

NOTE

Regiospecific Distribution of Highly Unsaturated Fatty Acids in Triacylglycerols of *Artemia* Nauplii Enriched with Marine Oils

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Abstract: Brine shrimp *Artemia* nauplii, an important live food used in aquaculture, were enriched with five marine oil triacylglycerols (TAG) in order to enhance n-3 highly unsaturated fatty acids (HUFA) essential for fish larvae. Positional distribution of fatty acids in TAG was determined for both of the enriched *Artemia* nauplii and dietary marine oils. In all of the enriched *Artemia* nauplii, docosahexaenoic acid was preferentially located in the *sn*-1,3 position followed by the *sn*-2 position. Icosapentaenoic acid was preferentially located in the *sn*-2 position. Distribution patterns of these fatty acids were not similar to those in dietary TAG of fish oil origin. Positional distribution of HUFA characteristic of marine fish TAG does not appear to hold for *Artemia* TAG during the HUFA enrichment.

Key words: *Artemia*, highly unsaturated fatty acid, positional distribution, regiospecific analysis, triacylglycerol

1 Introduction

Brine shrimp *Artemia* nauplii are used in aquaculture as an important live food for marine fish larvae. However, they are poor in essential n-3 highly unsaturated fatty acids (HUFA) and docosahexaenoic acid (DHA) in particular. In order to ensure successful production of fish larvae, the nauplii are reared on HUFA-containing oils for 18-24 h prior to being fed to fish larvae (1,2). This process is called HUFA enrichment of *Artemia* nauplii. Marine oils, such as ordinary cod liver oil and DHA-rich tuna orbital oil, have been used for enrichment as well as ethyl ester-type products.

During the enrichment of *Artemia* nauplii, n-3 HUFA mainly increase in triacylglycerols (TAG) of the nauplii (3-5). Recently it was revealed that DHA fed to *Artemia*

nauplii was preferentially esterified in the *sn*-3 position of TAG followed in sequence by the *sn*-1 and *sn*-2 positions (6). This result indicates that DHA was incorporated into the primary position [*sn*-1,3 position] of TAG rather than the secondary position [*sn*-2 position]. However, this positional distribution was obtained by enrichment with highly pure DHA ethyl ester (>99%) not used for practical enrichment.

In the present study, *Artemia* nauplii were enriched with TAG of five marine oils usable for practical enrichment. Differing from ethyl esters, n-3 HUFA in these TAG are esterified to the glycerol backbone with specific distribution patterns. The aim of the present work is to reveal whether positional distribution of n-3 HUFA in the *Artemia* TAG is different from those in the dietary marine oil TAG. For this purpose, both of the

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Artemia TAG and marine oil TAG were subjected to regiospecific analysis.

2 Experimental

2.1 Marine Oil TAG Used for *Artemia* Enrichment

TAG of fish oils [bonito head oil, tuna orbital oil, sardine oil, and HUFA-concentrated fish oil (Larodan Fine Chemicals, Malmö, Sweden)] and marine mammal oil (seal oil) were used for enrichment of *Artemia* nauplii. The TAG were isolated from the oils by column chromatography on Silicagel 60 (Merck, Darmstadt, Germany) with hexane/ethyl ether for elution.

2.2 Enrichment of *Artemia* Nauplii

The marine oil TAG were given to *Artemia* nauplii in the form of gelatin-acacia microcapsules (7). The microcapsules were prepared by the method described by Kondo (8) as follows. TAG (0.9 g) were added to a mixture of 10% (wt/wt) solution of gelatin (3 mL; Kanto Chemical Co. Inc., Tokyo), 10% (wt/wt) solution of acacia (3 mL; Wako Pure Chemical Industries, Ltd., Osaka) and 10% (wt/wt) solution of acetic acid (0.5 mL) maintained at 43°C. After the mixture was homogenized for 1 min, distilled water (14 mL) was added with homogenizing at 43°C and then the homogenate was immediately cooled down to 10°C in a ice-water bath. Resulting microcapsules were hardened in a refrigerator overnight in the presence of formalin (0.3 mL) at about pH 9 adjusted by adding 10% (wt/wt) sodium hydroxide. The microcapsules were neutralized by repeated centrifugation at 3,200 rpm for 5 min and resuspension in cold water, and then used in suspension for *Artemia* enrichment.

