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Author(s)	Tomita, Yuki; Ando, Yasuhiro
Citation	Fisheries Science, 75(2), 445-451 https://doi.org/10.1007/s12562-008-0048-y
Issue Date	2009-04
Doc URL	https://hdl.handle.net/2115/44612
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Type	journal article
File Information	FS08-4193.pdf



**Reinvestigation of positional distribution of tetracosahexaenoic acid in triacyl-*sn*-glycerols
of flathead flounder flesh**

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YUKI TOMITA AND YASUHIRO ANDO*

10 *Laboratory of Marine Bioresources Chemistry, Division of Marine Life Science,
Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan*

15 Running title: *Flathead flounder triacylglycerols*

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*Corresponding author: Tel: 81-138-40-8803. Fax: 81-138-40-8802.

Email: ando@fish.hokudai.ac.jp

ABSTRACT: Positional distribution of fatty acids in triacyl-*sn*-glycerols (TAG) of flathead flounder *Hippoglossoides dubius* has been reinvestigated in order to accurately determine the contents of tetracosahexaenoic acid (24:6n-3) in the *sn*-1, *sn*-2, and *sn*-3 positions. Flesh TAG obtained from three fish of the flounder were subjected to stereospecific analysis using a suitable
5 procedure for fish TAG analysis. The 24:6n-3 acid was found in the three positions at the concentrations of 0.3-5.5 mole% (the *sn*-1 position), 1.6-23.3 mole% (the *sn*-2 position), and 0.6-8.9 mole% (the *sn*-3 position). In contrast to previous analysis, the present analysis revealed that 24:6n-3 is preferentially esterified in the *sn*-2 position followed by the *sn*-3 and *sn*-1 positions. Other polyunsaturated fatty acids, docosahexaenoic acid (22:6n-3; DHA) and docosapentaenoic
10 acid (22:5n-3; DPA), showed a similar distribution pattern. These results indicate that general tendency observed for long-chain polyunsaturated fatty acids in marine fish TAG can be extended to the distribution of 24:6n-3 in flathead flounder TAG. Because the use of flathead flounder is entirely for human food, we intake 24:6n-3 concentrated in the *sn*-2 position of their TAG.

15 **KEY WORDS:** fatty acid, flathead flounder, *Hippoglossoides dubius*, stereospecific analysis, tetracosahexaenoic acid, triacylglycerol.

INTRODUCTION

Flathead flounder *Hippoglossoides dubius*, 'akagarei' in Japanese, is one of the most popular species of flatfish in Japan. About four thousands tons of this species are caught every year for human food. Lipids of flathead flounder are characterized by occurrence of 6,9,12,15,18,21-tetracosahexaenoic acid (24:6n-3) belonging to a family of very long-chain fatty acids.¹ This fatty acid is an intermediate for biosynthesis of docosahexaenoic acid (22:6n-3; DHA) from docosapentaenoic acid (22:5n-3; DPA),² and has anti-inflammatory and anti-allergic properties similar to those of 22:6n-3.³

In flathead flounder, 24:6n-3 was concentrated in triacyl-*sn*-glycerols (TAG) of edible flesh.⁴ This fact prompts us to research the positional distribution of this fatty acid in TAG, i.e., contents of this acid in the *sn*-1, *sn*-2, and *sn*-3 positions, because incorporation of fatty acids is influenced by the positions on glycerol backbone that the acids are esterified.⁵ The distributions of long-chain polyunsaturated fatty acids such as 22:6n-3, 22:5n-3, and eicosapentaenoic acid (20:5n-3; EPA) in fish TAG were detailed in many reports.⁶⁻⁸ Previously the authors analyzed flesh and liver TAG of flathead flounder, and reported that 24:6n-3 was located almost evenly among the three positions.⁴ However, the analytical procedure was later revealed to be unsuitable for the analysis of fish TAG.⁹ Therefore positional distribution of 24:6n-3 in fish TAG remains obscure.

In this study, we have reinvestigated the positional distribution of fatty acids in TAG of flathead flounder using a reliable analytical procedure. Because the previous samples were not available, new TAG samples prepared from edible flesh of flathead flounder were subjected to stereospecific analysis. The purpose of this study is to correctly characterize the distribution of 24:6n-3 in the flesh TAG.

