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ARTICLE

Effect of Chemical Randomization on Positional Distribution and Stability of Omega-3 Oil Triacylglycerols

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Randomization has been commonly used to modify the chemical and physical properties of natural fats and oils. In this study, seal blubber oil (SBO) and menhaden oil (MHO) were modified through chemical randomization using sodium methoxide, and the effect on positional distribution of fatty acids was investigated using gas chromatography (GC) and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The effect of randomization on the stability of the original oils and their randomized counterparts was analyzed by comparing conjugated dienes and thiobarbituric acid reactive substances (TBARS) values after accelerated oxidation at 60 °C for 4 days. The omega-3 polyunsaturated fatty acids (PUFA) were distributed more evenly among the terminal *sn*-1,3 positions and the middle *sn*-2 position in chemically randomized oils when compared to the starting oils. The effect was more pronounced for SBO with omega-3 PUFA attached preferentially to *sn*-1,3 positions of triacylglycerols before randomization, and it was less pronounced for MHO, which contained omega-3 PUFA more evenly distributed before randomization. However, different levels of commonly known omega-3 fatty acids, namely, docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA), and stearidonic acid (STA), were obtained in both original and randomized oils from GC and ^{13}C NMR spectroscopy. The stability of the randomized oils was also affected to different degrees, depending on the storage time.

KEYWORDS: Chemical randomization; sodium methoxide; seal blubber oil; menhaden oil; positional distribution; omega-3 fatty acids; ^{13}C nuclear magnetic resonance spectroscopy; oxidative stability

INTRODUCTION

The omega-3 polyunsaturated fatty acids (PUFA), especially *cis*-5,8,11,15,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA), offer a wide range of health benefits and play a critical role in many functions in the human body. Their role in alleviating cancer (*1*, *2*), cardiovascular disease (*3*, *4*), psychiatric disorders (*5*, *6*), Parkinson's disease (*7*), and inflammatory and cardiovascular ailments (*8*−*11*) has been well demonstrated in the literature during the past three decades. DHA and its biological significance in supporting the normal development of the brain, eyes, and nerves, especially for infants and fetuses, have been well recognized. Therefore, addition of DHA in the diet of pregnant and lactating women as well as infant formula is strongly recommended (*12*−*15*). Furthermore, EPA serves as a precursor of eicosanoids, including thromboxanes, prostacyclins, and leukotrienes, that play a vital role in inflammation, regulating blood flow, immune response, and ion transport, among others. In addition, omega-3 PUFA, especially EPA and DHA, can be converted to other anti-inflammatory molecules, such as protectins, resolvins, maresins, and omega-3-oxylipins, which may also explain the versatile health benefits of omega-3 oils (*16*).

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The most common sources of omega-3 PUFA are the liver of white lean fish such as cod and halibut, the flesh of fatty fish such as menhaden, tuna, and salmon, and the blubber of marine mammals such as seals and whales (*17*, *18*). Seal blubber oil (SBO) contains typically between 5.4 and 6.4% EPA, 4.6−4.9% docosapentaenoic acid (DPA), and 7.6−7.9% DHA, whereas menhaden oil (MHO) contains 10.4−13.2% EPA, 2.0−2.4% DPA, and 10.1−11.5% DHA (*19*−*21*). Positional distribution of fatty acids, especially omega-3 PUFA in triacylglycerols (TAG), may affect their absorption and deposition in the human body, as well as influence their oxidative stability (*22*, *23*). Omega-3 PUFA are primarily located at the terminal *sn*-1,3 positions of TAGs of marine mammals, including whale and seal oil (*17*, *18*). In fish oil, DHA is primarily located in the *sn*-2 position, whereas EPA varies. In fish oils with higher levels of EPA, the proportion of *sn*-2 chains is low (13−40%), whereas higher levels of *sn*-2 chains (up to 60%) are found for oils containing low levels of EPA (< 6%) (*24*, *25*).

Chemical redistribution of fatty acids in TAGs is a common means to change chemical and physical properties of the oils (*26*, *27*). The purpose of the present study was to study the effect of chemical redistribution on positional distribution of fatty acids in SBO and MHO, using gas chromatography (GC) and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. In addition, the effect of chemical randomization on the oxidative stability of

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the resultant oils was examined by comparing conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) values of the oils following accelerated oxidation at 60 °C for 4 days.

MATERIALS AND METHODS

Sodium methoxide, thiobarbituric acid, and $CDCl_3$ were obtained from Sigma-Aldrich (Mississauga, ON, Canada). Standards of fatty acid methyl esters (FAMEs; GLC-461) were purchased from Nu-Check (Elysian, MN). Porcine pancreatic lipase (EC 3.11.3), sodium taurocholate, and silica gel thin-layer chromatographic plates (TLC; 20 × 20 cm; 60 Å mean pore diameter, 2−25 μm mean particle size, 500 μm thickness, with dichlorofluorescein) were purchased from Sigma Chemical Co. (St. Louis, MO). All solvents used were of analytical grade and were purchased from Fisher Scientific (Nepan, ON, Canada).

