Oxidative Stability of Soybean Oil Products Obtained by Regioselective Chemical Interesterification[1]

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ABSTRACT: Oxidative stability of products produced as potential margarine basestock from soybean oil and methyl stearate by a novel chemical regioselective interesterification was evaluated. The oxidative stability of the products was evaluated by peroxide formation and volatile analysis during storage in the dark with oxygen at 60°C for 72 h. The product obtained by regioselective interesterification resulted in the lowest peroxide formation and volatile concentration sample in comparison with soybean oil and the randomized product of the regioselective interesterified product. Regioselective interesterification of soybean oil with methyl stearate produced a product with increased oxidative stability.
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KEY WORDS: Fatty acid position, interesterification, methyl stearate, oxidative stability, peroxide formation, randomization, regioselectivity, soybean oil, triacylglycerols, volatiles.

It is important to prevent oxidation of edible fats and oils and of foods that contain oils to maintain their quality and safety. Oxidation of fats and oils can be initiated by heat, light, and metals in the fats and oils. The oxidation products from oils, which include hydroperoxides and cyclic peroxides, decompose to produce a variety of volatile compounds, which result in undesirable flavors and odors in oils (1–6). Oils damaged by oxidation also have been reported to cause biological problems, such as diarrhea, growth depression, and tissue damage in living organisms (7).

The oxidative stability of an oil depends on the fatty acid (FA) composition and triacylglycerol (TAG) structure, as well as on non-TAG components, such as tocopherols, carotenoids, ascorbic acid, citric acid, free fatty acids, and sterols, which may prevent or promote oxidation (3,4,8–13). Several investigations have reported correlations of FA composition, TAG structure, and oxidative stability (14–20). Neff *et al.* (20) previously investigated the oxidative stability of purified TAG from soybean oil (SBO) in air in the dark at 60°C. The results of this work showed that oxidative stability in the dark correlated positively with a greater concentration of oleic acid (O) and lower concentrations of linoleic (L) and linolenic (Ln) acids of SBO TAG (20).

Hydrogenation has been a useful process to increase oxidative stability of edible oils because it changes polyunsaturated FA to monounsaturated and saturated FA (21–23). Hydrogenated oils can be suitable basestocks for margarine, shortening, and frying oil with improved stability and altered physical properties (24–27). But hydrogenation has not completely solved oxidative-stability problems, and there is increasing concern about the nutritional safety of partially hydrogenated oils (28–32).

Interesterification of edible oils is an important process for the modification of physical and functional properties, as are hydrogenation and fractionation (33–36). Many reports have been published on chemical and lipase-directed interesterification (33,34,37–41). Most individuals agree that chemical interesterification, catalyzed by sodium hydroxide, sodium methoxide, or sodium/potassium alloy, introduces FA randomly on the glycerol moiety, while some lipases exhibit interesterification regioselectivity (positional specificity). This latter reaction introduces FA mostly at the 1,3-positions of glycerol.

We previously studied reaction conditions that produced a regioselective chemical interesterification promoted by sodium methoxide (42). The ester exchange between SBO and methyl stearate at 1,3-positions progressed 1.7 times faster than at the 2-position at 30°C in hexane (42). The product obtained by the reaction had stearic acid mostly at the 1(3) carbon positions of the glycerol moiety.

In this current study, the oxidative stability of the product or margarine basestock obtained from SBO and methyl stearate by chemical interesterification with regioselectivity was evaluated and compared with that of the basestock from which FA were randomized.

