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# Effect of Enzymatic Randomization on Positional Distribution and Stability of Seal Blubber and Menhaden Oils

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**ABSTRACT:** In an effort to investigate the effect of positional distribution on oxidative stability of menhaden and seal blubber oils, Novozyme 435 was used as a random biocatalyst. Positional distribution of fatty acids was determined using gas chromatography. As some of the α-tocopherol was lost during randomization, its content was adjusted to the level prior to the process to eliminate this effect on oxidative stability of oils tested. Conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) were used as indicators of oxidative stability. The results showed that the polyunsaturated fatty acids were distributed predominantly at terminal positions in randomized menhaden oil, whereas they were distributed more evenly among all positions in enzymatically randomized seal blubber oil, compared to their unrandomized counterparts. Results of CD and TBARS values indicated that randomized menhaden oil was more stable than the original oil, whereas randomized seal blubber oil was more vulnerable to oxidation compared to its counterpart. Changes of oxidative stability after randomization were mainly due to positional redistribution of fatty acids, especially those of the polyunsaturated types.

**KEYWORDS:** enzymatic randomization, Novozyme 435, seal blubber oil, menhaden oil, positional distribution, omega-3 fatty acids, α-tocopherol, oxidative stability, conjugated dienes, thibarbituric acid reactive substances

## INTRODUCTION

The omega-3 oils of marine origin contain high levels of polyunsaturated fatty acids (PUFA). For instance, seal blubber oil (SBO) typically contains 5.4−6.4% eicosapentaenoic acid (EPA), 4.6−4.9% docosapentaenoic acid (DPA), and 7.6−7.9% docosahexaenoic acid (DHA), whereas menhaden oil (MHO) contains 10.4−13.2% EPA, 2.0−2.4% DPA, and 10.1−11.5% DHA.[1−3] These PUFA play a number of important roles in maintaining normal functions of the human body. Studies have shown the biological significance of DHA in supporting normal development of the brain, the eyes, and the nervous system, especially for infants and fetuses, and thus addition of DHA in the diet of pregnant and lactating women as well as infant formula is strongly recommended.[4−7] EPA serves as a precursor of signaling molecules, eicosanoids, which play a vital role in inflammation, regulating blood flow, immune response, and ion transport, among others. In addition, long-chain omega-3 PUFA 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.[8] The role of omega-3 oils in alleviating cancer,[9,10] cardiovascular disease,[11,12] psychiatric disorders,[13,14] Parkinson's disease,[15] and inflammatory disorders [16−18] has been extensively studied and well demonstrated in the literature during the past three decades.

Chemical and enzymatic randomizations are common means to modify the chemical and physical properties of the oils.[19,20] The effect of chemical randomization on positional distribution and stability of seal blubber and menhaden oils has already been studied.[21] The results 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 triacylglycerols (TAGs) of SBO and MHO, and 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 of the α-tocopherol present upon randomization due to esterification with the fatty acids present.[22] The contribution of the latter effect in previous studies was not eliminated, and hence results on the effect of positional distribution of fatty acids were not independent of this factor.[23]

The purpose of the present study was not only to examine the effect of enzymatic randomization on positional distribution of fatty acids in SBO and MHO, using gas chromatography (GC), but also to investigate the effect of positional distribution of fatty acids on the oxidative stability of the resultant oils. The conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) values were compared following accelerated oxidation at 60 °C for 3 days after α-tocopherol was added to its original level after randomization.

## MATERIALS AND METHODS

Novozyme 435, thiobarbituric acid, porcine pancreatic lipase (EC 3.11.3), α-tocopherol, tocopherol standards, 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 dichloroflurescein) were purchased from Sigma-Aldrich (Mississauga, ON, Canada). Standard of fatty acid methyl esters (FAMEs; GLC-461) were purchased from Nu-Check (Elysian, MN). All solvents used were of analytical grade and purchased from Fisher Scientific (Nepan, ON, Canada).

**Methods.** *Enzymatic Randomization of SBO and MHO Catalyzed by Novozyme 435 (Lipase Acrylic Resin from Candida antarctica).* Enzymatic randomization was carried out following the method of

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Criado et al.[24] with minor modification. SBO and MHO (25 g) were heated under a nitrogen blanket in round-bottom flasks at 75 °C. The randomization reaction was initiated with the addition of 10% (w/w) Novozyme 435 (Sigma-Aldrich) and maintained for 3 h at 75 °C ± 1 under a nitrogen blanket. The resultant mixtures were washed with sodium bicarbonate (0.05 M) solution to neutralize free fatty acids, and the soaps so produced were removed by hot water. Bentonite clay (1.5%, w/w; Sigma-Aldrich) was subsequently added to remove colorants and residual water, followed by vacuum filtration. The resultant oils were stored at −20 °C until use within 2 weeks.