Sodium Methoxide-Catalyzed Chemical Redistribution of SBO and MHO. Chemical redistribution was carried out following the method of Rousseau and Marangoni (*28*) with minor modifications. Starting oil (25 g) was heated under a nitrogen blanket in a round-bottom flask at 85 °C. Sodium methoxide (0.5%, w/w) was added to the oil to initiate the reaction. The temperature of the oil was increased to 88 ± 1 °C under a nitrogen blanket and kept there for 60 min. Sodium methoxide was removed after acidification with citric acid (20%, w/v), and then the mixture was washed with a sodium bicarbonate (0.05 M) solution to neutralize the residual acids. The soaps so produced were removed by hot water. Residual water and colorants were removed by adding bentonite clay (1.5%, w/w), and the recovered oils were stored at −20 °C until use.

Determination of Positional Distribution of Fatty Acids by GC Analysis. *Selective Hydrolysis Using Pancreatic Lipase*. The oil samples were hydrolyzed using pancreatic lipase as described by Christie (*29*) with minor modifications. The oil (25 mg) was weighed into a glass tube, and then 5.0 mL of Tris-HCl buffer (1.0 M, pH 8.0), 0.5 mL of calcium chloride (2.2%), and 1.25 mL of sodium taurocholate (0.05%) were added. Porcine pancreatic lipase (5.0 mg; EC 3.11.3) was added into the mixture after it had been kept in a water bath for 5.0 min at 40 °C. The glass tube was subsequently placed in a gyratory water bath shaker at 250 rpm under a blanket of nitrogen for 1 h at 40 °C. The enzymatic reaction was stopped by adding 5.0 mL of ethanol, followed by the addition of 5.0 mL of 6.0 M HCl.

Extraction and Separation of Hydrolytic Products. Diethyl ether (50 mL in total) was used to extract the hydrolytic products three times, and then the extract was washed twice with distilled water and dried over anhydrous sodium sulfate followed by removal of the solvent under reduced pressure at 30 °C. The hydrolytic products were separated on silica gel TLC plates. The plates were developed using a mixture of hexane/diethyl ether/acetic acid (70:30:1, v/v/v) for 45−55 min. The bands were located by viewing under short (254 nm) wavelength UV light (Spectraline, model ENF-240C, Spectronics Co., Westbury, NY). The free fatty acid bands were scraped off and lipids extracted into diethyl ether, which were then used for fatty acid analysis as described by Senanayake and Shahidi (*30*).

Fatty Acid Compositional Analysis of Hydrolytic Products. Fatty acid composition and positional distribution of the products were determined by their conversion to the corresponding methyl esters. The transmethylation reagent (2.0 mL) consisting of freshly prepared 6% sulfuric acid in methanol containing 15 mg of hydroquinone as an antioxidant was added to the sample vial, followed by vortexing. The mixture was incubated for 24 h at 60 °C and subsequently cooled to ambient temperature. Distilled water (1.0 mL) was then added to the mixture, after thorough mixing, and hydroquinone was added to each vial to prevent oxidation. The FAMEs were extracted three times, each with 1.5 mL of HPLC grade hexane. The combined hexane layers were then transferred to the test tube and washed twice with 1.0 mL of distilled water. The hexane was evaporated under a stream of nitrogen, and the extracted FAMEs were then dissolved in 1.0 mL of carbon disulfide and used for subsequent GC analysis. A Hewlett-Packard 5890 series II gas chromatograph (Agilent, Palo Alto, CA) equipped with a Supelcowax-10 column (30 m length, 0.25 mm diameter, 0.25 μm film thickness; Supelco Canada Ltd., Oakville, ON, Canada) was used to analyze the FAMEs. The oven temperature was first raised to 220 °C and kept there for 10.25 min and then raised to 240 °C at 30 °C/min and held there for 15 min. The injector and FID temperatures were 250 °C. Ultrahigh-purity helium was used as a carrier gas at a flow rate of 15 mL/min. Hewlett-Packard 3365 series II Chem-Station software (Agilent) was used for data handling and processing. The FAMEs were identified by comparing their retention times with those of a known standard mixture. Positional distribution of fatty acids at *sn*-1,3 positions was calculated as (% fatty acid at *sn*-1,3 positions/% fatty acid in triacylglycerols) × 100.