MATERIALS AND METHODS

Materials

Three fish of flathead flounder were caught in the water of Funka Bay and off Kushiro, Hokkaido, Japan in April 2007. These were obtained at food market in Hakodate, and stored in a refrigerator for 18 h before starting the analysis. Biological properties of the flounder are shown in Table 1. For convenience, the three fish were labeled as flathead flounder (A), (B), and (C).

Table 1

Lipid extraction and lipid class analysis

Flesh with skin of edible part was separated from each fish, and 20 g of the minced cake was subjected to lipid extraction. Total lipids (TL) were extracted by the method of Bligh and Dyer.¹⁰ Lipid content was determined by gravimetry.

As fundamental information on the TL, lipid class composition was analyzed by high-performance thin-layer chromatography (HPTLC) and scanning-densitometry based on the method of Olsen and Henderson.¹¹ After a silica gel 60 HPTLC plate (10 × 10 cm plate with 2.5 × 10 cm concentrating-zone, 0.2 mm thickness; Merck, Darmstadt, Germany) was pre-developed in chloroform, the TL in chloroform (1 mg/mL; 4-5 μL) were applied to the plate. Lipid standards were also applied to the same plate for construction of calibration curves. The plate was developed by methyl acetate/2-propanol/chloroform/methanol/water (25:25:25:10:9 by volume) to a distance of 3.7 cm from bottom of the separating-zone. Following dryness *in vacuo* for 2 min, the plate was developed by hexane/diethyl ether/acetic acid (80:20:2 by volume) to 7.0 cm. Lipid components were detected by spraying the plate with 10% cupric sulfate in 8% phosphoric acid and by charring at 150°C for 15 min.¹² Quantification was performed by scanning-densitometry using a scanner GT-7600U (Seiko Epson Corporation, Suwa, Japan) and software Scion Image (Scion Corporation, Frederick, USA).

Fatty acid analysis of TAG

TAG were isolated from the TL by preparative thin-layer chromatography (TLC) on a silica gel G plate (10 × 10 cm, 0.25 mm thickness; Analtech, Newark, USA) with hexane/diethyl ether (80:20 by volume) for development, and then further purified by column chromatography on silica gel 60 (Merck) with hexane/diethyl ether (85:15 by volume) for elution.

Fatty acid methyl esters were prepared by reacting a portion of the TAG (1 mg) in a mixture of dry dichloromethane (0.1 mL), methyl acetate (20 µL) and 1M sodium methoxide-methanol solution (20 µL) at 40°C for 20 min.¹³ After adding 4 µL of acetic acid, the solvents were evaporated in a stream of nitrogen. Resulting methyl esters were taken up in hexane from the residue.

Gas-liquid chromatography (GLC) of the methyl esters was performed with Shimadzu GC-17A (Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector and a column Omegawax 250 (30 m × 0.25 mm i.d., 0.25 µm film thickness; Supelco, Bellefonte, USA). The column temperature was 200°C. Injector and detector temperatures were 250 and 260°C, respectively. Helium was the carrier gas (250 kPa) with a split ratio of 50:1. Peaks were monitored with Shimadzu C-R3A integrator. Component peaks were identified by comparison of retention data of Supelco 37 FAME Mix (Supelco, Bellefonte, USA) and flathead founder fatty acid previously identified in the laboratory.¹ Under the GLC conditions, relative retention times of long-chain polyunsaturated fatty acids were 3.493 (20:5n-3), 6.639 (22:5n-3), 7.262 (22:6n-3), and 13.534 (24:6n-3) based on the retention time of 18:0 (≡1.000).

Stereospecific analysis of TAG

A method for stereospecific analysis involving boric acid TLC and chiral high-performance liquid chromatography (HPLC) of monoacylglycerols (MAG) intermediates^{8,9,14} was used as

follows. The TAG (5 mg) mixed with trionadecanoylglycerol (19:0-TAG; 0.5 mg) as an internal standard were dissolved in 0.23 mL of dry diethyl ether, and freshly prepared ethyl magnesium bromide in dry diethyl ether (0.1 mL of 1M solution) was added to degrade the TAG to MAG.¹⁵ The mixture was shaken for 25 s, and then 2 mL of acetic acid/diethyl ether (1:200 by volume) followed by water (1 mL) was added to stop the reaction. The ether layer was washed once with 2% aqueous sodium bicarbonate and then with water. The ether was evaporated at ambient temperature in a stream of nitrogen. Residual water was also evaporated in the presence of toluene. Resulting 1(3)- and 2-MAG were isolated by preparative HPTLC on a boric acid-impregnated silica gel HPTLC plate (10 cm × 5 cm, 0.2 mm thickness, boric acid 10 wt% to silica gel 60) developed in chloroform/acetone (96:4 by volume).¹⁶