Enrichment was carried out with 24-h-old *Artemia* nauplii (total length, about 1 mm) at a density of 150 nauplii/mL in 10 L tanks containing 9 L of well-aerated 2% (wt/wt) salinity artificial seawater at 20°C (6). After the TAG-containing microcapsule suspensions were added to the tanks, enriched *Artemia* nauplii samples were taken on a nylon mesh at 18 h, washed in distilled water, and stored at -35°C for lipid analysis. An initial sample was also taken just before start of the enrichment.

2.3 Fatty Acid Analysis of TAG

Total lipids (TL) were extracted from *Artemia* nauplii

by the method of Bligh and Dyer (9). TAG were separated from other lipids by column chromatography on Silicagel 60 with hexane/ethyl ether for elution, followed by preparative thin-layer chromatography (TLC) on Silicagel 60G plates (Merck) with hexane/ethyl acetate (90 : 10, v/v) for development. Fatty acid methyl esters were prepared by reacting a portion of TAG in a mixture of dry dichloromethane (0.6 mL), methyl acetate (25 µL) and 1 M sodium methoxide/methanol (25 µL) under nitrogen at room temperature overnight. After adding acetic acid (9 µL) and removing solvents, the products were taken up in hexane. Fatty acid methyl esters were analyzed by gas-liquid chromatography (GLC) on a Shimadzu GC-14A gas chromatograph (Shimadzu Co., Kyoto) equipped with a capillary column Omegawax 320 (30 m × 0.32 mm I.D., 0.25 mm film thickness; Supelco Inc., Bellefonte, USA) and a flame ionization detector. Column temperature was 200°C, and injector and detector temperatures were 250 and 260°C, respectively. Helium was the carrier gas.

2.4 Regiospecific Analysis of TAG

Positional distribution of fatty acids between the *sn*-1,3 and *sn*-2 positions of TAG was determined by regiospecific analysis of TAG. A part of the method for stereospecific analysis of fish oil TAG (10-12) was used as follows. TAG (40 mg), mixed with tridecanoylglycerol (5 mg) and tridodecanoylglycerol (40 mg), were dissolved in 3 mL of dry ethyl ether, and ethyl magnesium bromide in dry ethyl ether (0.33 mL of 3 M solution) was added. The mixture was shaken for 1 min, and then glacial acetic acid (0.1 mL) and water (3 mL) were added to stop the reaction. The products were extracted with ethyl ether, washed several times with 2% (wt/wt) aqueous sodium bicarbonate followed by water, and dried over anhydrous sodium sulfate. After removal of the solvent in a stream of nitrogen at room temperature, 1(3)- and 2-monoacylglycerols (MAG) were immediately isolated by preparative TLC on boric acid-impregnated silica gel plates [20 × 10 cm, 0.5 mm thickness, boric acid 10% (wt/wt) to Silicagel 60G] developed twice in chloroform/methanol (98:2, v/v) containing 0.002% of butylhydroxytoluene. Ether extracts of the 1(3)- and 2-MAG were washed with water and dried over anhydrous sodium sulfate. Fatty acid methyl esters were prepared from each of the 1(3)- and 2-MAG fractions and analyzed by GLC under

the conditions described above. Assignments of each fatty acid to the *sn*-1,3 and *sn*-2 positions of TAG were obtained from the peak area ratio of each fatty acid to 19:0, formed from the trionadecanoylglycerol internal standard, and percentages of each fatty acid in the *sn*-1,3 and *sn*-2 positions were calculated on the basis of the assignments.