*Determination of Positional Distribution of Fatty Acids by GC Analysis. (a) Selective Hydrolysis Using Pancreatic Lipase.* The oil samples were hydrolyzed using pancreatic lipase as described by Christie[25] 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 (New Brunswick Scientific, New Brunswick, NJ) 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.

*(b) 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-wavelength (254 nm) 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.[26]

*(c) Fatty Acid Compositional Analysis of Hydrolytic Products.* Fatty acid composition and positional distribution of the products were determined following their conversion to the corresponding methyl esters. The transmethylation reagent (2.0 mL) consisted of freshly prepared 6% sulfuric acid in methanol containing 15 mg of hydroquinone as an antioxidant, which 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 again to each vial to prevent oxidation. The FAMEs were extracted three times, each time 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 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 ChemStation 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.

*(d) Determination of Tocopherol Content by HPLC-MS.* Seal blubber oil and menhaden oil as well as their randomized counterparts were saponified according to the procedure described by Maguire et al.[27] with some modifications. The oil (0.5 g) was mixed thoroughly with 1 mL of 60% KOH (w/v) and 4 mL of 0.25% (w/v) ethanolic pyrogallol in screw-capped tubes fitted with Teflon-lined screw caps. Saponification progressed at 70 °C in a water bath for 45 min. The tubes were then cooled in ice, and unsaponified components were extracted three times with 2 mL of hexane. Hexane extracts were combined and evaporated under a stream of nitrogen and redissolved in 2 mL of hexane. Tocopherol standards were prepared by dissolving a known tocopherol mixture (consisting of four tocopherol analogues) in hexane and then by diluting to different concentrations. Prior to HPLC analysis, both samples and standards were filtered using a 0.45 μm syringe-filter (Whatman, Clifton, NJ).

Tocopherol content in prepared samples and standards was determined by normal phase high-performance liquid chromatography (HPLC)−mass spectrometry (MS). The analysis was performed using an Agilent 1100 HPLC system (Agilent) with a UV−diode array detector (UV-DAD). Separation was achieved on a Supelcosil LC-Si column (250 mm × 4.6 mm i.d., 5 μm, Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) coupled with a Supelcosil LC-Si guard column. Tocopherols were eluted using an isocratic solvent system containing hexane/diethyl ether (99:1, v/v) at a flow rate of 1.0 mL/min. Each tocopherol standard and sample (80 μL) was injected by using an autosampler. Tocopherols were detected at 295 nm by a UV detector and identified by comparing their retention times with those of known tocopherol standards. LC flow was analyzed online by a mass spectrometric detector system (LC-MSD-Trap-SL, Agilent) using positive ion atmospheric pressure chemical ionization (APCI). The operating conditions used were 121 V for the fragmentor voltage, drying temperature of 350 °C, APCI temperature of 400 °C, nebulizer pressure of 60 psi, and drying gas flow of 7 L/min. A standard curve was constructed for each tocopherol homologue (peak area versus concentration). Tocopherol concentrations in samples were obtained from the standard curve and expressed as milligrams of α-tocopherol per 100 g of oil.

*(e) Recovery of α-Tocopherol in Randomized Oils.* Standard α-tocopherol was dissolved in hexane and added to randomized SBO and MHO to replenish α-tocopherol contents to their initial levels. Hexane was removed from samples under a flow of nitrogen, and oils were stored under −20 °C for up to 2 weeks for oxidative stability tests.

*(f) 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 3 days. Each day (24 h) of storage under such conditions is equivalent 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, and 72 h, cooled to room temperature, flushed with nitrogen, capped, and stored at −20 °C for up to 7 days until analyzed. The experiments were carried out in triplicate. CD in the oils was determined according to IUPAC method 20505.[28] 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.[29] 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