Three fourths of the 1(3)-MAG obtained were dissolved in dry toluene (0.1 mL) and reacted with 3,5-dinitrophenylisocyanate (5 mg; Sumika Chemical Analysis Service, Osaka, Japan) for 1 h at room temperature in the presence of dry pyridine (1 μL) in order to convert the 1(3)-MAG to their bis-3,5-dinitrophenyurethane derivatives.^{8,9,14} Resulting 1(3)-MAG derivatives were resolved into *sn*-1- and *sn*-3-MAG fractions by chiral HPLC with a Shimadzu LC-6A pump, a Jasco 875-UV ultraviolet spectrophotometric detector (Japan Spectroscopic, Tokyo, Japan) and a Shimadzu C-R6A integrator. A column of Sumichiral OA-4100 (25 cm × 4.6 mm i.d., 5 μm particles; Sumika Chemical Analysis Service, Osaka, Japan) was used with hexane/1,2-dichloromethane/ethanol (40:12:3 by volume) as mobile phase at a flow rate of 1.5 mL/min at -10°C. Detection was done at 254 nm.

The 1(3)-, 2-, *sn*-1- and *sn*-3-MAG intermediates were converted to fatty acid methyl esters and analyzed by GLC in a manner similar to the analysis of original TAG except for the followings. The column temperature was programmed from 90 to 200°C at 10°C/min. The samples were loaded onto the column for 2 min under splitless conditions.

Assignments of each fatty acid to the *sn*-1, *sn*-2, and *sn*-3 positions of TAG were obtained

from peak area ratio of each fatty acid to 19:0 formed from the 19:0-TAG internal standard. Fatty acid composition of each position was calculated on the basis of the assignments. Results were presented in the form of mean and standard deviation of triplicate analyses.

5 RESULTS

Lipid content and lipid class composition

Lipid contents and lipid class compositions of the flathead flounder flesh are shown in Table 2.

Table 2

Lipid contents were 6.5% (A), 3.3% (B), and 1.9% (C) on wet-weight base. Concentrations of TAG were 74.4% (A), 71.8% (B), and 59.8% (C) of the TL, and these corresponded to 4.8, 2.4, and 1.1% of the flesh on wet-weight base, respectively. TAG were the most abundant lipid class. Phospholipids (phosphatidylethanolamines + phosphatidylinositols + phosphatidylcholines) accounted for 15.9% (A), 19.4% (B), and 32.1% (C) of the TL. Free fatty acids were 4.7, 2.7, and 1.1%. MAG were not detected in all samples.

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Fatty acid composition of TAG

Fatty acid compositions of the TAG are shown in Table 3 together with the results of stereospecific analysis. The principal fatty acids found at more than 5 mole% in the TAG were

Table 3

14:0, 16:0, 16:1n-7+16:1n-9, 18:1n-9+18:1n-11, 18:1n-7, 20:1n-11+20:1n-13, 20:5n-3, 22:6n-3, and 24:6n-3. The proportions of 24:6n-3 were 13.1 mole% (A), 7.6 mole% (B), and 0.9 mole% (C), varying widely among the flounder samples. The proportions of 22:6n-3, 22:5n-3, and 20:5n-3 were 2.1-9.0, 1.3-2.5, and 9.3-15.7 mole%, respectively.

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Positional distribution of fatty acids in TAG

25 The positional distributions are shown in Table 3 in the form of fatty acid compositions of the *sn*-

1, *sn*-2, and *sn*-3 positions. The flounder (A) contained 24:6n-3 at the concentrations of 5.5, 23.3 and 8.9 mole% in the *sn*-1, *sn*-2, and *sn*-3 positions, respectively. This fatty acid was located in the *sn*-2 position in preference to the *sn*-3 and *sn*-1 positions. Other polyunsaturated acids, 22:6n-3 and 22:5n-3, were also preferentially located in the *sn*-2 position followed by the *sn*-3 and *sn*-1 positions, whereas 20:5n-3 showed preference for the *sn*-3 position followed by the *sn*-2 and *sn*-1 positions.

