparing relative retention times (RRT) with those in the literature.

Analysis of ADAG. GLC of alkyldiacetylglycerols was done on a 3% OV-17 column. Oven temperature was programmed from 200 to 330°C at the rate of 8°C/min. Detector and injection port temperatures were 380 and 350°C, respectively. Nitrogen flow rate was 30 mL/min. Identification of the alkoxy compounds apart from GLC were based on (i) R_f values on TLC plate, which corresponded with those of the standards as described by Mangold (11) and Mangold and Bauman (18); (ii) the infrared (IR) spectrum, which showed absorption bands (19,20) at 1121 cm^{-1}, for -*O*-alkyl ether, -C-*O*-C-stretching and strong ester band at 1748 cm^{-1}; (iii) ^{1}H-NMR (300 MHz) spectrum, which showed signals at δ 5.18 (1H, *m*, -C*H*-O-), 4.15 (1H, *d*, J_{gem} = 12.0 Hz, J_{vic} = 6.5 Hz, H_A-3), 4.33 (1H, *d*, J_{gem} = 12.0 Hz, J_{vic} = 3.6 Hz, H_B-3), 3.54 (2H, *d*, J_{vic} = 5.2 Hz, H_2-1), 5.3 (*m*, -C*H*=C*H*-), 3.43 (2H, *m*, -OCH_2-CH_2-CH_2-), 1.54 (2H, *m*, -OCH_2-CH_2-CH_2-), 1.25 (broad *s*, x.-CH_2-), 2.04 (*m*, -CH_2-CH=CH-), 0.87 (3H, *t*, J_{vic} = 6.6 Hz, CH_3-CH_2-), 2.06, and 2.07 (3H each, *s*, -O-CO-CH_3), where *s*, *d*, *t*, and *m* indicate singlet, doublet, triplet, and multiplet, respectively. The ^{1}H NMR signal assignments were complemented by appropriate homo-decoupling experiments. The NMR spectra (δ, ppm) of ADAG were recorded on a 300 MHz Bruker (Fallanden, Switzerland) supercon NMR spectrometer (Model AM 300L) using $CDCl_3$ as solvent with tetramethylsilane (TMS) as internal standard. IR spectra were recorded in an instrument model MB-102, FT-IR by Bomem Inc., Québec, Canada with DTGS gain D detector, with a resolution of 50 scans in the range of 4000–400 cm^{-1}. A reference was first obtained with CsI powder in the Diamond Anvil Cell (DAC) and then with the neat sample (100–200 μg) and transmittance spectrum obtained at 4 cm^{-1} resolution. GLC identification of components was done by the techniques suggested by Misra and Ghosh (12).

Analysis of fatty acid methyl esters (FAME). FAME were analyzed by GLC on a 10% DEGS column. Oven tempera-

ture was kept isothermal at 196°C. Detector and injection port temperatures were 250°C. Nitrogen flow rate was 30 mL/min. Identification of fatty acids was made by (i) comparison of retention times of authentic standards, as well as with the fatty acids of cod liver oil, a secondary standard as suggested by Ackman and Burgher (21), (ii) semilogarithmic plots (22) of RRT against carbon chain lengths, (iii) comparison of equivalent chain length (ECL) value of the component acids with those reported in the literature (23,24), and (iv) comparison of the chromatogram of hydrogenated FAME with that of the original sample (25).

RESULTS AND DISCUSSION

Total lipids and various classes and subclasses obtained from the liver of *D. bleekeri* are presented in Table 1. The total lipid of the liver is considerably high (63.4%), which is in accordance with the many previous studies (5) on liver lipids of marine organisms. Early in the 20th century, with the development of vitamin chemistry, it was definitely established that night blindness and rickets are largely caused by a dietary deficiency in vitamins A and D, respectively. Both vitamins, A and D, are found in certain fish liver oils in various proportions (26). The most important raw materials for the production of liver oils come from the fisheries for cod, coalfish, and haddock (27). The livers of ling, tusk, several species of shark (such as dogfish, Greenland, and basking sharks), halibut whale, and tuna have been used in the production of liver oils.

Among the various classes of lipids separated by column chromatography, the proportion of NL was highest (91.8%), followed by GL (4.9%) and PL (3.3%). High levels of NL, particularly rich in TG, are very characteristic of liver oils of marine organisms (27). Among NL, TG are the major components (92.7%). Of the other components, hydrocarbons were the major constituents (3.3%). The total sterols and SE were 1.9%, whereas WE and ADAG were 0.8 and 1.3%, respectively.

