Influence of Interesterification on the Oxidative Stability of Marine Oil Triacylglycerols

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To understand the relationship between triacylglycerol structure of marine oils and their oxidative stability, peroxide values and absorbed oxygen levels of whale, sardine, cod liver and skipjack oils, interesterified by lipase and $NaOCH_3$, were compared with those of native oils during storage at 40°C. Triacylglycerol structures of marine oils were characterized by high-performance liquid chromatography and differential scanning calorimetry analyses. Enzymatically interesterified fish oils were more stable than native oils because the level of highly unsaturated triacylglycerols was decreased. However, the oxidative stability of interesterified whale oil was more susceptible to oxidation than the native oil.

KEY WORDS: Fish oil, interesterification, lipase, marine oil, oxidation, triacylglycerol, whale oil.

Marine oils, such as whale and fish oils, are rich sources of n-3 highly unsaturated fatty acids (HUFAs), such as eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6). The oils are attractive from the nutritional point of view because they are thought to provide specific physiological functions against thrombosis, cholesterol build-up and allergies. However, it is difficult to maintain the quality of marine oils applicable for functional foods and medicine because they are highly susceptible to oxidation and easily develop undesirable flavor during storage. Although antioxidants are generally available for prevention of lipid oxidation in foods, natural antioxidants, such as tocopherols, unfortunately show only minor effects in inhibiting oxidation of fish oils. It has been suggested that the autoxidation rates of polyunsaturated fatty acids depend upon varieties and structures of lipids containing them (1). Several researchers have investigated the effect of randomization on the oxidative stability of vegetable oils to understand the relationship between triacylglycerol structure and oxidative stability of oils. Raghuveer and Hammond (2), Lau *et al.* (3), and Wada and Koizumi (4) investigated the effects of randomization on the oxidative stability of corn and soybean oils, and found that randomized oils were oxidized faster than native oils. They suggested that the unsaturated fatty acids located at the 2-triacylglycerol position were more stable than those at the 1- or 3-position because the level of linoleic acid located at the 2-position in randomized vegetable oils was lower in comparison with native oils. In contrast, Park *et al.* (5) found no difference in autoxidative rates between randomized and native soybean oil triacylglycerols. Zalewski and Gaddis (6) also reported that randomization did not alter the oxidative stability of vegetable oils when measured at 60°C. Hoffmann *et al.* (7) studied the oxidative stability of synthetic unsaturated triacylglycerols, and concluded that their stability was not determined by total unsaturation ratio. Tautorus and McCurdy (8) reported that the procedure of randomization and storage temperature affected the oxidative stability of randomized vegetable oils. Unfortunately, reports are not consistent about effects of randomization and interesterification on the oxidative stability of oils. Especially, the effect of interesterification and randomization on the oxidative stability of marine oils, which contain EPA and DHA, has not been clarified yet.

In this study, enzymatic and chemical interesterifications were performed on fish and whale oils, and then their oxidative rates were assessed. Moreover, the triacylglycerol structures of marine oils were determined by high-performance liquid chromatography (HPLC) and differential scanning calorimetry (DSC). Based on these observations, we discuss the relationship between oxidative stability and triacylglycerol structure of marine oils.

MATERIALS AND METHODS

Materials. Sardine oil was obtained from Nippon Oil Co. (Tokyo, Japan). Cod liver and skipjack oils were obtained from Maruha Co. (Tokyo, Japan). Minke whale blubber oil was provided by The Institute of Cetacean Research (Tokyo, Japan).

Interesterification. Enzymatic interesterification was performed by continuously shaking a mixture of oil and 1% (w/w) nonspecific lipase (lipase TOYO; *Chromobacterium viscosum*; Toyo Brewer Co., Tokyo, Japan) or 10% (w/w) immobilized lipase [lipase (PG); *Pseudomonas fluorescens*; Amano Pharmacy Co., Nagoya, Japan] in *n*-hexane, saturated with water, at 40°C under N_2 gas for 24 h. Pure triacylglycerols from native and interesterified marine oils were prepared by florisil (5% moisture) column chromatography (9).

Chemical randomization was performed by adding 0.5% (w/w) sodium methoxide ($NaOCH_3$) to oils and heating at 80°C under N_2 at 5 mm Hg for 5 h. After the reaction, the interesterified oil was extracted with diethyl ether. Pure triacylglycerols of interesterified marine oils were prepared by florisil column chromatography.