Table 1. Percent Fatty Acid Distribution of Total Triacylglycerols Located at *sn*-1,3 and *sn*-2 Positions of Chemically Randomized Seal Blubber Oil Calculated by GC Analysis[a]

	sn-1,3[b]		*sn*-2[c]	
fatty acid	SBO	CRSBO	SBO	CRSBO
C14:0	58.70 ± 0.51 a	62.56 ± 0.19 b	41.30 ± 0.51	37.44 ± 0.19
C14:1ω9	58.44 ± 0.40 a	63.03 ± 0.16 b	41.56 ± 0.40	36.97 ± 0.16
C16:0	64.16 ± 0.35 a	66.25 ± 0.06 b	35.84 ± 0.35	33.75 ± 0.06
C16:1ω7	57.38 ± 0.78 a	64.69 ± 0.38 b	42.62 ± 0.78	35.31 ± 0.38
C17:1	66.41 ± 2.22	62.06 ± 0.21	33.59 ± 2.22	37.94 ± 0.21
C18:0	93.00 ± 1.31 a	77.63 ± 0.02 b	7.00 ± 1.31	22.37 ± 0.02
C18:1ω9	63.80 ± 0.56 a	65.84 ± 0.07 b	36.20 ± 0.56	34.16 ± 0.07
C18:1ω11	56.61 ± 1.76 a	62.20 ± 0.06 b	43.39 ± 1.76	37.80 ± 0.06
C18:2ω6	77.17 ± 1.48	67.41 ± 0.88	22.83 ± 1.48	32.59 ± 0.88
C18:3ω3	79.26 ± 2.56	75.76 ± 1.46	20.74 ± 2.56	24.24 ± 1.46
C18:4 ω3	70.58 ± 0.89	62.07 ± 0.64	29.42 ± 0.89	37.93 ± 0.64
C20:1ω9	68.50 ± 0.28	68.49 ± 0.23	31.50 ± 0.28	31.51 ± 0.23
C20:5ω3	68.44 ± 1.09 a	55.98 ± 0.13 b	31.56 ± 1.09	44.02 ± 0.13
C22:1ω11	67.34 ± 2.30	80.09 ± 0.21	32.66 ± 2.30	19.91 ± 0.21
C22:5ω3	90.16 ± 2.48 a	80.51 ± 0.01 b	9.84 ± 2.48	19.49 ± 0.01
C22:6ω3	76.11 ± 1.06	70.98 ± 0.15	23.89 ± 1.06	29.02 ± 0.15

[a] Values across a row not sharing a common letter are significantly different from one another ($P < 0.05$). SBO, seal blubber oil before randomization; CRSBO, seal blubber oil after randomization. [b] (% fatty acid at *sn*-1,3 positions/% fatty acid in triacylglycerols) × 100. [c] (% fatty acid at *sn*-2 position/% fatty acid in triacylglycerols) × 100.

^{13}C NMR Spectroscopy. Quantitative ^{13}C NMR spectra of natural oils and their chemically randomized counterparts were recorded under continuous ^{1}H decoupling at 20 °C at a ^{13}C frequency of 125 MHz in a Bruker Avance-500 MHz spectrometer on 100 mg samples dissolved in 0.7 mL of 99.8% $CDCl_3$. Experimental parameters were 128K complex data points, spectral width of 33784 Hz (268 ppm), and 15 s relaxation delay with collection of 1000 scans. The spectra were Fourier transformed and processed using the standard procedures of resolution and sensitivity enhancement. Carbonyl peak integrals were measured accurately using the standard deconvolution algorithm from TopSpin v 2.1 software, assuming a pure Lorentzian line shape. In all spectra, ^{13}C chemical shifts were expressed in parts per million (ppm) relative to $CDCl_3$ at 77.16 ppm (*31*).

Oxidative Stability Tests. The oxidative stability of randomized SBO and MHO as well as their original counterparts was determined under Schaal oven conditions at 60 °C for 4 days. Each day (24 h) of storage under such conditions is equaivalent to 1 month of storage at ambient temperatures. Oils (0.4−0.5 g) were placed in loosely capped test tubes (10 mm diameter and 4.0 cm height) and stored at 60 °C in a forced-air oven (Thelco, model 2; Precision Scientific Co., Chicago, IL). Samples were removed from the oven at 0, 6, 12, 24, 48, 72, and 96 h, cooled to room temperature, flushed with nitrogen, capped, and stored at −20 °C until analyzed. The experiments were carried out in triplicate. CD in the oils was determined according to IUPAC method 20505 (*32*). Oil samples (0.02−0.04 g) were weighed into 50 mL volumetric flasks, dissolved in iso-octane, and made up to the mark with the same solvent. The contents were thoroughly mixed, and the absorbance was read at 234 nm using a Hewlett-Packard model 845LA diode array spectrophotometer (Agilent).