EXPERIMENTAL PROCEDURES

Materials. SBO was obtained from a commercial source. Methyl stearate and sodium methoxide catalyst were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). The solid-phase extraction columns ("Mega Bond Elut," 60 mL vol, loaded with 10 g of silica), used for isolation of TAG

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from interesterification products, and the solid-phase extraction columns ("Bond Elut," 3 mL vol, loaded with 200 mg silica), used for resolution of lipolysis mixtures, were purchased from Varian Co. (Harbor City, CA). The solid-phase extraction columns (6.5 mL vol, loaded with 2.0 g silica), used for isolation of TAG from randomization products, were purchased from Baxter Health Care (Muskegon, MI). Pancreatic lipase (EC 3.1.1.3, type II, crude from porcine pancreas) used for positional distribution analysis of FA was obtained from Sigma Chemical Co. (St. Louis, MO). Potassium hydroxide, used as a catalyst for transmethylation of FA of TAG and 2-monoacylglycerol, was purchased from Aldrich Chemical Co., Inc. All organic solvents used in this research were high-performance liquid chromatography-grade.

METHODS

Interesterification. Regioselective interesterification between SBO and methyl stearate was conducted in the following manner and as described previously (42). The sodium methoxide-catalyzed ester interchange was carried out in 500 mL hexane in a temperature-controlled, bench-scale batch reactor (1 L vol; Parr Instrument Co., Moline, IL). The catalyst, 5 g sodium methoxide (5 wt%, wt/wt of reactants), was maintained in an atmosphere of carbon dioxide at −20°C overnight. The reactants, 20 g SBO and 80 g methyl stearate, were dried in the reactor at 60°C under vacuum for 30 min. Then, the catalyst was added to the reactants, followed by purging the reactor with nitrogen gas at 80 psi, which cooled the reactants. The mixture of reactants and catalyst was preheated to activate the catalyst. The mixture was heated to 60°C, maintained over a 15-min period and cooled down to 30°C. Based on previous work (42), the reaction started after the temperature of the reaction mixture reached 30°C. The reaction was maintained at 30°C for 24 h. Immediately after this reaction time, the product was diluted with 200 mL diethyl ether, washed with distilled water three times, and dried by anhydrous sodium sulfate. Diethyl ether and hexane were removed with a stream of helium gas.

Randomization. Randomization of a portion of the product produced by regioselective interesterification was conducted by the method of List *et al.* (43) to obtain the basestock with the same FA composition but distributed randomly on 1(3) and 2 carbon positions of the TAG glycerol moiety. Two grams of product was placed in a 10-mL round-bottomed flask, then placed on a rotary evaporator and heated for 30 min at 60°C under vacuum to remove traces of moisture from the product. Sodium methoxide (20 mg, 1.0 wt%, wt/wt of reactants) was added to the product in the round-bottomed flask. The flask was rotated to mix the reactants. After stirring, the product and catalyst were heated to 80°C for 30 min. Then the randomized product was diluted with 5 mL of diethyl ether/hexane (1:1, vol/vol), washed with distilled water three times to decompose the catalyst, and dried over anhydrous sodium sulfate. Diethyl ether and hexane were evaporated from the randomized product by a stream of helium gas.

Sample isolation. TAG was isolated from the reaction mixture by solid-phase extraction in "Mega Bond Elut" cartridges with solvent elution, as follows: 75 mL, diethyl ether/hexane (2:98, vol/vol), fraction 1, fatty acid methyl ester (FAME); 75 mL, diethyl ether/hexane (10:90, vol/vol), fraction 2, TAG; 50 mL, methanol, fraction 3, mono- and diacylglycerols. This procedure was repeated with 3 g of the product being added to the solid-phase extraction column for each purification operation. From 24 g of the product of regioselective interesterification, 17.5 g of fraction 1, 4.1 g of fraction 2, and 0.4 g of fraction 3 were obtained by solid-phase extraction. Percentage yield of FAME, TAG, and mono- and diacylglycerol were 79.5, 18.6 and 1.8%, respectively. TAG also was isolated from the randomization product by solid-phase extraction with "Bond Elut" cartridges and solvent elution, as described previously (42). Approximately 0.5 g of the product was placed on the column for each purification operation. Identification of each fraction was confirmed by thin-layer chromatography. An amount of 1.4 g of randomzed basestock was obtained from 1.5 g of regioselective basestock through randomization, followed by solid-phase extraction.