Analysis of marine oil triacylglycerols. Fatty acid composition was determined by gas–liquid chromatography (GLC) analysis after transesterification with $NaOCH_3$. A Shimadzu GC-8A (Kyoto, Japan), equipped with a flame-ionization detector and a glass column (3 mm × 2 m) packed with 10% Silar 10C on Uniport (100–120 mesh), was used for GLC analysis. Column temperature was programmed at 170 to 230°C (2°C/min), and the injection and detector temperature was 240°C.

The distribution of fatty acids in marine oil triacylglycerols was determined by a modified method of Luddy *et al.* (10). Triacylglycerols were hydrolyzed by porcine pancreatic lipase (EC.3.1.1.3; Sigma Chemical Co., St. Louis, MO), and the resulting 2-monoacylglycerols were separated by thin-layer chromatograhy (TLC) prior to GLC analysis. A silicic acid (Kieselgel 60; Merck, Darmstadt, Germany) plate and *n*-hexane/diethyl ether/acetic acid (80:30:1) mixture as a mobile phase were used for TLC.

Triacylglycerol molecular species were analyzed by reversed-phase HPLC on a Shodex C18-5B ODS column

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(4.6 mm × 250 mm; Showa Denko Co., Tokyo, Japan) as a reversed-phase column. Triacylglycerols were monitored with an Erma ERC-7510 refractive index (RI) detector (Erma Optical Works, Ltd., Tokyo, Japan). The mobile phase was acetone and acetonitrile (1:1) mixture, and the flow rate was 1.0 mL/min.

Melting points of marine oil triacylglycerols were analyzed by DSC according to the method described by Niiya *et al.* (11). A computer-programmable Mettler TA 3000 System, equipped with a DSC cell (Mettler Instrumente AG, Zürich, Switzerland), was used. After triacylglycerols (10 mg) were placed in an aluminum DSC pan, they were maintained at 60°C for 2 min and then cooled to −100°C (5°C/min). They were held at −100°C for 2 min and heated to 60°C. The transition temperature of triacylglycerols was given as a peak temperature.

Oxidation tests. Native and interesterified fish oils (50 mg) with 0.1% (w/w) α-tocopherol as an antioxidant were put in a 10-mL vial, sealed with a W-type rubber cap (Teraoka, Osaka, Japan) and then incubated at 40°C in the dark. The level of oxygen in the headspace gas of the vial was estimated by GLC in a Shimadzu GC-4A with a thermal conductivity detector and a stainless column (3 mm × 2 m) packed with molecular Sieve 5A (GL Science, Tokyo, Japan) (12). Column temperature was 70°C, and the injection and detector temperature was 90°C. The induction period was the number of days it took until 100 μL of oxygen was consumed by oxidation of the sample oil. Peroxide value was measured for sample oils after oxidation (13).

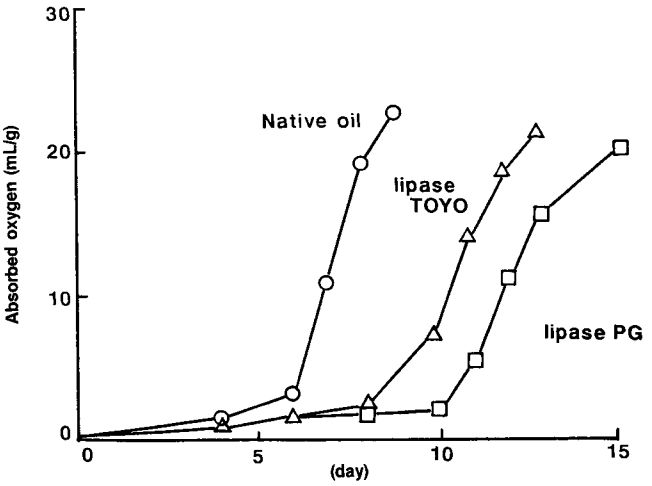

FIG. 1. Changes in level of oxygen absorbed during autoxidation of native and enzymatically interesterified sardine oils at 40°C. (○) Native oil; (△) lipase TOYO—interesterified oil (Toyo Brewer Co., Tokyo, Japan); (□) lipase PG—interesterified oil (Amano Pharmacy Co., Tokyo, Japan).