3 Results and Discussion

3.1 Positional Distribution of n-3 HUFA in Marine Oil TAG Used for *Artemia* Enrichment

Positional distributions of fatty acids in the marine oil TAG are shown in **Table 1** together with fatty acid compositions of the intact TAG. The positional distributions are expressed as fatty acid compositions of the *sn*-1,3 and *sn*-2 positions of TAG.

DHA contents in the marine oil TAG were 7.2-30.1 mole% of total fatty acids. Concentrations of this acid

Table 1 Positional Distribution of Fatty Acids in Marine Oil Triacylglycerols (TAG) Used for Enrichment of *Artemia Nauplii* (Mole%).

Fatty acid	Bonito head oil			Tuna orbital oil			Sardine oil			HUFA-conc. fish oil			Seal oil		
	TAG	<i>sn</i> -1,3	<i>sn</i> -2	TAG	<i>sn</i> -1,3	<i>sn</i> -2	TAG	<i>sn</i> -1,3	<i>sn</i> -2	TAG	<i>sn</i> -1,3	<i>sn</i> -2	TAG	<i>sn</i> -1,3	<i>sn</i> -2
14:0	3.2	2.7	4.1	4.0	3.2	5.6	8.6	7.8	10.0	1.0	0.8	1.4	5.9	2.9	11.1
14:1n-5	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.0	0.2	—	—	—	1.2	0.5	2.6
15:0	0.8	0.7	0.8	1.1	0.9	1.3	0.7	0.5	1.1	0.1	0.0	0.2	0.3	0.2	0.5
16:0	12.5	14.0	9.5	21.1	23.3	16.9	20.5	22.5	17.0	2.6	2.2	3.4	8.1	7.0	10.0
16:1n-7	7.4	8.2	5.8	6.4	6.7	5.8	6.5	6.3	6.8	5.4	4.9	6.3	21.6	14.2	34.9
16:2n-4+phytanic	1.9	1.4	2.9	1.9	1.4	3.0	1.4	1.0	2.0	1.7	1.5	2.2	1.1	0.9	1.4
17:0	0.5	0.7	0.2	1.0	1.4	0.5	0.6	0.6	0.6	0.1	0.1	0.1	0.1	0.1	0.1
16:3n-4	1.1	1.3	0.6	0.9	1.0	0.6	0.7	0.6	0.9	—	—	—	—	—	—
16:4n-1	—	—	—	—	—	—	0.7	0.5	1.2	—	—	—	—	—	—
18:0	1.8	2.3	0.9	4.5	6.1	1.5	2.7	3.4	1.5	0.6	0.5	0.9	0.9	1.0	0.8
18:1n-9 ^{a)}	15.9	20.8	6.5	14.8	19.1	6.8	10.0	12.5	5.4	7.6	7.4	8.0	18.3	17.3	20.2
18:1n-7	3.0	4.0	1.1	2.7	3.5	1.4	2.8	3.6	1.3	1.7	1.7	1.7	5.8	6.6	4.3
18:1n-5	0.2	0.3	—	0.2	0.3	0.1	0.5	0.5	0.3	—	—	—	0.6	0.6	0.6
18:2n-6	2.5	2.6	2.2	2.4	2.4	2.3	1.7	1.9	1.3	1.2	1.1	1.4	1.6	0.8	3.0
18:3n-3	0.7	0.8	0.5	0.6	0.6	0.5	1.2	1.2	1.2	0.7	0.7	0.7	0.4	0.3	0.7
18:4n-3	1.2	1.0	1.6	1.0	0.8	1.5	3.5	3.5	3.5	4.5	4.4	4.6	1.0	1.3	0.4
20:1n-11+20:1n-13	0.4	0.6	0.2	0.4	0.6	0.2	3.3	4.2	1.7	—	—	—	1.3	—	3.5
20:1n-9	1.0	1.4	0.3	1.0	1.3	0.3	1.8	2.0	1.3	0.4	0.4	0.5	7.3	10.8	1.2
20:1n-7	0.1	0.2	—	0.2	0.2	—	0.2	0.2	0.2	—	—	—	0.6	0.9	—
20:4n-6	2.2	1.9	2.6	1.7	1.4	2.2	0.7	0.6	0.8	1.9	1.9	1.9	0.4	0.6	—
20:4n-3	0.6	0.9	—	0.4	0.6	0.2	1.1	1.4	0.7	1.4	1.5	1.4	0.4	0.6	—
20:5n-3	8.3	8.3	8.4	6.2	6.0	6.5	11.2	11.8	10.1	41.2	42.1	39.2	6.7	10.0	0.9
22:1n-11+22:1n-13	0.5	0.7	—	0.5	0.6	0.2	3.5	4.7	1.3	0.8	0.8	0.7	2.5	3.8	0.3
22:1n-9	—	—	—	0.1	0.2	—	0.5	0.5	0.3	—	—	—	0.5	0.8	—
21:5n-3	0.3	0.4	—	0.2	—	0.6	0.4	0.4	0.5	1.6	1.6	1.5	0.3	0.5	—
22:5n-3	1.5	1.4	1.8	1.1	1.0	1.4	1.6	1.0	2.8	4.2	4.4	3.9	4.3	6.5	0.5
22:6n-3	30.1	20.9	48.1	22.7	15.3	36.5	11.1	5.0	22.2	21.0	21.6	19.5	7.2	10.7	1.0
24:1n-9	—	—	—	—	—	—	0.8	0.8	0.9	—	—	—	—	—	—
Others ^{b)}	2.3	2.6	1.7	2.9	2.3	4.0	2.0	1.2	3.2	0.4	0.4	0.5	1.7	1.3	2.4