Analysis. FA analysis of TAG (fraction 2) by gas chromatography (GC), FA positional distribution on glycerol carbons by 1,3-specific lipolysis followed by GC, and composition of TAG molecular species (TAGMS) by reversed-phase high performance liquid chromatography (RP–HPLC) with flame-ionization detection were described previously (44,45).

Oxidation of products. The product from regioselective interesterification (regioselective product), the randomized product (random product), and the original SBO were purified before oxidation by solid extraction chromatography, as described previously (20). Purified TAG samples (225 mg each) were placed in capped vials and wrapped with aluminum foil to exclude light. Vials were purged with oxygen in the headspace, and kept in the dark at 60°C for 24, 48, and 72 h. Two samples per oil type were prepared for each oxidation time. Oxidation was monitored by following peroxide value (PV) and volatile formation by static headspace–gas chromatography (20). Samples (15 mg) for PV determination and 50-mg samples for volatile analysis were removed, in duplicate, from each oxidized sample per time period, including zero time. Because of insufficient sample, a volatile analysis was not conducted on the randomized product at zero time. The data results were averaged for oxidation of duplicate samples.

RESULTS AND DISCUSSION

FA composition and positional distribution on glycerol carbons of the original SBO, regioselective product, and random product are shown in Table 1. Also shown are the degree of unsaturation or average number of double bonds and oxidizability calculated from the FA composition as: $(\{0.02 \times [O\%] + [L\%] + 2 \times [Ln\%]\}/100)$ (19).

The original SBO had a FA composition typical of SBO, with 3.1% stearic acid and 9.7% palmitic acid, located mostly on glycerol carbons 1,3. The regioselective product was ob-

TABLE 1
Fatty Acid Composition, Positional Distribution on Glycerol Carbons (Refs. 20,44), Degree of Unsaturation, and Oxidizability of the Original Soybean Oil, the Regioselective Product and the Random Product

	Fatty acid (%)						
Sample/positions[a]	P	S	O	L	Ln[b]	Degree of unsaturation	OX[c]
Soybean oil						1.61	0.74
1,2,3	9.7	3.1	22.0	56.6	8.6		
1,3	14.4	4.6	21.9	48.9	10.2		
2	0.3	0.1	22.2	72.0	5.4		
Regioselective product						1.39	0.64
1,2,3	9.6	14.6	19.7	48.7	7.4		
1,3	11.6	18.0	19.1	43.1	8.2		
2	5.6	7.8	20.9	60.0	5.7		
Random product						1.39	0.64
1,2,3	9.7	14.4	19.7	49.1	7.1		
1,3	10.1	14.7	19.2	48.5	7.5		
2	9.0	13.7	20.6	50.3	6.4		

[a]Glycerol carbon numbers of triacylglycerol.
[b]P, palmitic; S, stearic; O, oleic; L, linoleic; Ln, linolenic acid.
[c]Oxidizability = (0.02 × [O%] + [L%] + 2 × [Ln%])/100 (Ref. 19).

tained by interesterification of original SBO and methyl stearate. Stearic acid concentration was increased from 3.1 to 14.6% by interesterification. Consequently, the degree of unsaturation and the oxidizability dropped from 1.61 to 1.39 and from 0.74 to 0.64, respectively. Stearic acid from methyl stearate was incorporated by intermolecular ester exchange to yield 14.8% stearic acid in the product. The stearic acid was distributed 82.2% on carbon 1,3 and 17.8% on carbon 2 of the TAG glycerol moiety. The increase of stearic acid on each of carbons 1 and 3 was 1.7 times greater than that on carbon 2. In the original SBO, palmitic and linolenic acids were concentrated on glycerol carbons 1(3), and linoleic acid was concentrated on carbon 2. Those FA were still unevenly distributed on the carbons of regioselective products. This indicated that complete intramolecular ester exchange did not occur during the interesterification.