The determination of TBARS was carried out as described in AOCS method Cd 19-90 (*33*). Oil samples (0.05−0.10 g) were weighed into 25 mL volumetric flasks, dissolved in a small volume of 1-butanol, and then made up to the mark with the same solvent. This solution (5 mL) was transferred into a screw-capped test tube, and then 5 mL of freshly prepared TBA reagent (0.5 g of TBA in 250 mL of *n*-butanol) was added. The mixture was thoroughly mixed, and then the test tube was placed in a water bath at 95 °C for 2 h. Heated samples were cooled in an ice bath, and the absorbance of the resultant colored complex was read at 532 nm using a Hewlett-Packard diode array model 8452 A spectrophotometer (Agilent).

Table 2. Percent Fatty Acid Distribution of Total Triacylglycerols Located on *sn*-1,3 and *sn*-2 Positions of Chemically Randomized Menhaden Oil Calculated by GC Analysis[a]

	sn-1,3[b]		*sn*-2[c]	
fatty acid	MHO	CRMHO	MHO	CRMHO
C14:0	67.97 ± 0.67 a	73.16 ± 0.26 b	32.03 ± 0.67	26.84 ± 0.26
C14:1ω9	69.88 ± 0.71	71.9 ± 0.91	30.12 ± 0.71	28.1 ± 0.91
C16:0	67.22 ± 0.64 a	71.52 ± 0.24 b	32.78 ± 0.64	28.48 ± 0.24
C16:1ω7	69.68 ± 0.65	73.3 ± 0.16	30.32 ± 0.65	26.7 ± 0.16
C17:1	65.26 ± 0.30	64.16 ± 0.29	34.74 ± 0.30	35.84 ± 0.29
C18:0	78.78 ± 0.34	73.46 ± 0.39	21.22 ± 0.34	26.54 ± 0.39
C18:1ω9	76.37 ± 0.28	73.92 ± 0.04	23.63 ± 0.28	26.08 ± 0.04
C18:1ω11	76.07 ± 1.47	76.03 ± 0.82	23.93 ± 1.47	23.97 ± 0.82
C18:2ω6	83.35 ± 0.47	78.67 ± 0.03	16.65 ± 0.47	21.33 ± 0.03
C18:3ω3	74.69 ± 1.33	67.6 ± 0.83	25.31 ± 1.33	32.4 ± 0.83
C18:4 ω3	60.27 ± 0.61	57.83 ± 0.32	39.73 ± 0.61	42.17 ± 0.32
C20:1ω9	88.6 ± 1.69 a	70.16 ± 0.42 b	11.4 ± 1.69	29.84 ± 0.42
C20:5ω3	49.46 ± 0.13	47.58 ± 0.13	50.54 ± 0.13	52.42 ± 0.13
C22:5ω3	56.81 ± 1.49	55.24 ± 0.82	43.19 ± 1.49	44.76 ± 0.82
C22:6ω3	50.97 ± 1.06 a	55.61 ± 0.92 b	49.03 ± 1.06	44.39 ± 0.92

[a] Values across a row not sharing a common letter are significantly different from one another ($P < 0.05$). MHO, menhaden oil before randomization; CRMHO, menhden oil after randomization. [b] (% fatty acid at *sn*-1,3 positions/% fatty acid in triacylglycerols) × 100. [c] (% fatty acid at *sn*-2 position/% fatty acid in triacylglycerols) × 100.

RESULTS AND DISCUSSION

Fatty Acid Positional Distributions by GC Analyses. The positional distributions of the major fatty acids of SBO, MHO, and their chemically randomized counterparts CRSBO and CRMHO, respectively, calculated from the fatty acid profiles of hydrolytic products, are shown in **Tables 1** and **2**, respectively.

Seal Bubbler Oil before and after Chemical Randomization. In the original SBO, unsaturated fatty acids, especially PUFA such as C18:2ω6, and those of the omega-3 class C18:3, C18:4, C22:5 and C22:6 were preferentially located on the *sn*-1,3 positions (**Table 1**). The important omega-3 fatty acid C20:5 was distributed nearly evenly among *sn*-1,3 (68%) and *sn*-2 positions (32%, **Table 1**). It is worth noting that equal distribution in this work is defined as a *sn*-1,3 regiospecificity of 66.67 mol % and a *sn*-2 regiospecificity of 33.33 mol %.

Saturated residues are assembled differently in TAG of the original SBO; for instance, C18:0 residues reside mostly in the terminal *sn*-1,3 positions (93%) and C16:0 appears to be close to equally distributed, whereas C14:0 showed a slight preference for the *sn*-2 position (41%).

The monounsaturated fatty acids C17:1 and C18:1n-9 appeared to be nearly equally distributed (66 and 64%, respectively) on the *sn*-1,3 positions. The other monounsaturated residues C14:1ω9, C16:1ω7, and C18:1ω11 were in slight excess on the *sn*-2 positions, judging from the *sn*-2 area percentages, which were close to 43% in each case (**Table 1**).