RESULTS

Oxidative stability of enzymatically interesterified fish oils. Native sardine oil was rapidly oxidized and consumed more oxygen in a vial in comparison with enzymatically interesterified oils (Fig. 1). Induction periods, determined by absorbed oxygen levels of native oil and lipase TOYO- and lipase PG-interesterified oils of sardine were 6, 8 and 10 d, respectively. These observations indicated that enzymatic interesterification enhanced the oxidative stability of sardine oil triacylglycerols. Sardine oil interesterified with lipase PG showed the highest oxidative stability among the three sardine oil samples.

There were significant differences in the distribution of fatty acids between native and enzymatically interesterified sardine oils (Table 1). Especially, the level of DHA (22:6) at the 2-triacylglycerol position in native sardine oil decreased after enzymatic interesterification, while the level of oleic acid (18:1) at the 2-position increased in interesterified oils. There were also differences in the distribution of fatty acids between lipase PG- and lipase TOYO-interesterified oils. Most fatty acids were equally distributed at each position of triacylglycerol in sardine oil interesterified by lipase PG because catalytic activity of lipase PG was higher for not only monounsaturated and saturated fatty acids but also for HUFAs. The fatty acid specificity of lipases may thus affect the oxidative stability of lipase-interesterified sardine oils.

In the HPLC analyses of native and interesterified sardine oil triacylglycerols (Fig. 2), some peaks, due to nonpolar triacylglycerols, appeared in sardine oils interesterified by lipase TOYO and lipase PG at retention times of 60 to 70 min, while other peaks (peaks 1–4), due to highly polar triacylglycerols, below 10 min were reduced

TABLE 1

Fatty Acid Composition of Enzymatically Interesterified Sardine Oils

	Fatty acid (mole %)								
	14:0	16:0	16:1	18:0	18:1	20:1	22:1	20:5	22:6
Native									
Total	6.3	15.5	8.0	3.2	14.5	11.1	15.0	11.5	6.1
2-Position	8.4	16.4	6.8	0.9	8.4	10.0	10.4	11.4	15.9
Lipase TOYO[a]									
Total	4.6	14.6	7.3	2.4	16.6	14.3	20.7	8.8	5.9
2-Position	6.6	16.3	8.0	0.7	10.2	14.0	10.8	9.7	10.7
Lipase PG[b]									
Total	3.7	11.7	7.0	2.7	15.8	15.4	19.9	9.8	5.5
2-Position	2.8	7.5	6.0	1.9	13.2	12.2	23.2	13.3	5.2

[a]Toyo Brewer Co., Tokyo, Japan.
[b]PG, prostaglandin (Amano Pharmacy Co., Nagoya, Japan).

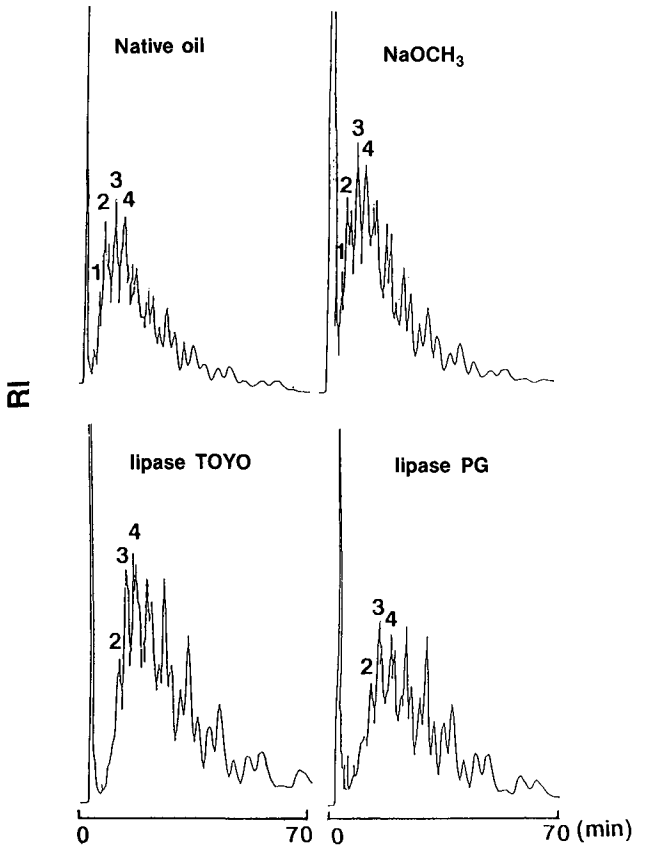

FIG. 2. High-performance liquid chromatography analysis of native and enzymatically interesterified sardine oil triacylglycerols. RI, refractive index.