TABLE 1
Composition of Various Classes of Lipids Obtained from the Liver of *Dasyatis bleekeri*

	% w/w
Lipids	
Total lipids (TL)[a]	63.4
Neutral lipids (NL)[b]	91.8
Glycolipids (GL)[b]	4.9
Phospholipids (PL)[b]	3.3
Neutral lipids	
Hydrocarbons (HC)[c]	3.3
Wax esters (WE)[c]	0.8
Steryl esters (SE)[c]	1.7
1-*O*-Alkyl-2,3-diacylglycerols (ADAG)[c]	1.3
Triacylglycerols (TG)[c]	92.7
Sterols (ST)[c]	0.2

[a]Expressed as percentage (w/w) of wet tissue.
[b]Expressed as percentage (w/w) of total lipid.
[c]Expressed as percentage (w/w) of neutral lipid.

Hydrocarbon composition. Hydrocarbons from the liver lipids of *D. bleekeri,* as obtained by separating the NL fraction by preparative TLC, is presented in Table 2. Among hydrocarbons, *n*-alkanes were the major components (67.3%), whereas the branched chain alkanes, *viz.* iso- and anteiso-components, were 12.8 and 19.9%, respectively.

Chain lengths of the components were from C_{19} to C_{36} for *n*-alkanes, whereas for iso- and anteiso- components the chain lengths were from pristane (C_{19}) to C_{36} and from C_{20} to C_{32}, respectively. Shorter-chain *n*-alkanes between C_{19} and C_{30} predominated, with C_{22} being the major component.

Among the branched-chain hydrocarbons, pristane was the major component, being 6.7% of the total hydrocarbons. Squalene, an unsaturated branched-chain hydrocarbon, was present at the level 3.5%. Occurrence of squalene was confirmed by comparison of GLC retention time with that of authentic squalene, as well as by catalytic reduction of the hydrocarbon band and rechromatography, which showed disappearance of the squalene peak and appearance of the squalane peak.

Although hydrocarbons are only minor components of most marine animals, squalene forms a large part of the liver lipids of many sharks living at the surface (28) or near the surface (29) and many deep-living sharks (29). The other hydrocarbon which occurs in significant amounts in cartilaginous fish is pristane (28), while phytane was undetectable (28). However, occurrence of pristane and phytane in marine organisms was conclusively established by Ackman (30).

Alcohol composition. Alcohol compositions are presented in Table 3. Chain lengths of alcohols were from C_{15} to C_{32}, including normal and iso-chain components. The *n*-alkanols were 97.6%, the rest being branched-chain alcohols. Of all the alco-

TABLE 2
Hydrocarbon Compositions in the Lipids of the Liver of *Dasyatis bleekeri*

Carbon number	Anteiso-chain[a]	Iso-chain[a]	Normal chain[a]
Pristane	—	6.7	—
19	—	0.4	5.0
20	1.2	—	5.6
21	0.8	1.1	5.8
22	1.1	1.1	6.3
23	1.1	0.4	5.6
24	1.1	0.4	5.6
25	1.1	0.6	5.2
26	1.3	0.6	5.3
27	1.3	0.6	5.3
28	1.4	0.8	4.8
Squalene	—	3.5	—
29	—	—	3.3
30	—	1.9	3.4
31	1.9	0.5	2.1
32	0.5	0.7	2.2
33	—	0.3	0.7
34	—	0.1	0.6
35	—	0.2	0.2
36	—	—	0.3
Total	12.8	19.9	67.3

[a]Expressed as percentage (w/w) of total hydrocarbons.

TABLE 3
Composition of Alcohols Obtained from Wax Esters of the Liver of *Dasyatis bleekeri*

Carbon number	Normal chain[a]	Iso-chain[a]
15	0.2	—
16	0.6	—
17	1.1	—
18	1.6	—
19	0.3	0.3
20	0.3	—
21	8.5	—
22	0.6	—
23	0.6	0.1
24	0.2	—
25	0.4	0.5
26	0.1	—
27	2.3	—
28	76.5	—
29	4.0	1.3
30	0.2	0.2
32	0.1	—
Total	97.6	2.4

[a]Expressed as % w/w of total alcohols obtained by hydrolysis of wax ester.