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

| | *sn*-1 + *sn*-3[b] | | *sn*-2[c] | |
|---|---|---|---|---|
| fatty acid | SBO | ERSBO | SBO | ERSBO |
| C14:0 | 58.70 ± 0.51a | 63.54 ± 0.28b | 41.30 ± 0.51 | 36.46 ± 0.28 |
| C14:1ω-9 | 58.44 ± 0.40a | 67.73 ± 0.34b | 41.56 ± 0.40 | 32.27 ± 0.34 |
| C16:0 | 64.16 ± 0.35a | 65.72 ± 0.23b | 35.84 ± 0.35 | 34.28 ± 0.23 |
| C16:1ω-7 | 57.38 ± 0.78a | 63.29 ± 0.36b | 42.62 ± 0.78 | 36.71 ± 0.36 |
| C17:1 | 66.41 ± 2.22a | 58.96 ± 1.68b | 33.59 ± 2.22 | 41.04 ± 1.68 |
| C18:0 | 93.00 ± 1.31a | 78.28 ± 0.83b | 7.00 ± 1.31 | 21.72 ± 0.83 |
| C18:1ω-9 | 63.80 ± 0.56a | 66.18 ± 0.17b | 36.20 ± 0.56 | 33.82 ± 0.17 |
| C18:1ω-11 | 56.61 ± 1.76a | 61.43 ± 0.87b | 43.39 ± 1.76 | 38.57 ± 0.87 |
| C18:2ω-6 | 77.17 ± 1.48 | 69.00 ± 5.20 | 22.83 ± 1.48 | 31.00 ± 5.20 |
| C18:3ω-3 | 79.26 ± 2.56a | 86.23 ± 1.03b | 20.74 ± 2.56 | 13.77 ± 1.03 |
| C18:4 ω-3 | 70.58 ± 0.89a | 59.49 ± 1.65b | 29.42 ± 0.89 | 40.51 ± 1.65 |
| C20:1ω-9 | 68.50 ± 0.28a | 69.89 ± 0.11b | 31.50 ± 0.28 | 30.11 ± 0.11 |
| C20:5ω-3 | 68.44 ± 1.09a | 52.98 ± 0.53b | 31.56 ± 1.09 | 47.02 ± 0.53 |
| C22:1ω-11 | 67.34 ± 2.30a | 75.80 ± 2.01b | 32.66 ± 2.30 | 24.20 ± 2.01 |
| C22:5ω-3 | 90.16 ± 2.48a | 75.52 ± 0.23b | 9.84 ± 2.48 | 27.48 ± 0.23 |
| C22:6ω-3 | 76.11 ± 1.06a | 63.80 ± 0.02b | 23.89 ± 1.06 | 36.20 ± 0.02 |

[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; ERSBO, 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.

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

| | *sn*-1 + *sn*-3[b] | | *sn*-2[c] | |
|---|---|---|---|---|
| fatty acid | MHO | ERMHO | MHO | ERMHO |
| C14:0 | 67.97 ± 0.67a | 61.73 ± 0.25b | 32.03 ± 0.67 | 38.27 ± 0.25 |
| C14:1ω-9 | 69.88 ± 0.71a | 65.87 ± 0.44b | 30.12 ± 0.71 | 34.13 ± 0.44 |
| C16:0 | 67.22 ± 0.64a | 63.01 ± 0.25b | 32.78 ± 0.64 | 36.99 ± 0.25 |
| C16:1ω-7 | 69.68 ± 0.65a | 62.04 ± 0.22b | 30.32 ± 0.65 | 37.96 ± 0.22 |
| C17:1 | 65.26 ± 0.30a | 60.57 ± 0.51b | 34.74 ± 0.30 | 39.43 ± 0.51 |
| C18:0 | 78.78 ± 0.34a | 72.69 ± 0.48b | 21.22 ± 0.34 | 27.31 ± 0.48 |
| C18:1ω-9 | 76.37 ± 0.28a | 72.66 ± 0.34b | 23.63 ± 0.28 | 27.34 ± 0.34 |
| C18:1ω-11 | 76.07 ± 1.47a | 65.01 ± 1.53b | 23.93 ± 1.47 | 34.99 ± 1.53 |
| C18:2ω-6 | 83.35 ± 0.47a | 74.76 ± 2.19b | 16.65 ± 0.47 | 25.24 ± 2.19 |
| C18:3ω-3 | 74.69 ± 1.33 | 73.99 ± 0.88 | 25.31 ± 1.33 | 26.01 ± 0.88 |
| C18:4 ω-3 | 60.27 ± 0.61 | 62.17 ± 0.45 | 39.73 ± 0.61 | 37.83 ± 0.45 |
| C20:1ω-9 | 88.6 ± 1.69 | 90.75 ± 0.69 | 11.4 ± 1.69 | 9.25 ± 0.69 |
| C20:5ω-3 | 49.46 ± 0.13a | 61.28 ± 0.33b | 50.54 ± 0.13 | 38.72 ± 0.33 |
| C22:5ω-3 | 56.81 ± 1.49a | 71.97 ± 0.51b | 43.19 ± 1.49 | 28.03 ± 0.51 |
| C22:6ω-3 | 50.97 ± 1.06a | 66.07 ± 0.44b | 49.03 ± 1.06 | 33.93 ± 0.44 |

[a] Values across a row not sharing a common letter are significantly different from one another ($P < 0.05$). MHO, menhaden oil before randomization; ERMHO, 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.