In the flounder (B), the contents of 24:6n-3 in the *sn*-1, *sn*-2 and *sn*-3 positions were 2.5, 14.6 and 5.2 mole%, respectively. This fatty acid was highest in the *sn*-2 position followed by the *sn*-3 and *sn*-1 positions. Other polyunsaturated fatty acids, 22:6n-3, 22:5n-3 and 20:5n-3, were also highest in the *sn*-2 position, although the last one was found in the *sn*-3 position at the level similar to that in the *sn*-2 position.

In the flounder (C), the contents of 24:6n-3 were 0.3, 1.6 and 0.6 mole% in the *sn*-1, *sn*-2 and *sn*-3 positions, respectively. Distribution patterns of 24:6n-3 and other polyunsaturated fatty acids, 22:6n-3, 22:5n-3 and 20:5n-3, were similar to those of the flounder (B). The three fish analyzed in this study preferentially contained 24:6n-3 in the *sn*-2 position followed by the *sn*-3 and *sn*-1 positions.

DISCUSSION

In this study, three fish of flathead flounder were subjected to the lipid analyses. The lipid class analysis showed that these fish were low in free fatty acids and MAG in their flesh TL (Table 2). It is probable that hydrolysis of TAG did not remarkably occur in the fish during transport and storage before the analysis.

The three fish samples varied in the 24:6n-3 concentrations (0.9-13.1 mole%; Table 3). Ota *et al.* reported wide variety of 24:6n-3 content in flatfish.¹ In their report, two samples collected

in Funka Bay contained 24:6n-3 at concentrations of 9.27 and 6.32% in flesh TL, whereas this fatty acid was not detected in the fish collected in the Sea of Japan. They assumed that 24:6n-3 is primarily incorporated into the flatfish through their diets.¹

Previously the authors reported positional distribution of 24:6n-3 in TAG of flathead flounder flesh and liver.⁴ The flesh TAG contained 24:6n-3 at the concentrations of 9.0, 7.8 and 7.1 mole% in the *sn*-1, *sn*-2 and *sn*-3 positions, respectively. The liver TAG had 1.5, 1.2 and 1.7 mole% in the *sn*-1, *sn*-2 and *sn*-3 positions, respectively. In both TAG, 24:6n-3 was distributed almost evenly among the three positions. This distribution pattern is different from that observed in the present study.

Later, the analytical procedure used in the previous analysis was pointed out to be unsuitable for the analysis of 22:6n-3-containing fish oil TAG.⁹ In the analysis, 1(3)- and 2-MAG intermediates were converted to bis-3,5-dinitrophenylurethane derivatives and then separated to 1(3)- and 2-MAG fractions by silicic acid TLC. However, the 1(3)- and 2-MAG derivatives made up of both short- and long-chain lengths cannot be correctly resolved by the TLC. The 1(3)- and 2-MAG must be resolved by boric acid TLC prior to derivatization.⁹ When tuna orbital oil TAG were analyzed by the unsuitable procedure, 22:6n-3 were found in the *sn*-1, *sn*-2, and *sn*-3 positions at concentrations of 24.7, 5.5, and 33.2 mole%, respectively.¹⁷ When the same TAG were analyzed by using boric acid TLC, 22:6n-3 was found at the concentrations of 3.9 (*sn*-1 position), 33.5 (*sn*-2 position), and 26.6 (*sn*-3 position) mole%.⁹ Difference in the two sets of data was attributed to unsuitable TLC separation of 1(3)- and 2-MAG derivatives in the former analysis. In the present study, boric acid TLC was used in the analysis of flathead flounder flesh TAG. The results showed that 24:6n-3 was highest in the *sn*-2 position followed by the *sn*-3 and *sn*-1 positions. It is probable that the previous data on the distribution of 24:6n-3 should be corrected, although the TAG samples were not the same between the present and previous investigations.