a) Including significant amount of 18:1n-11 in the seal oil TAG.

b) Less than 0.5 mole% of total fatty acids.

in the *sn*-1,3 and *sn*-2 positions were 5.0-21.6 and 1.0-48.1 mole%, respectively. In the bonito head oil TAG, DHA contents in the *sn*-1,3 and *sn*-2 positions were 20.9 and 48.1 mole%, respectively. Proportion of DHA in the *sn*-2 position was much higher than that in the *sn*-1,3 position. Similar distribution pattern was found for the tuna orbital oil TAG (15.3 vs. 36.5 mole%) and sardine oil TAG (5.0 vs. 22.2 mole%). In the HUFA-con-

centrated fish oil TAG, proportions of DHA were almost even between the *sn*-1,3 position (21.6 mole%) and the *sn*-2 position (19.5 mole%). In the seal oil TAG, DHA was found in the *sn*-1,3 position (10.7 mole%) at higher concentration than in the *sn*-2 position (1.0 mole%). Distribution pattern of DHA in the seal oil TAG was opposite to those in the bonito head, tuna orbital, and sardine oil TAG.

Table 2 Positional Distribution of Fatty Acids in Triacylglycerols (TAG) of *Artemia* Nauplii Enriched with Marine Oil TAG (Mole%).