The random basestock obtained by chemical randomization of a portion of the regioselective product had approximately the same FA composition as did the regioselective product. Therefore, calculated oxidizability and degree of unsaturation of the random product, 0.64 and 1.39, respectively, are the same values as those of the regioselective product. In the random product, however, all FA were distributed approximately equally on the 1(3) and 2 glycerol carbons of the TAG. The fatty acid distribution on carbons was the result of migration of stearic and palmitic acids from carbons 1(3) to carbon 2, and the converse migration of linoleic and linolenic acids from carbon 2 to carbons 1(3). No *trans* isomers of FA were observed in the products prepared in this study.

RP–HPLC analysis indicated 26 TAGMS in the products and the original SBO. Amounts of TAGMS in the oils are presented in Table 2. The RP–HPLC chromatogram of the regioselective product is given in Figure 1. In the regioselective product, LLS, LOS, SLP, SLS, and SOP contents were increased greatly compared with those in the original SBO. Also, the contents of LLL, LLO, LOP, OOO, and POO were decreased. Therefore, stearic acid was incorporated mostly in the soybean TAG that previously contained linoleic and oleic acids in the original SBO. These data, combined with the FA positional distribution of the original SBO and the regioselective product, indicated that stearic acid was located mostly on the 1(3) glycerol carbons of TAG.

TABLE 2
Selected Major Triacylglycerol Molecular Species (TAGMS) Contents[a] of Original Soybean Oil (SBO), Regioselective Product, and Random Product

TAGMS[b]	Original SBO (%)	Regioselective product (%)	Random product (%)
LnLL	6.0	5.6	4.5
LLL	15.4	13.4	10.6
LnLO	4.7	4.4	4.2
LnLP	2.9	2.5	2.1
LLO	15.6	13.9	13.7
LLP	12.2	11.4	9.6
LOO	8.4	6.2	6.4
LLS	3.7	8.4	9.3
LOP	9.0	7.3	7.9
PLP	2.1	2.0	2.1
OOO	3.2	1.5	1.2
LOS	3.2	6.2	8.5
POO	2.4	1.4	1.2
SLP	1.4	3.4	4.4
SOO	0.9	1.2	1.7
SLS	0.3	2.1	2.7
SOP	0.4	1.0	1.6
PPS	0.1	0.7	1.1

[a]Determined by reversed-phase high-performance liquid chromatography–flame-ionization detection (20).
[b]See Table 1 for other abbreviations.

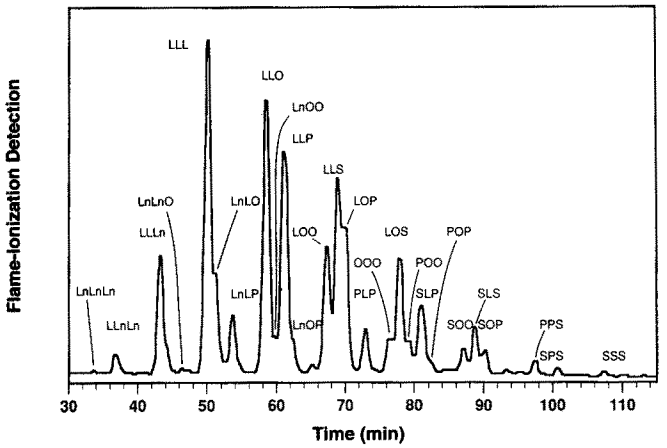

FIG. 1. Reverse-phase high-performance liquid chromatography analysis of purified regioselective product triacylglycerols. Sample size: 0.5–1.0 mg; 5 μ C-18 column (0.49 × 50 cm); 120-min solvent gradient acetonitrile/methylene chloride (70:30 to 40:60, vol/vol); flow rate, 0.8 mL/min; flame-ionization detector. Column cleaned with methylene chloride after analysis. L, linoleic; Ln, linolenic; O, oleic; P, palmitic; S, stearic.