Brockerhoff *et al.* studied the positional distribution of fatty acids in marine fish TAG, and pointed out the general tendency of longer-chain polyunsaturated fatty acids to be preferentially esterified in the *sn*-2 position.⁶ Litchfield reported that the positional distribution of 22:6n-3 and 22:5n-3 in fish TAG can be predicted by the following proportionality equations:⁷ $y_1 = 0.28x$,
5 $y_2 = 2.06x$, and $y_3 = 0.66x$, where x shows the mole% of 22:6n-3 or 22:5n-3 in the total TAG ($0 < x < 30$); and y_1 , y_2 , and y_3 show the mole% of these fatty acids in the *sn*-1, *sn*-2, and *sn*-3 positions, respectively. These relationships indicate that about 10, 70, and 20% of 22:6n-3 and 22:5n-3 were esterified in the *sn*-1, *sn*-2, and *sn*-3 positions, respectively. In the report by Ando *et al.*, 50-85% of 22:6n-3 and 22:5n-3 were located in the *sn*-2 position.⁸ In farmed salmon oils analyzed by ¹³C-
10 NMR, 60-70% of 22:6n-3 was found in this position.¹⁸

The general tendency can be extended to include the case of 24:6n-3 in the flathead flounders. In this study, assignments of 24:6n-3 to the *sn*-1, *sn*-2, and *sn*-3 positions were calculated to be (A) 15, 62 and 24 %, respectively; (B) 11, 65 and 23 %, respectively; and (C) 12, 64 and 24 %, respectively. This result indicates that the general tendency and prediction rule can
15 be applied to 24:6n-3 of flathead flounder flesh TAG.

The general tendency also held for 22:6n-3 and 22:5n-3 in the flathead flounder. In this study, 71-75% of 22:6n-3 was found in the *sn*-2 position, followed by the *sn*-3 (16-22%) and *sn*-1 (9-10%) positions. Assignments of 22:5n-3 were 66-86 % (the *sn*-2 position), 12-22 % (the *sn*-3 position), and 2-12 % (the *sn*-1 position). In the previous analysis, opposite result were observed
20 for 22:6n-3 in the flesh and liver TAG.⁴ Assignment of 22:6n-3 to the *sn*-2 position was less than 40 %. This result seems to be caused by unsuitable analytical procedure in the previous study.

In contrast, 20:5n-3 was highest in the *sn*-3 position rather than the *sn*-2 position in the flounder (A) (Table 3). The flounder (B) and (C) also showed high concentrations of 20:5n-3 in both of the *sn*-2 and *sn*-3 positions. This fatty acid seems to vary in positional distribution among
25 fish samples. Lower assignments of 20:5n-3 to the *sn*-2 position were also observed for the other

fish TAG, e.g., tuna orbital oil (36%),⁹ bonito head oil (33-35%),⁹ and farmed salmon oil (25-35%).¹⁸

In conclusion, a very long-chain polyunsaturated fatty acid characteristic of the flounder, 24:6n-3, showed high preference for the *sn*-2 position of TAG followed by the *sn*-3 and *sn*-1 positions. Similar distribution pattern was also found for 22:6n-3 and 22:5n-3. These results do not differ from general tendency of long-chain polyunsaturated fatty acids in marine fish TAG. Flathead flounder is entirely used for human food, and therefore we intake 24:6n-3 in the form of TAG concentrating this fatty acid in the *sn*-2 position together with 22:6n-3.

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Table 1 Biological properties of flathead flounder labeled as A, B, and C

	Flathead flounder		
	(A)	(B)	(C)
Date	April 17, 2007	April 15, 2007	April 18, 2007
Origin	Off Kushiro	Funka Bay	Funka Bay
Sex	Male	Male	Male
Body length (cm)	27.6	29.6	24.8
Body weight (g)	348.7	369.1	218.4
Head (g)	58.6	55.3	50.1
Trunk and tail [†] (g)	263.8	280.6	151.2
Gonad (g)	8.7	8.2	1.6

[†]Without viscera.

Table 2 Lipid content and lipid class composition of the flathead flounder flesh

	Flathead flounder		
	(A)	(B)	(C)
Lipid content (% , wet-weight base)	6.5	3.3	1.9
Lipid class composition [†] (wt%)			
Sterol esters	1.1	1.1	1.7
Triacylglycerols (TAG)	74.4	71.8	59.8
Free fatty acids	4.7	2.7	1.1
Sterols	4.0	5.0	5.4
Monoacylglycerols (MAG)	0.0	0.0	0.0
Phosphatidylethanolamines	4.9	6.6	10.8
Phosphatidylinositols	1.7	2.4	3.3
Phosphatidylcholines	9.3	10.4	18.0

[†]Mean of duplicate analyses ($n=2$).