Fatty acid	Initial ^{a)}			Bonito head oil ^{b)}			Tuna orbital oil ^{b)}			Sardine oil ^{b)}			HUFA-conc. fish oil ^{b)}			Seal oil ^{b)}		
	TAG	<i>sn</i> -1,3	<i>sn</i> -2	TAG	<i>sn</i> -1,3	<i>sn</i> -2	TAG	<i>sn</i> -1,3	<i>sn</i> -2	TAG	<i>sn</i> -1,3	<i>sn</i> -2	TAG	<i>sn</i> -1,3	<i>sn</i> -2	TAG	<i>sn</i> -1,3	<i>sn</i> -2
TL (wt%) ^{c)}	13.7			17.1			27.3			16.4			20.3			15.3		
TAG (wt%) ^{c)}	5.0			6.1			6.6			4.2			4.7			5.1		
14:0	1.2	0.7	2.0	1.0	1.2	0.7	1.3	1.3	1.1	1.9	2.1	1.6	0.7	0.7	0.7	1.7	1.8	1.6
iso-15:0	1.7	2.4	0.5	1.1	1.5	0.2	1.2	1.8	0.4	1.6	2.1	0.6	0.9	1.2	0.3	1.3	1.7	0.5
anteiso-15:0	1.0	1.4	0.3	0.6	1.0	0.0	0.7	1.1	0.0	0.9	1.4	0.0	0.5	0.8	0.0	0.7	1.1	0.0
iso-16:0	1.0	1.3	0.4	0.7	0.9	0.5	0.8	1.0	0.4	1.0	1.2	0.6	0.6	0.7	0.4	0.8	0.8	0.8
16:0	13.0	18.2	4.5	10.8	13.3	6.1	11.8	14.2	7.6	13.2	16.8	7.0	8.5	10.4	5.0	10.7	13.0	6.6
16:1n-7	3.2	3.5	2.6	3.7	3.7	3.6	3.7	3.4	4.1	4.2	4.3	4.1	3.2	3.2	3.3	9.8	9.8	9.7
iso-17:0	0.8	1.3	0.0	0.7	1.0	0.2	0.7	0.9	0.2	0.9	1.2	0.3	0.6	0.8	0.4	0.8	0.9	0.5
anteiso-17:0+16:2n-6	1.9	2.7	0.5	1.4	0.9	0.5	1.4	2.0	0.6	1.7	2.4	0.6	1.3	1.7	0.7	1.5	2.0	0.7
16:2n-4+phytanic	—	—	—	1.2	1.1	1.2	1.2	1.2	1.4	0.8	0.6	1.1	0.8	0.9	0.5	0.6	0.6	0.6
17:0	0.7	0.9	0.2	0.6	0.7	0.3	0.6	0.7	0.3	0.6	0.8	0.2	0.4	0.5	0.2	0.5	0.6	0.3
16:3n-4	0.9	1.2	0.5	0.9	1.1	0.6	0.9	1.0	0.7	0.9	1.1	0.5	0.8	0.9	0.5	0.8	0.9	0.6
16:3n-3+17:1	1.0	1.4	0.3	0.6	0.8	0.2	0.6	0.7	0.5	0.7	0.9	0.2	0.6	0.8	0.2	0.6	0.8	0.2
16:4n-3	0.5	0.8	0.1	0.3	0.5	0.0	0.4	0.6	0.0	0.3	0.5	0.0	0.5	0.6	0.1	0.3	0.5	0.0
18:0	3.3	4.8	0.8	3.1	4.3	1.0	3.1	4.4	1.0	3.2	4.4	1.0	2.7	3.8	0.8	2.6	3.6	0.9
18:1n-9	18.3	26.1	5.4	19.7	26.7	7.1	19.6	26.6	7.6	18.9	25.8	6.8	14.8	20.5	4.6	21.4	28.1	9.4
18:1n-7	4.3	5.9	1.7	4.6	6.0	2.0	4.2	5.6	1.9	4.7	6.3	1.9	3.9	5.1	1.7	5.5	7.2	2.4
18:2n-6	5.3	4.5	6.6	4.3	3.3	6.0	4.4	3.4	6.1	4.5	3.4	6.3	3.6	2.9	4.7	4.0	2.9	6.0
18:3n-3	34.4	14.3	67.8	24.8	9.0	53.4	24.3	8.5	51.0	25.6	9.5	53.7	23.0	9.2	47.8	21.7	7.7	46.9
18:4n-3	3.2	3.7	2.3	2.1	2.2	1.9	2.2	2.3	2.0	2.3	2.4	2.0	2.7	2.9	2.3	1.8	1.9	1.7
20:1n-11+20:1n-13	—	—	—	0.2	0.3	0.0	—	—	—	0.9	1.1	0.5	0.1	0.1	0.1	0.5	0.6	0.3
20:1n-9	0.5	0.4	0.6	0.7	1.2	0.0	0.9	1.4	0.0	0.8	1.3	0.0	0.5	0.8	0.1	2.4	3.5	0.4
20:4n-6	0.4	0.5	0.3	1.4	1.5	1.2	1.2	1.4	0.9	0.6	0.7	0.5	1.3	1.6	0.9	0.5	0.5	0.4
20:3n-3	0.7	1.0	0.2	0.6	0.7	0.6	0.6	0.5	0.7	0.5	0.2	1.0	0.6	0.7	0.4	0.4	0.3	0.5
20:4n-3	0.6	0.8	0.4	0.7	0.7	0.7	0.6	0.7	0.5	0.7	0.7	0.7	1.0	1.1	0.8	0.5	0.5	0.5
20:5n-3	1.2	0.9	1.7	5.1	4.2	6.8	4.2	3.4	5.4	4.0	3.0	5.7	16.6	15.3	19.0	3.4	2.6	5.0
22:1n-11+22:1n-13	—	—	—	—	—	—	—	—	—	0.7	1.0	0.1	0.3	0.4	0.2	0.6	0.9	0.0
22:5n-3	—	—	—	0.7	1.0	0.0	0.6	0.9	0.0	0.5	0.5	0.4	1.8	2.1	1.1	1.4	1.6	1.2
22:6n-3	—	—	—	7.5	8.9	4.9	7.6	9.2	4.8	1.9	2.1	1.5	7.0	9.3	2.9	1.5	1.9	0.8
Others ^{d)}	1.1	1.5	0.5	1.0	2.3	0.4	1.5	1.9	0.8	1.9	2.4	1.1	0.9	1.1	0.6	1.9	2.0	1.7