As a result of the regioselective reaction, LOS, SLP, LLS, SOO, SLS, and SOP contents were increased moderately, and the LLL, LLP, and LnLL contents were decreased. The changes in TAGMS composition were the result of random rearrangement of FA concentrated on the 1(3) or 2 carbons of regioselective product TAG.

The oxidative stability of the original SBO and products were monitored by peroxide and volatile analyses. Plots of PV vs. oxidation time for the oxidation of TAG of the three samples are shown in Figure 2. PV of the original SBO increased with increased oxidation time, and the rate of increase accelerated gradually with oxidation time. The slope, obtained by linear regression analysis (20), of the PV vs. time plot for SBO was 0.61 meq/kg/h (correlation coefficient = 0.988). The regioselective product showed a linear increase of PV, and the slope of PV increase was 0.31 meq/kg/h (correlation coefficient = 0.999). When stearic acid was incorporated into SBO TAG by the regioselective reaction, both the degree of unsaturation and oxidizability decreased by 14%. The slope for the regioselective product's PV increase was reduced by 49%. Regioselective interesterification depressed the PV increase more than expected from the drop of degree of unsaturation and calculated oxidizability. The PV of the random product increased with oxidation time similarly to that of the original SBO before 48 h. After 48 h, however, the rate of PV increase was reduced. The slope of PV for the random product was 0.46 meg/kg/h (correlation coefficient = 0.970), greater than that of the regioselective product but less than that of the original SBO. The possibility of an effect by pro- or antioxidants on the oxidative stability of the randomized and regioselective products is not likely due to the removal of these compounds by solid-phase extraction before the oxidation studies. Thus, incorporation of stearic acid into SBO TAG resulted in a slow increase of PV with time, and the effect was greater in the product produced by regioselective interesterification than by randomization.

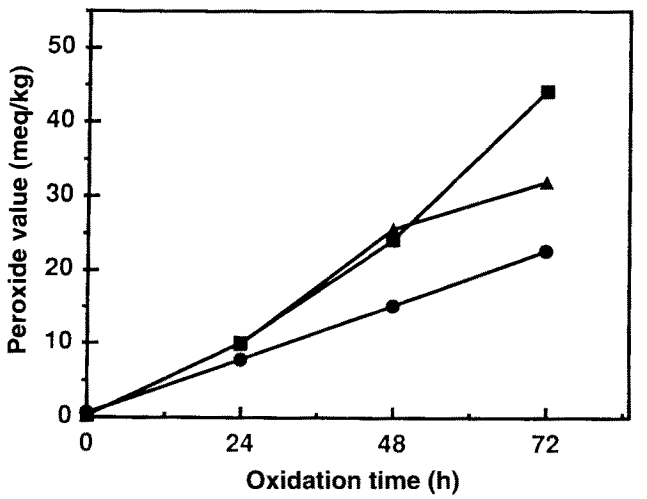

FIG. 2. Oxidation in the dark at 60°C of original soybean oil and basestocks. ■, original soybean oil; ●, regioselective product; ▲, random product.

The major volatile compounds detected from the original SBO and the regioselective and random products were pentane, pentanal, hexanal, and heptenal, which are derived from linoleic acid (L-derived volatiles) (20), and propanal and heptadienal, which are derived from linolenic acid (Ln-derived volatiles)(20). Plots of total selected volatiles, of L-derived volatiles, and of Ln-derived volatiles generated from oxidized TAG vs. oxidation time are given in Figure 3. The amount of total selected volatiles from original SBO did not increase greatly after 24 h (Fig. 3a). The regioselective product increased in total volatiles until 48 h and had lower total volatile generation than did SBO. The random product showed an increase of total volatile generation during the oxidation time examined in this study. The amounts of total volatiles, L-derived volatiles, and Ln-derived volatiles from the random product were greater than from original SBO and the regioselective product (Fig. 3b). L-derived volatiles showed a major contribution to the amount of total selected volatiles for all samples. The original SBO initially generated less Ln-derived volatiles than did the regioselective product (Fig. 3c). The regioselective and random products showed an increase in the Ln-derived volatiles in the same manner as the total selected and L-derived volatiles.