Table 3 Positional distribution of fatty acids in TAG of the flathead flounder flesh (mole%)

Fatty acid	Flathead flounder (A)				Flathead flounder (B)				Flathead flounder (C)			
	TAG	<i>sn</i> -1	<i>sn</i> -2	<i>sn</i> -3	TAG	<i>sn</i> -1	<i>sn</i> -2	<i>sn</i> -3	TAG	<i>sn</i> -1	<i>sn</i> -2	<i>sn</i> -3
14:0	7.7	7.1 ± 1.4	12.2 ± 1.9	3.6 ± 0.7	8.8	7.7 ± 0.2	12.5 ± 0.4	6.1 ± 0.3	5.7	6.5 ± 0.9	4.7 ± 0.3	5.9 ± 0.7
iso-15:0	0.8	0.9 ± 0.1	1.3 ± 0.1	0.3 ± 0.1	0.7	0.7 ± 0.1	1.1 ± 0.0	0.4 ± 0.1	0.3	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
anteiso-15:0	0.4	0.4 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.3	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
15:0	0.8	0.8 ± 0.0	1.0 ± 0.1	0.4 ± 0.1	0.6	0.6 ± 0.0	0.8 ± 0.0	0.5 ± 0.1	0.6	0.8 ± 0.1	0.5 ± 0.0	0.7 ± 0.0
iso-16:0	0.4	0.4 ± 0.1	0.5 ± 0.0	0.2 ± 0.0	0.4	0.4 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.2	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:0	10.0	13.4 ± 0.3	11.0 ± 0.4	5.9 ± 0.3	10.2	12.7 ± 0.3	10.9 ± 0.2	6.9 ± 0.6	15.0	21.4 ± 0.9	9.1 ± 0.5	14.2 ± 0.9
16:1n-7+16:1n-9	7.6	10.4 ± 0.2	5.0 ± 0.4	8.0 ± 0.4	10.8	12.6 ± 0.6	7.3 ± 0.3	12.7 ± 0.7	10.0	10.6 ± 0.7	6.0 ± 0.2	13.1 ± 0.3
16:1n-5	0.3	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3	0.3 ± 0.0	0.2 ± 0.0	0.5 ± 0.0
iso-17:0	0.7	1.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.5	0.8 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5	0.7 ± 0.0	0.3 ± 0.1	0.4 ± 0.0
anteiso-17:0+16:2n-6	0.4	0.8 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.5	0.6 ± 0.2	0.5 ± 0.0	0.4 ± 0.2	0.5	0.8 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
16:2n-4+phytanic	1.2	1.3 ± 0.1	0.8 ± 0.0	1.4 ± 0.0	1.6	1.2 ± 0.3	1.4 ± 0.4	2.2 ± 0.3	1.3	0.9 ± 0.2	1.1 ± 0.3	1.8 ± 0.0
17:0	0.4	0.7 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3	0.5 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4	0.6 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
16:3n-4+17:1	0.7	0.0 ± 0.0	1.8 ± 0.0	0.0 ± 0.0	0.9	0.3 ± 0.4	1.5 ± 0.1	0.8 ± 0.4	0.8	0.0 ± 0.0	2.5 ± 0.2	0.1 ± 0.1
iso-18:0	0.4	0.9 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.3	0.4 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2	0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
16:4n-1	0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.7	0.2 ± 0.1	1.0 ± 0.0	0.8 ± 0.1	0.4	0.1 ± 0.0	0.8 ± 0.0	0.4 ± 0.0
18:0	2.8	6.5 ± 0.2	0.6 ± 0.1	1.9 ± 0.0	2.7	5.9 ± 0.0	0.6 ± 0.0	1.8 ± 0.1	2.6	5.4 ± 0.3	0.4 ± 0.0	1.8 ± 0.0
18:1n-13	1.3	2.5 ± 0.1	0.3 ± 0.0	1.2 ± 0.0	0.7	1.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.2	0.3 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