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).

## RESULTS AND DISCUSSION

**Fatty Acid Positional Distributions by GC Analysis.** The positional distribution of the major fatty acids of SBO, MHO, and their enzymatically randomized counterparts ERSBO and ERMHO, 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 C18:2ω-6, and those of the omega-3 class including C18:3ω-3, C18:4ω-3, C22:5ω-3, and C22:6ω-3 were preferentially located at the *sn*-1,3 positions (terminal positions) (Table 1). The important omega-3 fatty acid C20:5 was distributed nearly evenly among *sn*-1,3 (68%) and *sn*-2 (32%) positions (Table 1). It is important to understand that equal distribution in this study is defined as a *sn*-1,3 regiospecificity of 66.67 mol % and a *sn*-2 regiospecificity of 33.33 mol %.

Saturated fatty acids are located differently in TAG of the original SBO; for instance, C18:0 resides mostly in the terminal *sn*-1,3 positions (93%) and C16:0 appears to be fairly 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) at the *sn*-1,3 positions. The other monounsaturated residues, C14:1ω-9, C16:1ω-7, and C18:1ω-11, were in slight excess at the *sn*-2 positions, judging from the *sn*-2 area percentages, which were close to 43% in each case (Table 1).

The distribution of PUFA at the *sn*-2 position in the glycerol backbone of the TAGs of chemically randomized seal blubber oil was recognized except for C18:3ω-3 (Table 1). For instance, 8% of C18:3ω-3 was relocated from the *sn*-1,3 positions to the *sn*-2 position during randomization (Table 1). Distribution of C18:4ω-3 at *sn*-1,3 positions decreased from 71 to 59%, whereas that of C20:5ω-3 decreased from 68 to 53% in ERSBO. These fatty acids were nearly equally distributed among terminal positions and middle position in randomized oils. Meanwhile, the proportion of C22:5ω-3 and C22:6ω-3 chains decreased about 15 and 13% in ERSBO, respectively. However, C22:5ω-3 and C22:6ω-3 were still predominantly located at terminal positions of ERSBO. The C18:3ω-3 was the only PUFA that increased its distribution on terminal positions in ERSBO, and 7% of it was relocated from middle position to the terminal positions.

The positional distribution of saturated fatty acids was affected to different degrees by enzymatic randomization. For instance, myristic acid (C14:0) and palmitic acid (C16:0) residues increased 5 and 2% on terminal positions, respectively (Table 1). Although C18:0 remained attached preferentially to the *sn*-1,3 positions of the resultant oil, it decreased by 15% at terminal positions.

Monounsaturated residues, including C16:1, C18:1, C20:1, and C21:1, increased by 1−9% on the terminal *sn*-1,3 positions, with the exception of C17:1, which was relocated (7%) from the *sn*-1,3 positions to the *sn*-2 position in ERSBO.

*Menhaden Oil before and after Chemical Randomization.* In the original MHO, omega-3 PUFA C18:4, C20:5, C22:5, and C22:6 were attached preferentially on the *sn*-2 position, and

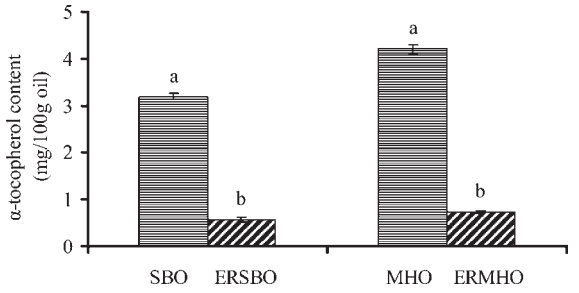


**Figure 1.** α-Tocopherol contents in randomized seal blubber and menhaden oils and their counterparts. SBO, seal blubber oil before randomization; ERSBO, seal blubber oil after randomization; MHO, menhaden oil before randomization; ERMHO, menhaden oil after randomization.

other PUFA, including C18:2ω6 and C18:3ω3, were esterified preferentially on the *sn*-1,3 positions of glycerol, as judged from the *sn*-1,3 percentages of 83 and 75%, respectively (Table 2).