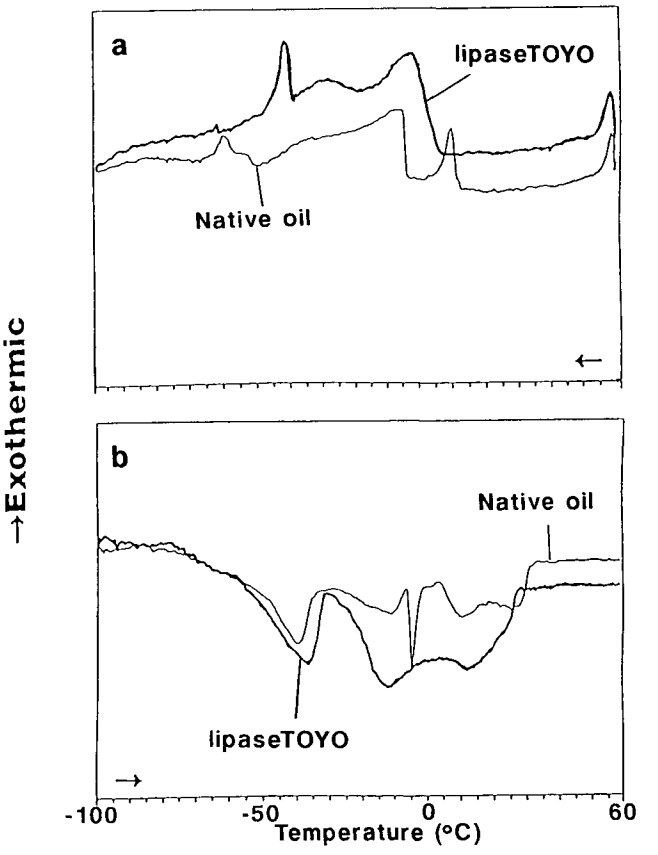

FIG. 3. Differential scanning calorimetry analysis of native and enzymatically interesterified sardine oils. a, Cooling curve; b, heating curve.

in enzymatically interesterified oils. These highly polar triacylglycerols are regarded as highly unsaturated triacylglycerols that contained three molecules of EPA or DHA (peaks 1 and 2) or two molecules of HUFAs (peaks 3 and 4). HPLC analysis showed that enzymatic interesterification decreased the ratio of highly unsaturated triacylglycerols in sardine oils.

DSC analysis of native and enzymatically interesterified sardine oil triacylglycerols is shown in Figure 3. In the cooling curve, native sardine oil had an exothermic peak of a very low melting point (−60°C) due to highly unsaturated triacylglycerols, while the corresponding peak in interesterified oil was at −40°C.

These observations suggested that the higher oxidative stability of enzymatically interesterified sardine oils was due to the reduction of highly unsaturated triacylglycerols.

Table 2 shows the induction period of cod liver and skipjack oils as well as sardine oils, as determined by levels of absorbed oxygen when the oils were oxidized at 40°C. Induction periods of native oil and lipase TOYO- and lipase PG-interesterified oils of cod liver were 14, 17 and over 30 d, respectively. The induction period of cod liver oil interesterified with lipase PG was the longest among the three oils. The induction period of skipjack oil was also extended from 5 to 6 and 8 d after lipase TOYO- and lipase PG-interesterifications, respectively. This suggests that the degree of enzymatic interesterification is correlated with the oxidative stability of fish oils. HPLC and DSC analyses suggested that the higher oxidative stability of enzymatically interesterified cod liver and skipjack oils resulted from the reduction of highly unsaturated triacylglycerols.

Our results, however, were not in agreement with those from several researchers (2,4,14) who have suggested that unsaturated fatty acids located at the 2-triacylglycerol position in lard and vegetable oils were more stable than those at the 1- or 3-position because the oxidative stability of oils was lowered by randomization. The most important factor in determining the oxidative stability of fish

TABLE 2

Induction Periods of Enzymatically Interesterified Fish Oils

	Induction period (day)[a]		
Fish oil	Native	Lipase TOYO	Lipase PG
Sardine	6	8	10
Cod liver	14	17	>30
Skipjack	5	6	8

[a]The day until 100 μL of oxygen was consumed in a vial. See Table 1 for company sources.