Table 2 reflects that PUFA were more equally distributed in the glycerol backbone of the TAGs of chemically randomized seal blubber oil, as a result of relocation from the *sn*-1,3 positions to the *sn*-2 position (**Table 1**). For instance, 9% of each of the C18:4ω3 and C22:5ω3 chains was relocated from the *sn*-1,3 positions to the *sn*-2 position (**Table 1**). The proportion of *sn*-1,3 chains of C18:3ω3 and C22:6ω3 decreased 3 and 5%, respectively, and from the resulting *sn*-1,3 area percentages of 76 and 71%, respectively, it was concluded that these fatty acids remained attached preferentially to the *sn*-1,3 positions in chemically randomized seal blubber oil (CRSBO, **Table 1**).

The chemical randomization also redistributed C18:2ω-6 chains, relocating them from mostly the *sn*-1,3 positions (77%) in natural SBO to equally distributed in CRSBO (67% *sn*-1,3). Meanwhile, C20:5ω3 moieties that were distributed evenly among *sn*-1,3 (67%) and *sn*-2 (33%) positions in natural SBO were relocated (12%) to the *sn*-2 position (44% area *sn*-2) upon chemical randomization (CRSBO, **Table 1**).

The positional distributions of saturated residues were affected in different fashion upon chemical randomization. For instance, palmitic acid (C16:0) residues were unaffected; they remained equally distributed in CRSBO (**Table 1**). C14:0 residues increased (14%) in the terminal *sn*-1,3 positions (to 63% area), whereas the proportion of *sn*-1,3 chains of C18:0 decreased 15%, although from the resultant area percentage (78%), it remained attached preferentially to the *sn*-1,3 positions.

For the most part, monounsaturated residues increased by 5−8% in the terminal *sn*-1,3 positions with the exception of C18:1ω-9, which remained unaffected, and of C17:1, which was relocated (4%) from the terminal *sn*-1,3 positions to the middle *sn*-2 position.

Menhaden Oil before and after Chemical Randomization. In the original MHO, the PUFA C18:2ω6 and C18:3ω3 were esterified preferentially in the *sn*-1,3 positions of glycerol, as judged from the *sn*-1,3 area percentages of 83 and 75%, respectively. The other omega-3 PUFA, C18:4, C20:5, C22:5, and C22:6, were attached preferentially to the *sn*-2 position (**Table 2**).

Of the three major saturated fatty acids of MHO, C14:0 and C16:0 were equally distributed, and the third one, C18:0, resided mostly in the *sn*-1,3 positions (**Table 2**). Of the six monounsaturated fatty acids analyzed, five displayed *sn*-1,3 area percentages of 70% or higher, and the sixth one (C17:1) was close to being evenly distributed (65% *sn*-1,3).

The PUFA distribution was largely unaffected by the randomization process, but higher changes were observed for C18:3ω3, C18:2ω6, and C22:6ω3. In the first two, the proportion of *sn*-1,3 chains decreased by 7 and 5%, respectively. For C22:6ω3, the proportion of *sn*-1,3 chains increased by 5%. Relocation of C18:3ω3 residues from the *sn*-1,3 positions to the *sn*-2 position resulted in an equal distribution; C18:2ω6 remained mostly attached to the *sn*-1,3 positions (79%), whereas C22:6ω3 residues increased to 55.6% on the *sn*-1,3 positions from its redistribution. With the exception of C18:2ω6, the *sn*-1,3 area percentage decreased by 4%, and the distribution of the remaining omega-3 acids C18:4, C20:5, and C22:5 remained almost unaffected upon chemical randomization.

Chemical randomization changed (5% increase) the distribution of C14:0 and C16:0 in the *sn*-1,3 positions from near equal over the *sn*-1,2 and 3 positions in the original oil. Meanwhile, 6% of C18:0 residues that resided mostly in the *sn*-1,3 positions of MHO were relocated to the *sn*-2 position, but the overall statistical distribution was unchanged. On chemical randomization, almost 20% of *sn*-1,3 chains of the monounsaturated residue C20:1ω9 were relocated from the *sn*-1,3 positions to the *sn*-2 position, although from the resultant *sn*-1,3 area percentage of 70, this fatty acid remained attached mostly to the terminal *sn*-1,3 positions. The positional distribution of the remaining monounsaturated fatty acids remained almost unchanged.

Fatty Acid Positional Distributions by ^{13}C NMR Analyses. *Seal Blubber Oil before and after Chemical Randomization*. The NMR spectral analysis of original SBO indicated that the omega-3 PUFA C18:4, C22:5, and C22:6 were attached only to the terminal *sn*-1,3 positions, and none of them were found in the middle *sn*-2 position (**Figure 1**). These results differ from those obtained by GC analysis, which revealed that these omega-3 PUFA were preferentially, but not uniquely, located in the terminal *sn*-1,3 positions (**Table 1**). From GC analysis it was established that the omega-3 acid C20:5 was evenly distributed (**Table 1**).