Of the three major saturated fatty acids of MHO, C14:0 and C16:0 equally resided among middle position and terminal positions, whereas C18:0 distributed mostly on the *sn*-1,3 positions (Table 2). Of the six monounsaturated fatty acids examined, five of them present *sn*-1,3 percentages of 70% or higher, and the sixth one (C17:1) was close to evenly distributed (65% *sn*-1,3).

The PUFA were distributed more evenly on terminal and middle positions of ERMHO compared to their positional distribution in the original oil. For instance, the proportion of *sn*-1,3 chains of C18:2ω-6 decreased (9%) from 83 to 74%, whereas that of C18:3ω-3 decreased (1%) from 74 to 73%; such results indicated a trend of equal distribution (66.67% on terminal positions) in ERMHO (Table2). Other PUFA, including C18:4 ω-3, C20:5ω-3, C22:5ω-3, and C22:6ω-3, which were preferentially located on the *sn*-2 position in the original oil, exhibit an increased distribution on terminal positions in the randomized oil. Relocation of C18:4 ω-3, C20:5ω-3, C22:5ω-3, and C22:6ω-3 residues from the *sn*-2 position to *sn*-1,3 positions resulted in an equal distribution. C18:4ω-3 residues increased to 62% on the *sn*-1,3 positions upon its redistribution, whereas the proportion of C20:5ω-3 on the *sn*-1,3 positions increased to 61%, indicating a significant relocation (12%). C22:5ω-3 and C22:6ω-3 residues showed a 15% increase at the *sn*-1,3 positions and final contributions of 72 and 66%, respectively.

The enzymatic randomization of menhaden oil resulted in a decreased distribution of C14:0, C16:0, and C18:0 on the *sn*-1,3 positions from 4 to 6%. The C14:0 residue at *sn*-1,3 positions decreased from 68 to 62%, whereas that of C16:0 decreased from 67 to 63%. There was a 6% decrease in the proportion of C18:0 on the *sn*-1,3 positions in ERMHO. Monounsaturated residues, including C16:1, C17:1, C18:1, C20:1, and C21:1, decreased by 4−11% on the *sn*-1,3 positions, with the exception of C21:1, which was relocated (2%) from the *sn*-1,3 positions to the *sn*-2 position in ERMHO.

*Tocopherol Content.* α-Tocopherol is the natural antioxidant in marine oils, and it is the only tocopherol homologue detected in SBO and MHO.[30,31] Figure 1 shows changes in the content of α-tocopherol following randomization. A significant ($p < 0.05$) decrease in the content of α-tocopherol was noted in randomized oils, 82% for both SBO and MHO. Formation of tocopherol esters in randomized oil during enzymatic randomization

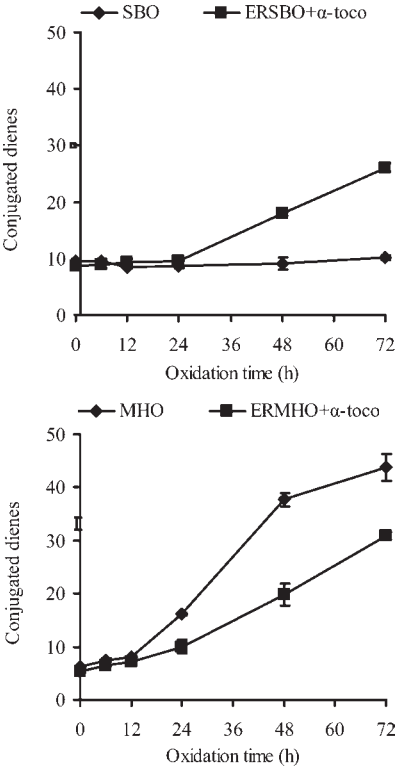


**Figure 2.** Conjugated dienes values of SBO (top) and MHO (bottom) before and after enzymatic randomization and storage under Schaal oven condition at 60 °C. SBO, seal blubber oil before randomization; ERSBO+α-toco, seal blubber oil after randomization; MHO, menhaden oil before randomization; ERMHO+α-toco, menhaden oil after randomization with added α-tocopherol.

as well as their loss or oxidation may be responsible for the decrease in the α-tocopherol content. α-Tocopherol acts as a natural antioxidant in fish and seal blubber oils. Thus, commercial α-tocopherol was added in randomized oils to adjust its content to the original levels prior to conducting the oxidative stability tests in order to eliminate the effect of lost α-tocopherol on the oxidative stability of the tested oils.