TABLE 4
Composition of Free and Esterified Sterols from the Liver of *Dasyatis bleekeri,* Analyzed as Acetates by Gas-Liquid Chromatography

	Percentage[a]	RRT
Sterols (free)		
Unidentified (8 components)	40.9	<0.87
cis-22-Dehydrocholesterol	15.7	0.87
Cholesterol	13.1	1.00
Ergosterol	4.3	1.22
Sitosterol	5.9	1.42
$\Delta^{7,25}$-Stigmastadienol	2.9	1.57
Unidentified	8.1	2.36
Unidentified	3.1	2.85
Unidentified	6.0	3.55
Sterols (esterified)		
Unidentified (8 components)	21.3	<1.00
Cholesterol	67.9	1.00
Ergosterol	1.1	1.22
Sitosterol	1.5	1.42
$\Delta^{5,7}$-Cholestadienol	0.8	1.09
Campesterol	1.0	1.34
4α-Methyl-$\Delta^{8(9),14}$-ergostadienol	5.4	1.50
4α-Methyl-$\Delta^{8(9),14}$-stigmastadienol	0.5	1.87
Citrostadienol	0.5	2.13

[a]Expressed as percentage (w/w) of total sterols obtained from free and esterified sterols of the sample. RRT, relative retention time.

hols, *n*-C_{28} was unusually high (76.5%). Of the other *n*-alkanols, mention can be made of C_{21} (8.5%) and C_{29} (4.0%).

WE have attracted considerable interest from marine chemists. WE are formed by esterification of long-chain fatty acids with long-chain alcohols. Their presence in fish was first reported by Nevenzel *et al.* (31). The possibility that WE in fish may not necessarily require a dietary source has led to extensive studies of their biosynthesis in fresh water fish (32), but studies on marine organisms have been limited because of culture problems. *In vivo* and *in vitro* synthesis of WE from acetate, long-chain alcohol or fatty acid precursors has been found to occur in mesopelagic fish, with the hepatopancreas and gut being more active for the *in vitro* synthesis (33), which suggests that these lipids are not merely persistent dietary survivors in the food web but may be, to some extent, specifically synthesized by midwater and deep-living fish to meet their own requirements. More recent work by Nevenzel and Menon (34) has certainly indicated that the direct source of WE present in some Gonastomatoids was unlikely to be merely dietary.

Sterol composition. Sterol composition of free and esterified compounds is presented in Table 4. Cholesterol, ergosterol, and sitosterol were the common constituents in the two samples, of which the level of cholesterol was exceptionally high (67.9%) in the esterified form. On the other hand, cholesterol was only 13.1%. Considerable amounts of sterols could not be identified following the method of Patterson (17). The stereochemistry of the sterols identified in the present study was not determined.

The subcellular distribution of sterols in marine organisms has not been extensively investigated, but in terrestrial organisms the amount of sterols in eukaryotic membranes varies from 2 to 25% of the total membrane lipid (35). The few detailed analyses which have been reported so far, while agreeing that cholesterol is by far the major sterol, suggest that this molecule best performs the structural function of sterols in membranes (35).

Alkyl chain composition of ADAG. Inspection of Table 5 reveals that chainlengths of ADAG were from C_{10} to C_{30} with

TABLE 5
Composition of Alkyl Chains Obtained from 1-*O*-Alkyl-2,3-diacylglycerols of the Liver of *Dasyatis bleekeri*

Carbon number	Unsaturated chain[a]	Anteiso-chain[a]	Iso-chain[a]	Normal chain[a]
10	—	—	—	0.6
11	—	0.2	0.4	0.1
12	—	—	0.3	—
13	—	—	—	0.1
14	—	—	—	0.9
15	—	—	1.2	1.3
16	—	—	1.0	23.4
17	—	0.9	0.7	3.2
18	—	—	0.1	44.4
19	—	0.1	0.2	0.8
20	—	0.2	2.2	5.1
21	3.5	—	0.2	0.2
22	—	0.1	1.4	1.4
23	—	1.2	0.2	0.7
24	—	—	0.2	0.6
25	—	0.2	0.8	0.3
26	—	0.3	0.2	0.1
27	—	0.1	0.4	0.1
28	—	—	—	0.1
29	—	—	—	0.1
30	—	—	0.1	0.1

[a]Expressed as percentage (w/w) of total alkyl chains obtained from 1-*O*-alkyl-2,3-diacylglycerol.

odd and even carbon chains, and contained saturated, iso-, anteiso-, and unsaturated moieties which were similar to the alkyl chains isolated from the cod fish muscle as reported by Ratnayake *et al.* (36). The major components found by the deacylation of ADAG were $C_{16:0}$ (chimyl alcohol) and $C_{18:0}$ (batyl alcohol), percentages being 23.4 and 44.4, respectively. Of the other components, $C_{17:0}$, $C_{20:i}$, $C_{20:0}$, and $C_{21:0}$ were present in appreciable quantities. Among the four classes, *n*-alkyl chains were the major components (83.6%), whereas the proportions of other three classes were between 3.5 and 9.6%. Lipids with long alkyl chains are widely distributed in human and animal tissue (18), as well as in some microorganisms, but they are not found in plants. Some naturally occurring and synthetic ether lipids have been shown to exhibit a variety of physiological activities (37). Several of these compounds are used in clinical diagnosis and medical research, as well as in therapy of cancer (38).