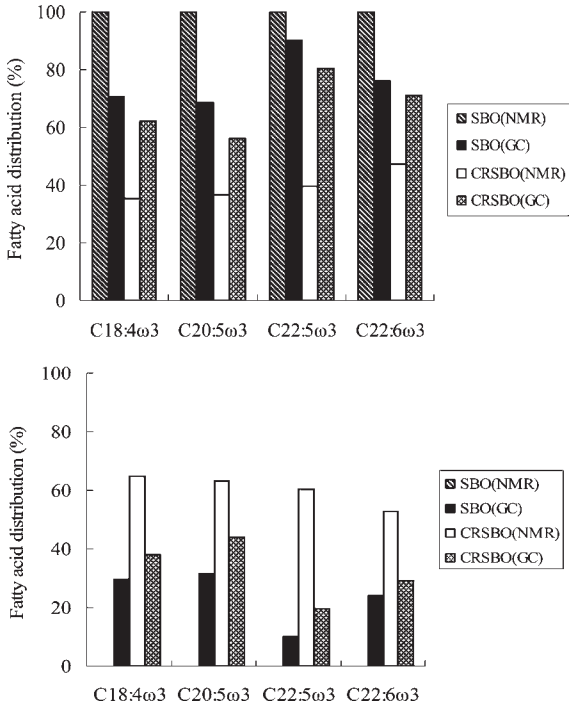

Figure 1. Percent fatty acid distribution of total TAG located on the *sn*-1,3 positions (top) and the *sn*-2 position (bottom) of chemically randomized seal blubber oil. SBO(NMR) indicates data obtained for SBO using ^{13}C NMR, SBO(GC) data obtained for SBO using GC, CRSBO(NMR) data obtained for CRSBO using ^{13}C NMR, and CRSBO(GC) data obtained for CRSBO using GC. Note that the % *sn*-2 distribution of omega-3 PUFA in SBO was zero, as determined by ^{13}C NMR.

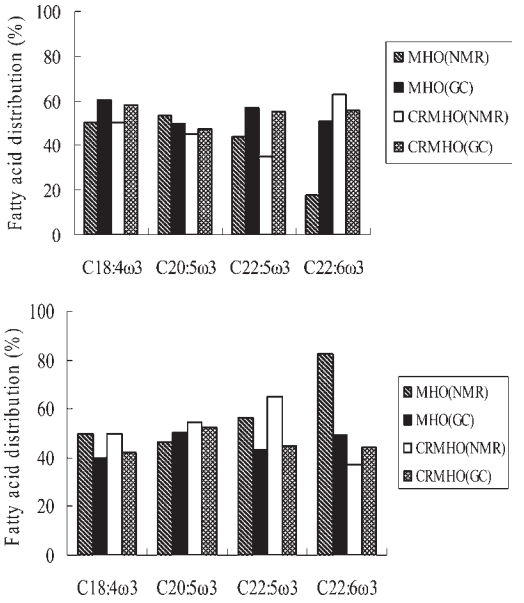

Figure 2. Percent fatty acid distribution of total TAG located on the *sn*-1,3 positions (top) and the *sn*-2 position (bottom) of chemically randomized menhaden oil. MHO(NMR) indicates data are obtained for MHBO using ^{13}C NMR, MHO(GC) data obtained for MHO using GC, CRMHO(NMR) data obtained for MHOBO using ^{13}C NMR, and CRMHO(GC) data obtained for MHO using GC.

However, NMR spectral data revealed that C20:5 was attached only to the *sn*-1,3 positions of MHO (**Figure 2**).

In chemically randomized SBO, both GC and NMR spectral analyses revealed that the omega-3 acids C18:4, C20:5, C22:5, and C22:6 were distributed more equally in the middle and terminal positions (**Figure 1** and **Table 1**). In fact, NMR spectral analysis indicated that with the exception of C22:6, half of the total amount of these acids was relocated to the middle *sn*-2 position on chemical randomization (**Figure 1**). For C22:6, the amounts of *sn*-1,3 chains relocated to the *sn*-2 position were lower, 37%. With the exception of C22:6, the omega-3 PUFA in CRSBO displayed a preference for the *sn*-2 position; C22:6 appears to be close to equally distributed (**Figure 1**).