**Oxidative Stability.** The CD value of lipids, measured by 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.[32] Figure 2 shows the CD contents of enzymatically randomized SBO and its original 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 hydroperoxides as primary oxidation products.[33] Formation of lipid hydroperoxides coincides with that of CD upon oxidation.[34] 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 the oxidation chain reaction. As the storage time was extended to 48 h, large amounts of primary oxidation products were produced due to the abundance of free radicals and the oxidation chain reaction, and this sharply increased CD values (Figure 2). CD values reached the highest values at 72 h. Values obtained from the 0, 6, 12, and 24 h tests did not show any significant

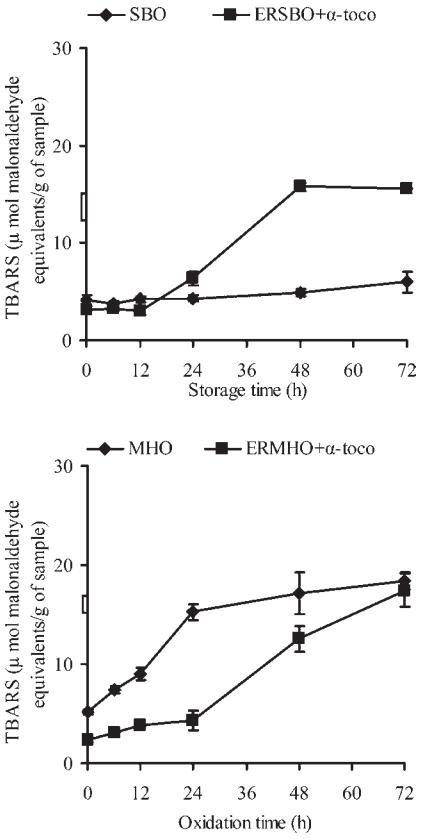


**Figure 3.** TBARS values of SBO (top) and MHO (bottom) before and after enzymatic randomization and storage under Schaal oven condition at 60 °C. SBO, seal blubber oil before randomization; ERSBO+α-toco, seal blubber oil after randomization with added α-tocopherol; MHO, menhaden oil before randomization; ERMHO+α-toco, menhaden oil after randomization with added α-tocopherol.

difference between the randomized and original oils. However, CD values from the original SBO increased sharply from 48 h, and randomized SBO showed a significantly lower CD value afterward, including 48 and 72 h, than its unrandomized counterpart; thus, randomized SBO was more stable compared to its counterpart. Positional distribution of fatty acids, especially PUFA, is thought to influence oxidative stability. Fewer PUFA, including C18:2, C18:4, C20:5, C22:5, and C22:6 (Figure 2), were located on the terminal positions in the randomized oil, and this contributes to the enhanced oxidative stability of the resultant products.

The CD values of both original MHO and its randomized counterpart were also increased during the oxidation period, and the highest values appeared at 72 h. By comparison of each tested point with the original oil, randomized MHO had generally higher CD values, which is an indicator of lower oxidative stability. The reason for the differences of CD value between the two groups of oil samples is due to the fact that more PUFA, including C18:4, C20:5, C22:5, and C22:6 (Figure 2), were located on the terminal positions, which are considered to be less protected from oxidation as they are more exposed to the oxygen compared to those located at the middle position.

The TBA test has been commonly used to assess the degree of lipid oxidation by measuring the content of secondary oxidation products. The rate of formation of secondary oxidation products is mainly affected by three factors, including the amount of hydroperoxides as primary oxidation products, the level of α-tocopherol, and the nature of hydroperoxides, which may be decomposed to secondary oxidation products at different rates under the same conditions.[35,36] The type of hydroperoxide is dictated by the nature of the fatty acid oxidized. The results shown in Figure 3 summarize TBARS values and demonstrate their progressively increased content until reaching their highest values during the storage period in both SBO and its randomized counterpart. Compared to SBO, randomized SBO had higher TBARS values from 24 to 72 h, but lower values at other tested points without significant difference TBARS values of randomized SBO increased sharply after 12 h and reached the highest value at 72 h, when more primary oxidation products were broken down to secondary oxidation products; the values may decrease or increase once the oxidation period is extended. On the other hand, TBARS values of original SBO increased slowly through the whole oxidation period and reached the highest value until 72 h. By comparison of these two groups of TBARS values, the original oil showed a steadier oxidative stability than the randomized SBO during the tested period. The decreased oxidative stability in randomized oil was possibly caused by changes in positional distribution of fatty acids, especially PUFA (Table 1). The results obtained for MHO samples also showed that both randomized MHO and original MHO followed an increasing trend for TBARS values during the oxidation period. Randomized MHO had generally lower TBARS values throughout the entire experimental period, thus indicating its better oxidative stability during the 72 h of storage, and such results are also caused by redistribution of unsaturated fatty acids.