18:1n-9+18:1n-11	10.1	10.9 ± 0.2	5.4 ± 0.2	14.3 ± 0.2	12.7	15.7 ± 0.4	7.7 ± 0.3	15.0 ± 0.4	15.0	15.7 ± 0.3	15.2 ± 0.4	14.2 ± 0.5
18:1n-7	4.2	4.9 ± 0.1	1.8 ± 0.1	6.1 ± 0.3	4.6	5.7 ± 0.1	2.1 ± 0.0	6.0 ± 0.1	7.1	8.9 ± 0.2	4.0 ± 0.1	8.4 ± 0.2
18:1n-5	0.4	0.5 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.3	0.4 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.5	0.6 ± 0.1	0.3 ± 0.1	0.6 ± 0.1
18:2n-6	0.4	0.4 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.5	0.6 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	1.1	1.1 ± 0.0	0.8 ± 0.0	1.3 ± 0.0
18:2n-4	0.2	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
18:3n-3	0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.7	0.6 ± 0.0	0.7 ± 0.0	0.9 ± 0.1
18:4n-3	0.4	0.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.9	0.4 ± 0.0	1.2 ± 0.0	0.9 ± 0.0	1.3	0.5 ± 0.0	2.3 ± 0.0	1.1 ± 0.0
20:1n-11+20:1n-13 [†]	8.7	13.1 ± 0.2	3.3 ± 0.3	10.5 ± 0.1	10.9	13.6 ± 0.3	6.5 ± 0.1	12.8 ± 0.5	4.1	5.5 ± 0.5	3.2 ± 0.0	3.7 ± 0.3
20:1n-7	1.6	2.0 ± 0.0	0.6 ± 0.1	2.2 ± 0.0	1.7	2.0 ± 0.1	0.8 ± 0.0	2.4 ± 0.1	1.3	1.9 ± 0.2	0.6 ± 0.0	1.4 ± 0.1
20:1n-5	0.3	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.5	0.5 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:4n-6	1.5	1.0 ± 0.0	1.2 ± 0.0	2.3 ± 0.0	1.7	1.2 ± 0.4	1.6 ± 0.1	2.2 ± 0.5	1.5	1.0 ± 0.1	1.9 ± 0.0	1.6 ± 0.1
20:4n-3	0.2	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.5	0.3 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
20:5n-3	15.7	6.8 ± 0.3	15.3 ± 0.7	24.0 ± 0.5	9.3	3.5 ± 0.2	13.1 ± 0.1	11.2 ± 0.4	11.6	6.0 ± 0.4	14.7 ± 0.1	14.0 ± 0.9
22:1n-11+22:1n-13	1.2	2.3 ± 0.1	0.4 ± 0.1	0.9 ± 0.0	2.4	3.2 ± 0.3	1.5 ± 0.0	2.5 ± 0.2	1.1	1.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.0
22:1n-9	1.0	1.6 ± 0.0	0.5 ± 0.0	1.0 ± 0.0	0.9	1.1 ± 0.1	0.5 ± 0.0	1.0 ± 0.1	0.4	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
21:5n-3	0.3	0.0 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3	0.1 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.4	0.2 ± 0.0	0.7 ± 0.0	0.4 ± 0.0
22:5n-3	1.3	0.1 ± 0.1	3.1 ± 0.1	0.5 ± 0.2	1.5	0.1 ± 0.1	3.6 ± 0.1	0.5 ± 0.0	2.5	0.9 ± 0.1	5.0 ± 0.2	1.7 ± 0.1
22:6n-3	2.9	0.8 ± 0.0	6.0 ± 0.1	1.4 ± 0.2	2.1	0.6 ± 0.1	4.7 ± 0.2	1.0 ± 0.1	9.0	2.4 ± 0.4	19.1 ± 0.7	6.0 ± 0.2
24:1n-9	0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3	0.5 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.4	0.5 ± 0.1	0.2 ± 0.1	0.3 ± 0.0
24:6n-3	13.1	5.5 ± 0.6	23.3 ± 1.2	8.9 ± 0.9	7.6	2.5 ± 0.5	14.6 ± 0.8	5.2 ± 0.4	0.9	0.3 ± 0.1	1.6 ± 0.2	0.6 ± 0.1
Others (<0.2%)	0.9	1.4	0.5	0.7	1.1	1.3	0.6	1.4	1.4	1.3	1.2	1.5

Values for the distribution are mean ± standard deviation of the triplicated stereospecific analyses.

[†]Including 20:1n-9.