Based on the peroxide and volatile content, the regioselective product was less susceptible to oxidation than the original SBO and the random product. The improved stability of the regioselective product was presumably due to incorporation of stearic acid into TAG that contained linoleic acid. Linoleic acid was stabilized with stearic acid incorporated on the 1(3) carbon positions of the TAG glycerol moiety by regioselective interesterification. Compared with our previous investigations, which studied the oxidation mechanism of polyunsaturated TAG and the effect of TAG composition and unsaturated FA structure on oxidative stability (15–18,20),

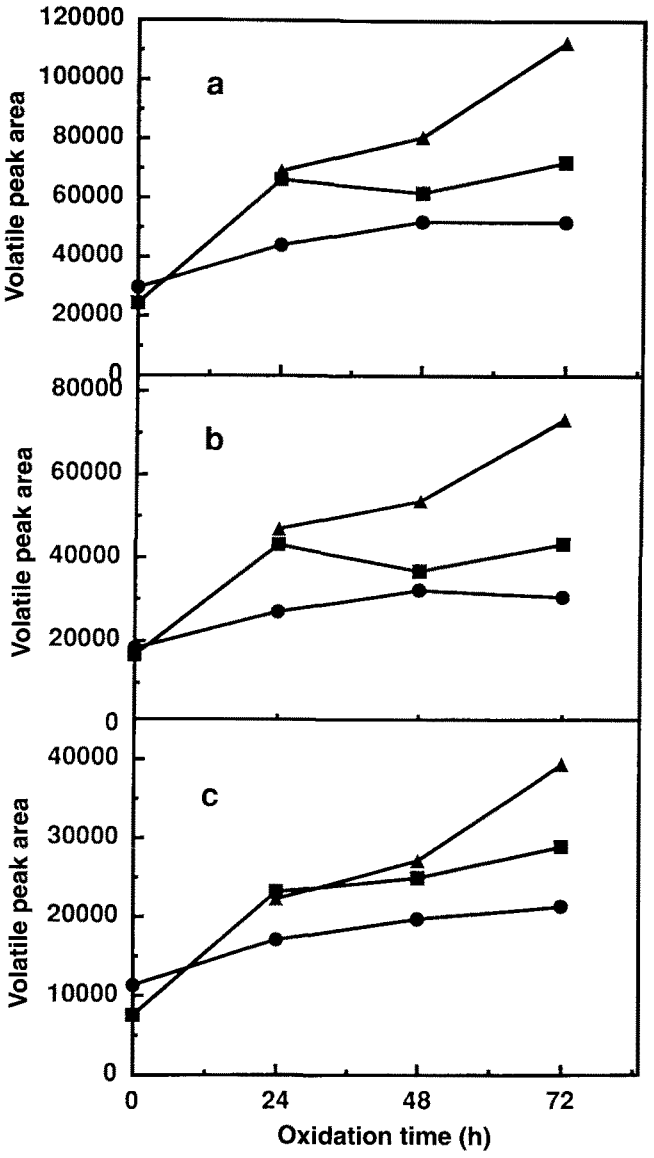

FIG. 3. Selected volatiles generated from oxidized original soybean oil basestocks, which were thermally decomposed in the static headspace analyzer (140°C, 20 min) with respect to oxidation time, a, total selected volatiles; b, volatiles derived from linoleic acid; c, volatiles derived from linolenic acid). There was not enough random product to conduct volatile studies at zero time. Symbols are the same as in Figure 2.

this study focused more on TAG with a high stearic acid composition on the 1(3) glycerol carbons.

The results presented in this study support regioselective interesterification as a useful fat modification procedure to obtain edible basestocks with high oxidative stability while maintaining polyunsaturated FA content and eliminating *trans* FA isomers.

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