**Conclusions.** Fatty acids were redistributed among the stereoisomeric *sn*-1,3 and *sn*-2 positions of the glycerol moiety of TAGs of SBO and MHO at different levels, and this led to the changes of oxidative stability of these omega-3 oils. Redistribution of fatty acids, in particular, unsaturated fatty acids, is confirmed to be one of the major factors that affect the oxidative stability of omega-3 oils; higher distribution of PUFA on terminal positions leads to weaker oxidative stability of the oil.

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## REFERENCES

(1) Wanasundara, U. N.; Shahidi, F. Positional distribution of fatty acids in triacylglycerols of seal blubber oil. *J. Food Lipids* **2007**, *4*, 51–64.

(2) Senanayake, S. P. J. N.; Shahidi, F. Enzyme-catalyzed synthesis of structured lipids via acidolysis of seal (*Phoca groenlandica*) blubber oil with capric acid. *Food Res. Int.* **2002**, *8*, 745–752.

(3) Kim, I.-H.; Hill, C. G., Jr. Lipase-catalyzed acidolysis of menhaden oil with pinolenic acid. *J. Am. Oil Chem. Soc.* **2006**, *83*, 109–115.

(4) Simopoulos, A. P. Omega-3 fatty acids in health and disease and growth and development. *Am. J. Clin. Nutr.* **1991**, *54*, 438–463.

(5) Denomme, J.; Stark, K. D.; Holub, B. J. Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations. *J. Nutr.* **2005**, *135*, 206–211.