Menhaden Oil before and after Chemical Randomization. For the most part, NMR spectral analysis of omega-3 PUFA distribution in MHO is in agreement with GC analysis. For instance, C22:5 and C22:6 were preferentially esterified in the *sn*-2 position (62 and 66%, respectively); C18:4 residues were also attached primarily to the *sn*-2 position, but in lesser amounts (46%) (**Figure 2**). C20:5 was the exception; whereas GC analysis indicated a preference for the middle *sn*-2 position (51% area; **Table 2**), NMR spectral analysis suggested a slight preference for the *sn*-1,3 positions (71% area; **Figure 2**).

For CRMHO, the NMR spectral data agree only partially with those from GC analysis. For instance, whereas GC analysis suggested that the PUFA distribution was almost unaltered on chemical randomization (**Table 2**), NMR analysis indicated that this occurred only for C18:4ω-3 and C20:5ω-3 residues (**Figure 2**). For the omega-3 PUFA C22:5 and C22:6, drastic changes were observed. The former was relocated from being primarily in the *sn*-2 position (62%) in the original MHO to almost equally distributed in chemically randomized MHO (35% *sn*-2; **Figure 2**). The latter was relocated from being primarily in the *sn*-2 position (66%) in MHO to a slight excess in the *sn*-1,3 positions (70%) in CRMHO (**Figure 2**).

Oxidative Stability. The CD value of lipids, reflected in their absorption at 234 nm, is an indicator of primary oxidation products as conjugated dienes are formed due to a shift in the double-bond positions upon oxidation of lipids that contain dienes or polyenes (*34*). **Figure 3** shows the CD contents of randomized SBO and its unrandomized counterpart under accelerated oxidation conditions at 60 °C. Both randomized and original SBO followed an increasing trend in their CD levels throughout the experimental period due to the formation and accumulation of lipid hydroeroxides as primary oxidation products (*35*). Formation of lipid hydroperoxides coincides with that of CD upon oxidation (*36*, *37*). CD values increased slowly from 0 to 24 h when primary oxidation products, hydroperoxides, were generated and released during lipid oxidation. However, the amount of hydroperoxide formed was fairly low at the beginning of oxidation chain reaction. As the storage time was extended to 96 h, large amounts of primary oxidation products were produced due the abundance of free radicals and the oxidation chain reaction, and this sharply increased CD values. CD values reached a peak within 96 h. Randomized SBO had a significantly higher CD value on each tested point except at the beginning point than its unrandomized counterpart. Positional distribution of fatty acids, especially unsaturated fatty acids, and the level of α-tocopherol (major antioxidant in fish and seal blubber oils) are thought to influence oxidative stability. Fewer PUFA, including C18:2, C18:3, C18:4, C20:5, C22:5, and C22:6, were located on the terminal positions in the randomized oil, and this should contribute a better oxidative stability to the resultant products. However, the decreased oxidative stability shown in the tested randomized oil was primarily due to the loss of α-tocopherol, the main natural antioxidant present in seal blubber oil. Formation of tocopheryl esters in randomized oil during chemical randomization as well as their oxidation may be responsible for the observed effect (*38*).

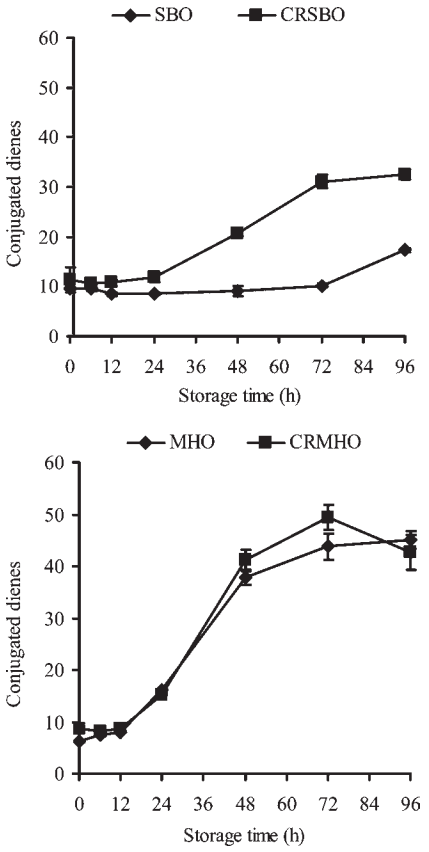

Figure 3. Conjugated diene values of SBO (top) and MHO (bottom) before and after chemical randomization and storage under Schaal oven condition at 60 °C. SBO, seal blubber oil before randomization; CRSBO, seal blubber oil after randomization; MHO, menhaden oil before randomization; CRMHO, menhaden oil after randomization.