TABLE 3

Fatty Acid Composition of Chemically Interesterified Fish Oils

	Fatty acid (mole %)								
	14:0	16:0	16:1	18:0	18:1	20:1	22:1	20:5	22:6
Sardine									
Total	7.0	16.5	7.2	3.3	16.1	10.6	9.4	11.9	8.1
2-Position	7.0	16.3	6.2	1.0	9.6	11.8	11.9	12.3	15.0
Sardine (interesterified)									
Total	6.7	16.6	8.1	3.6	14.7	10.6	10.8	11.9	7.4
2-Position	6.9	18.7	7.6	3.6	16.5	11.2	11.9	10.2	5.2
Cod liver									
Total	4.1	10.0	6.3	2.4	16.1	19.2	19.1	9.1	6.9
2-Position	5.0	8.5	4.1	0.7	9.1	14.6	20.4	14.0	16.5
Cod liver (interesterified)									
Total	4.5	8.0	8.1	2.1	18.6	17.2	17.2	10.7	7.7
2-Position	5.2	10.5	6.0	2.4	16.9	18.6	19.1	8.4	3.6
Skipjack									
Total	2.9	17.8	7.9	6.8	19.4	3.2	1.6	6.0	22.4
2-Position	4.8	15.7	6.5	2.1	9.4	1.5	0.6	3.0	32.8
Skipjack (interesterified)									
Total	3.3	17.8	7.9	6.6	18.6	3.1	1.6	6.1	22.1
2-Position	3.3	14.9	7.0	6.7	21.4	3.9	2.5	3.0	16.9

oils might be the level of highly unsaturated triacylglycerols but not the position of HUFAs present in the triacylglycerol molecule. This would explain that enzymatically interesterified fish oils were more stable than native oils.

Oxidative stability of chemically interesterified fish oils. The effects of chemical interesterification on the oxidative stability of fish oils were also investigated. The distributions of fatty acids in triacylglycerol of the fish oils before and after chemical interesterification are shown in Table 3. No great change was seen in the total fatty acid composition of any oils after interesterification. Although most of the HUFAs, such as EPA and DHA, are generally located at the 2-triacylglycerol position in fish oils (15), the level of HUFAs located at the 2-position decreased in chemically interesterified fish oils. The distribution of fatty acids in the triacylglycerols of chemically interesterified fish oils was almost similar to that of the enzymatically interesterified oils.

The chemically interesterified cod liver and skipjack oils were oxidized slower than the native oils (Fig. 4). This result was consistent with that of enzymatically interesterified oils except for sardine oils. Chemical interesterification also improved the oxidative stability of fish oils as well as enzymatic interesterification. However, chemically interesterified sardine oil was oxidized faster than the native oil. Possibly, the oxidative stability of chemically interesterified sardine oil was lower because of a prooxidative factor produced by the treatment with $NaOCH_3$ at high temperature (80°C).

Oxidative stability of chemically interesterified minke whale oil. Minke whale blubber oil, a marine mammal oil, was chemically interesterified. Contrary to fish oils, HUFAs were concentrated at the 1- or 3-triacylglycerol positions of minke whale oil (Table 4). HUFAs, such as EPA and DHA, were distributed not only to their 1- or 3-triacylglycerol positions but also to the 2-position in minke whale oil after interesterification.