a) *Artemia* nauplii recovered just before start of enrichment.

b) *Artemia* nauplii enriched with TAG of each marine oil for 18 h.

c) Total lipids (TL) and TAG contents in the *Artemia* nauplii determined by gravimetry (dry-weight base).

d) Less than 0.5 mole% of total fatty acids.

Icosapentaenoic acid (IPA) contents of the marine oil TAG were 6.2-41.2 mole% of total fatty acids. Highest content of this acid was observed for the HUFA-concentrated fish oil TAG. IPA was found at almost similar concentrations in the *sn*-1,3 and *sn*-2 positions of TAG originated from fish oils, i.e., 8.3 vs. 8.4 mole% (bonito head oil); 6.0 vs. 6.5 mole% (tuna orbital oil); 11.8 vs. 10.1 mole% (sardine oil); and 42.1 vs. 39.2 mole% (HUFA-concentrated fish oil). In the seal oil TAG, IPA was concentrated in the *sn*-1,3 position (10.0 mole%) at higher level than in the *sn*-2 position (0.9 mole%).

3·2 Positional Distribution of n-3 HUFA in TAG of the Enriched *Artemia* Nauplii

Positional distributions of fatty acids in TAG of the initial and enriched *Artemia* nauplii are shown in **Table 2**. TAG contents in the enriched *Artemia* nauplii (4.2-6.6%, dry-weight base) were close to that in the initial (5.0%). However, these values were higher than that observed for 18-h starved nauplii without enrichment (TAG, 1.9% and TL, 10.7%; data not shown in **Table 2**). In the initial *Artemia*, DHA was not detected in TAG. IPA was found at the level of 1.2 mole% of total fatty acids. After enrichment with the marine oil TAG, DHA and IPA contents in *Artemia* TAG increased to 1.5-7.6 and 3.4-16.6 mole%, respectively.

In the *Artemia* nauplii enriched with bonito head oil TAG, DHA was found at concentrations of 8.9 and 4.9 mole% in the *sn*-1,3 and *sn*-2 positions, respectively. DHA was found in the *sn*-1,3 position at higher concentration than in the *sn*-2 position. Similar distribution pattern was observed for *Artemia* nauplii enriched with other marine oils: 9.2 vs. 4.8 mole% (tuna orbital oil); 2.1 vs. 1.5 mole% (sardine oil); 9.3 vs. 2.9 mole% (HUFA-concentrated fish oil); and 1.9 vs. 0.8 mole% (seal oil). With the sardine oil and seal oil TAG enrichments, DHA contents in the *Artemia* nauplii were not high. However, distribution pattern of DHA was similar to those observed for the enrichments with bonito head oil, tuna orbital oil, and HUFA-concentrated fish oil TAG. All of the enriched *Artemia* nauplii showed higher concentration of DHA in the *sn*-1,3 position than in the *sn*-2 position.