(6) Haag, M. Essential fatty acids and the brain. *Can. J. Psychiatry* **2003**, *48*, 195–203.
(7) Craig-Schmidt, M. C.; Stieh, K. E.; Lien, E. L. Retinal fatty acids of piglets fed docosahexaenoic and arachidonic acids from microbial sources. *Lipids* **1996**, *31*, 53–59.
(8) Serhan, C. N.; Yang, R; Martinod, K.; Kasuga, K; Pillai, P. S; Porter, T. F.; Oh, S. F.; Spite, M. Maresins: a novel macrophage mediators with potent anti-inflammatory and proresolving actions. *J. Exp. Med.* **2008**, *206*, 13–23.
(9) De Deckere, E. A. Possible beneficial effect of fish and fish *n*-3 polyunsaturated fatty acids in breast and colorectal cancer. *Eur. J. Cancer Prev.* **1999**, *8*, 213–221.
(10) Chen, Y. Q. Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. *J. Clin. Invest.* **2007**, *117*, 1866.
(11) Marchioli, R. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the GISSI-Prevenzione. *Circulation* **2002**, *105*, 1897–1903.
(12) Wang, C. C.; Harris, W. S.; Chung, M.; Lichtenstein, A. H.; Balk, E. M.; Kupelnick, B.; Jordan, H. S.; Lau, J. *n*-3 Fatty acids from fish or fish-oil supplements, but not α-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am. J. Clin. Nutr.* **2006**, *84*, 5–17.
(13) Puri, B. K. High-resolution magnetic resonance imaging sinc-interpolation-based subvoxel registration and semi-automated quantitative lateral ventricular morphology employing threshold computation and binary image creation in the study of fatty acid interventions in schizophrenia, depression, chronic fatigue syndrome and Huntington's disease. *Int. Rev. Psychiatry* **2006**, *18*, 149–154.
(14) Nemets, H.; Nemets, B.; Apter, A.; Bracha, Z.; Belmaker, R. H. Omega-3 treatment of childhood depression: a controlled, double-blind pilot study. *Am. J. Psychiatry* **2006**, *163*, 1098–1100.
(15) Bousquet, M.; Saint-Pierre, M.; Julien, C.; Salem, N.; Cicchetti, F.; Calon, F. Beneficial effects of dietary omega-3 polyunsaturated fatty acid on toxin-induced neuronal degeneration in an animal model of Parkinson's disease. *FASEB J.* **2008**, *22*, 1213–1225.
(16) Chandrasekar, B.; Troyer, D. A.; Venkatraman, J. T.; Fernandes, G. Dietary omega-3 lipids delay the onset and progression of autoimmune lupus nephritis by inhibiting transforming growth factor beta mRNA and protein expression. *J. Autoimmun.* **1995**, *8*, 381–393.
(17) Fisher, M.; Levine, P. H.; Weiner, B. H. Dietary n-3 fatty acid supplementation reduces superoxide production and chemiluminescence in a monocyte-enriched preparation of leukocytes. *Am. J. Clin. Nutr.* **1990**, *51*, 804–808.
(18) De Caterina, R.; Zampolli, A. n-3 Fatty acids: antiatherosclerotic effects. *Lipids* **2001**, *36*, S69–S78.
(19) Marangoni, A. G.; Rousseau, D. Engineering triacylglycerols: the role of interesterification. *Trends Food Sci. Technol.* **1995**, *6*, 329–335.
(20) Rousseau, D.; Marangoni, A. G. Chemical interesterification. In *Food Lipids*; Akoh, C., Min, D., Eds.; Dekker: New York, 1997; pp 251−281.
(21) Wang, J.; Reyes Suárez, E.; Kralovec, J.; Shahidi, F. Effect of chemical randomization on positional distribution and stability of omega-3 oil triacylglycerols. *J. Agric. Food Chem.* **2010**, *58*, 8842–8847.
(22) Criado, M.; Hernandéz-Martins, E.; Lopéz-Hernandéz, O. A.; Otero, C. Enzymatic interesterification of extra virgin olive oil with a fully hydrogenated fat: characterization of the reaction and its products. *J. Am. Oil Chem. Soc.* **2007**, *84*, 717–726.
(23) Christie, W. W. Structural analysis of lipids by means of enzymatic hydrolysis. In *Lipid Analysis*; Christie, W. W., Ed.; Pergamon Press: New York, 1982; pp 155−166.
(24) Senanayake, S. P. J. N.; Shahidi, F. Concentration of docosahexanoic acid (DHA) from algal oil *via* urea complexation. *J. Food Lipids* **2000**, *7*, 51–61.
(25) Maguire, L. S.; O'Sullivan, S. M.; Galvin, K.; O'Connor, T. P.; O'Brien, N. M. Fatty acid profile, tocopherol, squalene, and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *Int. J. Food Sci. Nutr.* **2004**, *55*, 171–178.
(26) IUPAC. *Standard Methods and Recommended Practices of the American Oil Chemists' Society*, 4th ed.; AOCS: Champaign, IL, 1987.
(27) AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th ed.; AOCS: Champaign, IL, 1990.
(28) Ackman, R. G.; Courmier, A. α-Tocopherol in some Atlantic fish and shellfish with parWular reference to live-holding without food. *J. Fish. Res. Board Can.* **1967**, *24*, 357–373.
(29) Kinsella, J. E. *Seafoods and Fish Oils in Human Health and Disease*; Dekker: New York, 1987; pp 231−237.
(30) Jackson, H. W. Techniques for flavour and odour evaluation of soy oil. *J. Am. Oil Chem. Soc.* **1981**, *58*, 227–231.
(31) Logani, M. K.; Davies, R. E. Lipid oxidation: biologic effects and antioxidants—a review. *Lipids* **1980**, *15*, 485–495.
(32) Hamam, F.; Shahidi, F. Acidolysis reactions lead to esterification of endogenous tocopherols and compromised oxidative stability of modified oils. *J. Agric. Food Chem.* **2006**, *54*, 7319–7323.
(33) Farmer, E. H.; Sutton, D. A. Peroxidation in relation to olefinic structure. *Trans. Faraday Soc.* **1946**, *42*, 228–232.
(34) Nevdakh, O. P.; Agabekov, V. E.; Mitskevich, N. I. Kinetics of the formation and decomposition of hydroperoxide in the oxidation of 8-pentadecanone. *React. Kinet. Catal. Lett.* **1984**, 225–230.
(35) Strange, E. D.; Konstance, R. P.; Lu, D.; Smith, P. W.; Onwulata, C. I.; Holsinger, V. H. Oxidative and functional stability during storage of butter oil encapsulated with sucrose or flour. *J. Food Lipids* **1997**, *4*, 245–260.
(36) Senanayake, S. P. J. N.; Shahidi, F. Chemical and stability of structured lipids from borage (*Borage officinalis* L.) and evening primrose (*Oenothera biennis* L.) oils. *J. Food Sci.* **2001**, *67*, 2039–2045.