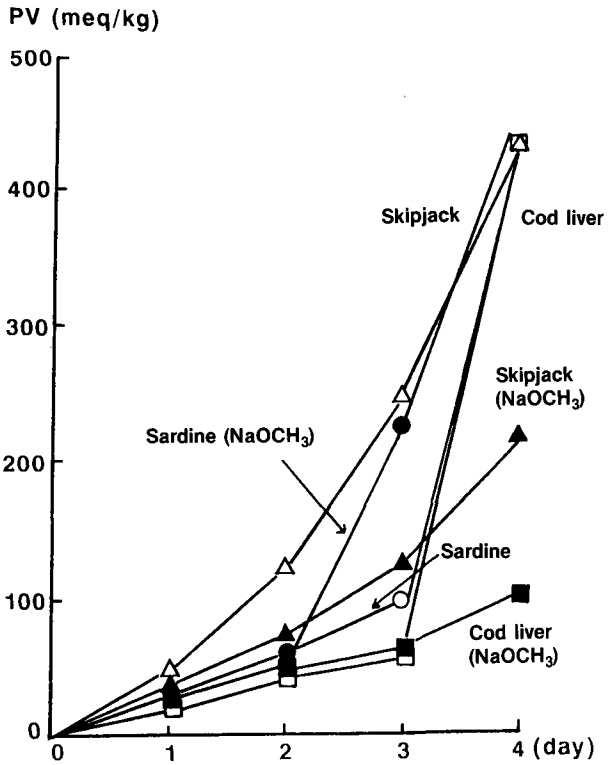

FIG. 4. Changes in peroxide value (PV) of native and chemically interesterified sardine, skipjack and cod liver oils during autoxidation at 40°C. (○) Native sardine oil; (●) interesterified sardine oil; (△) native skipjack oil; (▲) interesterified skipjack oil; (□) native cod liver oil; (■) interesterified cod liver oil.

Changes in peroxide value showed that chemically interesterified whale oil was rapidly oxidized in comparison with native oil (Fig. 5). There were no significant

TABLE 4

Fatty Acid Composition of Chemically Interesterified Minke Whale Oils

	Fatty acid (mole %)								
	14:0	16:0	16:1	18:0	18:1	20:1	22:1	20:5	22:6
Native oil									
Total	8.3	13.8	15.6	2.7	35.7	3.2	1.2	5.8	4.3
2-Position	15.6	6.6	24.6	0.9	37.7	1.8	1.1	1.4	—
Interesterified oil									
Total	8.2	13.7	15.4	2.9	35.2	3.3	1.1	5.9	4.0
2-Position	10.5	16.8	15.5	2.2	37.5	2.5	1.5	4.2	1.9

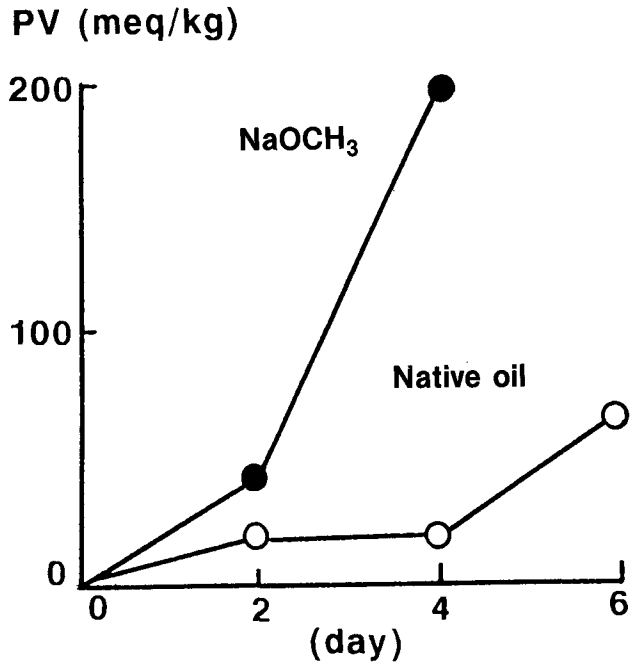

FIG. 5. Changes in peroxide value (PV) of native and chemically interesterified mink whale blubber oils during autoxidation at 40°C. (○) Native whale oil; (●) interesterified whale oil.

differences in HPLC and DSC analyses between native and chemically interesterified minke whale oil (data not shown). Furthermore, whale oil did not contain highly unsaturated triacylglycerols as observed in fish oils. Thus, the lower oxidative stability of chemically interesterified whale oil seemed to be due to dispersion of HUFAs located at the 1- or 3-triacylglycerol position to the 2-position.

Frankel *et al.* (16–18) synthesized triacylglycerols that contained linoleate (L) and linolenate (Ln) in specific positions and measured their oxidative rates. They have reported that LnLLn was more stable than LnLnL. These observations indicated that the oxidative rate of a triacylglycerol containing two linolenic acids was higher when they were located at the 1,2- or 2,3-position. HUFAs located at the 1,3-triacylglycerol position in whale oil also might become more susceptible to free radical oxidation when they are transferred to the 1,2- or 2,3-position.

In the present studies, marine oils containing HUFAs were chemically and enzymatically interesterified, and the oxidative rates were estimated to understand the relationship between their triacylglycerol structure and oxidative stability. We found that enzymatic interesterification could improve the oxidative stability of fish oils because highly unsaturated triacylglycerols present in them were reduced by interesterification. Chemical interesterification also improved oxidative stabilities of cod liver and skipjack oils but not for sardine oil. On the other hand, oxidative stability of minke whale blubber oil was reduced after interesterification.

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