Fatty acid compositions of NL, GL, and PL. Fatty acid compositions of GL, PL, NL, and their different fractions of the liver of *D. bleekeri* are presented in Table 6. Unsaturated fatty acids predominated over the saturates in NL, TG, GL, and PL, whereas WE, SE, and ADAG were rich in saturates. The n-3 fatty acids were higher in PL, NL, TG, and GL, but WE and SE were rich in n-6 fatty acids, of which the proportions of 24:5n-6 in both latter fractions is noteworthy. Considerably high levels of n-3 polyunsaturated fatty acids, particularly 20:5n-3 and 22:6n-3, in the liver lipid fractions is characteristic of marine lipids. The biological and pharmacological roles of n-3 PUFA are of considerable interest, since it has been indicated that 20:5n-3 eicosapentaenoic acid (EPA) is very effective in the treatment and prevention of some cardiovascular diseases and disorders (39). EPA has a protective effect against thrombosis, atherosclerosis, and some inflammatory diseases (39,40). EPA reduces the concentration of cholesterol and TG in the plasma by lowering the rate of synthesis of low-density lipoprotein and very low density lipoprotein by the liver and vascular tissue (41). Similar studies with docosahexaenoic acid, 22:6n-3, indicate that it is effective in treatment of skin disorders, aids brain development, and also forms a good part of the retina (40).

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TABLE 6
Fatty Acid Composition of Neutral Lipids, Its Four Different Sub-classes, Glycolipids and Phospholipids of the Liver of *D. bleekeri*.

Fatty acid[a,b,c]	NL[b,d]	WE[b,d]	SE[b,d]	ADAG[b,d]	TG[b,d]	GL[b,d]	PL[b,d]
14:0	4.0	6.6	6.5	1.2	1.3	1.0	0.6
15:0	—	—	—	—	—	0.1	—
16:0	23.2	31.5	33.5	32.4	22.0	29.9	25.2
16:1 n-9	24.1	4.7	3.1	12.0	21.0	20.5	7.0
16:2 n-6	1.2	0.6	0.9	0.6	2.9	1.0	0.4
18:0	7.1	14.8	18.4	11.5	9.5	9.0	16.2
18:1 n-9	11.8	18.2	13.0	18.3	13.6	10.5	7.5
18:2 n-6	1.0	4.5	2.3	1.4	1.2	1.5	0.6
20:0	—	0.9	1.1	0.2	1.1	0.4	0.1
18:3 n-3	4.7	1.3	1.1	7.6	5.0	4.5	1.5
18:4 n-3	0.5	0.2	0.3	0.1	0.6	0.4	0.1
20:3 n-9	0.4	0.1	0.1	0.5	0.6	0.3	0.3
22:0	0.4	2.0	2.4	1.0	0.4	1.0	0.5
20:4 n-6	1.9	3.0	2.5	2.1	1.9	4.6	10.5
22:2 n-6	0.4	0.6	1.0	2.0	0.4	0.9	0.3
20:5 n-3	9.8	0.1	0.1	1.2	9.8	5.7	4.6
24:0	—	—	—	1.5	—	0.9	0.2
22:4 n-6	1.2	1.3	1.7	0.5	1.1	1.4	1.4
22:5 n-6	1.2	1.7	1.1	2.2	1.2	1.0	4.1
22:5 n-3	2.1	0.5	0.7	0.4	2.1	1.5	2.6
22:6 n-3	4.5	1.3	1.9	2.2	4.2	3.5	15.4
24:5 n-6	0.5	6.1	8.3	1.1	0.6	0.4	0.9
Saturates/unsaturates	0.53	1.26	1.62	1.1	0.52	0.73	0.74
n-3	21.6	3.4	4.1	11.5	21.7	15.6	24.2
n-6	7.4	17.8	17.8	9.9	9.3	10.8	18.2

[a]First and second figures represent carbon chainlength/number of double bonds. The n-values represent the number of carbon atoms by which terminal methyl is far from the closest olefinic carbon atom.
[b]Expressed as % w/w of total fatty acids present in each component.
[c]Components below 0.1% w/w of total fatty acids present were not reported in the table.
[d]NL, neutral lipids; WE, wax esters; SE, steryl esters; ADAG, 1-*O*-alkyl-diacylglycerols; TG, triacylglycerols; GL, glycolipids; PL, phospholipids.

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