IPA contents in the *sn*-1,3 and *sn*-2 positions were 4.2 and 6.8 mole% (bonito head oil), 3.4 and 5.4 mole% (tuna orbital oil), 3.0 and 5.7 mole% (sardine oil), 15.3 and 19.0 mole% (HUFA-concentrated fish oil), and 2.6 and 5.0 mole% (seal oil), respectively. In all of

the enriched *Artemia* nauplii, IPA was found in the *sn*-2 position at higher concentration than in the *sn*-1,3 position.

3·3 Comparison of the Distribution Patterns of n-3 HUFA between the Marine Oils and Enriched *Artemia* Nauplii

In the present study, positional distributions of fatty acids were determined for both TAG of the marine oils and *Artemia* nauplii. There has been no previous report on the positional distribution of fatty acids in TAG of *Artemia* nauplii enriched with marine oil TAG. From the data of **Tables 1** and **2**, distribution patterns of DHA and IPA are summarized as follows: for DHA,

sn -1,3 < sn -2 (bonito head, tuna orbital and sardine oils),

sn -1,3 \approx sn -2 (HUFA-concentrated fish oil), and

sn -1,3 > sn -2 (seal oil and all of the enriched *Artemia* nauplii);

and for IPA,

sn -1,3 > sn -2 (seal oil),

sn -1,3 \approx sn -2 (bonito head, tuna orbital, sardine and HUFA-concentrated fish oils), and

sn -1,3 < sn -2 (all of the enriched *Artemia* nauplii)

Distribution pattern of DHA in the *Artemia* nauplii TAG was not similar to those of TAG originated from fish oils (including HUFA-concentrated one). Compared with the fish oils TAG, *Artemia* nauplii TAG showed higher preference of DHA for the *sn*-1,3 position. This distribution pattern resembled that of the seal oil TAG. Distribution pattern of IPA in the *Artemia* nauplii was not similar to those in all of the marine oils used for the enrichment. IPA showed preference for the *sn*-2 position in the *Artemia* nauplii.

Marine oils of fish origin, such as cod liver oil and tuna orbital oil, are used for practical HUFA enrichment of *Artemia* nauplii (1,2). TAG of such fish oils generally contain DHA at the highest concentration in the *sn*-2 position (11-14). Bonito head oil, tuna orbital oil, and sardine oil TAG used in the present study were also no exception to this tendency. However, high preference of DHA in the *sn*-2 position was not entirely reflected to TAG of the enriched *Artemia* nauplii. When *Artemia* nauplii were enriched with DHA ethyl ester (>99 %) for 18 h, concentrations of DHA in the *sn*-1, *sn*-2, and *sn*-3 positions were 8.6, 6.0, and 25.3 mole%, respectively (6). Concentration in the *sn*-1,3 position is calculated to be 17.0 mole%, indicating higher concentration of

DHA in the *sn*-1,3 position than in the *sn*-2 position [i.e., *sn*-1,3 > *sn*-2]. This distribution pattern was the same as that observed in the present study. It is probable that most of TAG fed to the nauplii are hydrolyzed to release free form of DHA and then DHA is incorporated into the *Artemia* TAG in the same manner as DHA ethyl ester (6). In marine mammal TAG (e.g., seal oil TAG), DHA are generally esterified in the *sn*-3 position followed by the *sn*-1 position (13,14). This distribution pattern coincides with that of the enriched *Artemia* nauplii. By contrast, distribution pattern of n-3 HUFA characteristic of marine fish TAG does not appear to hold for *Artemia* TAG during the n-3 HUFA enrichment.

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