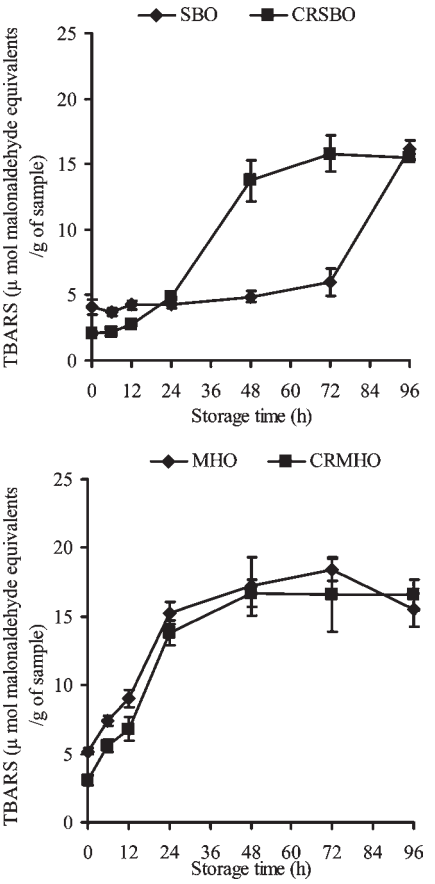

Figure 4. TBARS values of SBO (top) and MHO (bottom) before and after chemical randomization and storage under Schaal oven condition at 60 °C. SBO, seal blubber oil before randomization; CRSBO, seal blubber oil after randomization; MHO, menhaden oil before randomization; CRMHO, menhaden oil after randomization.

CD values of both original MHO and its randomized counterpart were also increased, except that the peak value of randomized MHO appeared at 72 h. Comparison of each tested point with the original oil revealed that randomized MHO had generally higher CD values, brought about by redistribution of unsaturated fatty acids and loss of α-tocopherol. However, results in **Table 2** show a decreased distribution of major unsaturated fatty acids on the terminal positions in randomized menhaden oil. The reason for the differences of CD value between two groups of oil samples may possibly be due to the fact that α-tocopherol exerts a stronger influence on oxidative stability of MHO than that of positional distribution of unsaturated fatty acids. The loss of α-tocopherol directly led to decreased oxidative stability of randomized oil, although there were more unsaturated fatty acids located on the middle position, which are expected to be better protected from oxidation.

The TBA test has been widely used for assessing the content of secondary oxidation products of lipids. The rate of formation of secondary oxidation products is affected not only by the amount of hydroperoxides as primary oxidation products, and the level of α-tocopherol, but also by the nature of hydroperoxides, which may be decomposed to secondary oxidation products at different rates under the same conditions (*39*). The type of hydroperoxide is dictated by the nature of the fatty acid oxidized. The results given in **Figure 4** summarize TBARS values and demonstrate that their content increases progressively until reaching peak values during the storage period in both SBO and its randomized counterpart. Compared to SBO, randomized SBO had lower TBARS values at 0, 6, 12, and 96 h, but higher values at other tested points. TBARS values of randomized SBO increased sharply after 24 h, reached a peak value at 72 h because more primary oxidation products were broken down to secondary oxidation products(*40*, *41*), and then decreased at 96 h with a lower value. Meanwhile, TBARS values of unrandomized SBO started to increase dramatically after 72 h and reached the highest level at 96 h. Thus, randomized SBO was more stable at the early storage period and less stable afterward. The difference in TBARS values between the original and the randomized oil was possibly caused by a combination of loss of α-tocoherol and changes in positional distribution of fatty acids. The overwhelming influence of α-tocoperol as compared to positional distribution effect of unsaturated fatty acid on oxidative stability in oils was demonstrated as the original oil is considered to be more stable than its randomized counterpart by comparison of levels of the difference of TBARS values between early storage period and later period. The differences of TBARS value between randomized and original oils were much higher at the later storage period compared with the early period. The results also show that both randomized MHO and its original counterpart followed an increasing trend for TBARS values. Randomized MHO had generally lower TBARS values throughout the entire experimental period, thus indicating its better oxidative stability during the first 72 h of storage, and such results are also caused by loss of α-tocopherol and redistribution of unsaturated fatty acids, but additional work is required to shed light on this observation.

Conclusions. This study demonstrated that chemical randomization leads to the redistribution of fatty acids among the stereoisomeric *sn*-1,3 and *sn*-2 positions of the glycerol moiety

of TAGs of SBO and MHO. However, the effect of randomization on positional distribution of each fatty acid occurred at different levels in the oils tested. Furthermore, whereas results obtained from GC and ^{13}C NMR spectral analysis were different when positional distributions of C18:4, C20:5, C22:5, and C22:6 in seal blubber and menhaden oils were compared before and after randomization, they followed a similar trend. Redistribution of fatty acids, especially unsaturated fatty acids, is expected to lead to changes in the chemical and physical properties of the oils and hence in their oxidative stability. The changes in stability of the oils are also partially due to the loss of some α-tocopherol upon randomization, which is a subject of interest for future research.

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