Stability of Furanoid Fatty Acids in Soybean Oil[1]

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ABSTRACT: Analysis of the positional distribution of the furanoid fatty acids, 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid (F20) and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (F22), in soybean oil (SBO) indicated that they were preferentially esterified with the primary –OH groups of glycerol molecules. Hydrogenation of SBO reduced the concentrations of F20 and F22 somewhat. During exposure of SBO to daylight, F20 and F22 were completely degraded within two days, whereas linoleic acid and linolenic acid were not affected. β-Carotene inhibited both the degradation of the furanoid fatty acids and their oxidation to the odorant 3-methylnonane-2,4-dione (MND), which contributes strongly to the light-induced off-flavor of SBO. A model experiment indicated that two days of light exposure of SBO, followed by filtration through silica gel and further refining, prevented the formation of MND during subsequent storage of the oil.
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KEY WORDS: β-Carotene, furanoid fatty acids, hydrogenation, light exposure, α- and γ-tocopherol, refining.

A beany and green odor, the so-called reversion flavor, can develop in oxidized soybean oil (SBO) (1,2). In SBO stored in daylight for 30 d we observed a beany, strawy, green flavor that was somewhat different from the reversion flavor formed in darkness (3). The odorants that caused this light-induced off-flavor have recently been screened by aroma extract dilution analysis (AEDA) (4). Quantitative measurements and calculation of odor activity values (OAV, ratio of concentration to odor threshold) showed that 3-methylnonane-2,4-dione (MND) was mainly responsible for this off-flavor (3,5). Potent odorants formed by autoxidation of linoleic and linolenic acid, e.g., 1-octen-3-one, hexanal, (*Z*)-1,5-octadien-3-one and (*Z*)-3-hexenal, were additionally identified in the SBO sample (4), but they did not significantly contribute to the off-flavor because their OAV were much lower than the OAV of MND (5).

Search for the precursors of MND led to the detection of small amounts of two furanoid fatty acids, 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid (F20) and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (F22), in SBO (6). A model experiment showed that MND was formed when F20 was exposed to light in the presence of a sensitizer (6).

It has been speculated (7) that lipoxygenase is involved in the biosynthesis of furanoid fatty acids. However, an investigation of soybeans that lacked lipoxygenase isoenzymes (L-1, L-2, L-3) did not support this hypothesis (8) because there was no correlation between lipoxygenase activity in soybeans and the concentrations of F20 and F22 in the oil extracted from these seeds.

The objectives of this study were to determine the position at which the furanoid fatty acids F20 and F22 are bound to glycerol molecules in SBO and to determine the stability of F20 and F22 during hydrogenation. The stability of the two furanoid fatty acids was investigated as well as the effect of β-carotene and α- and γ-tocopherols on the production of both MND and hexanal in SBO exposed to daylight. Finally, model experiments were performed to study whether light exposure of SBO before the refining process prevented the formation of MND during storage.

EXPERIMENTAL PROCEDURES

Materials. Sample A was a commercially refined and deodorized SBO; its fatty acid composition is detailed in Table 1. The following three batches of SBO sample B were obtained from a producer of hydrogenated SBO: sample B1 was neutralized and bleached, sample B2 (melting point 35°C) was partially hydrogenated, and sample B3 (melting point 65°C) was fully hydrogenated. The catalyst used for the hydrogenation process was nickel (15%, w/w) on carrier (25%, w/w) embedded in hydrogenated fat (60%, w/w). The fatty acid composition of samples B1 to B3 is summarized in Table 1.

Samples of pure α- and γ-tocopherol were gifts from Hoffman La Roche (Basel, Switzerland), β-carotene was from Fluka (Neu-Ulm, Germany), 2- and 3-*tert*-butyl-4-hydroxyanisol (BHA) from Aldrich (Steinheim, Germany) and porcine pancreatic lipase (type VI-S, 70% protein, activity 20,000–100,000 units per mg protein) from Sigma (München, Germany). Kaolin (Aldrich) and silica gel 60 (0.063–0.2 mm; Merck, Darmstadt, Germany) were washed with concentrated HCl and water (9); the water content was adjusted to 6.1% (kaolin) and to 1.5% (silica gel). Hexane, pentane, diethyl ether, and methanol were from Merck (HPLC grade) and redistilled prior to use.

Quantitative methods. Definite amounts of the internal standard 3-[$^2H_3$]methylnonane-2,4-dione, prepared from nonane-2,4-dione and [$^2H_3$]methyl iodide (4,5), were added

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TABLE 1
Fatty Acid Composition of the Soybean Oil (SBO) Samples

Fatty acid	Sample[a] (wt%)				
	A1	A2	B1	B2	B3
16:0	9.7	10.7	9.8	10	10
18:0	4.1	4.0	3.9	10.8	90
18:1	23.7	24.8	22	71.7	<0.1
18:2	55.2	53.5	56.3	7.0	<0.1
18:3	7.0	6.9	7.8	<0.1	<0.1

[a]A1, before storage; A2, after storage for 48 h (30 h daylight, 18 h darkness) at a window facing north; 2000–3000 lux. B1, neutralized and bleached; B2 (melting point 35°C), partially hydrogenated; B3 (melting point 65°C), fully hydrogenated.

to the SBO samples. After stirring for 45 min, the oil was diluted with diethyl ether (50 mL). The volatiles, including the solvent, were distilled off in high vacuum (temperature, 50°C, pressure 3 mPa) with the apparatus reported earlier (4). The solution containing the volatiles was concentrated to a volume of 0.1 mL by distilling off the diethyl ether. MND was determined in an aliquot of the sample by mass chromatography (μS 8230; Finnigan, Bremen, Germany) on an SW-10 capillary column (30 m × 0.32 mm fused-silica capillary, Supelcowax 10; Supelchem, Sulzbach, Germany) (3,5) and by using the chemical ionization mode (3). The amount of MND was calculated from the integrated abundances of the protonated molecular ions at *m/z* 171 (MND) and *m/z* 174 (3-[$^2H_3$]methylnonane-2,4-dione) (3).

Hexanal. Hexanal was determined in the oil samples by using [3,4-$^2H_4$]hexanal as internal standard (3,10).

Furanoid fatty acids. F20 and F22 were quantitated in SBO and in SBO fractions on the basis of F20-d_2 as internal standard (6). The procedure consisted of the following steps: After transesterification of the SBO sample (1 g) with sodium methoxide in methanol, the internal standard F20-d_2 (about 100 μg) was added in the form of its methyl ester derivative. Then, the methyl esters of F20, F20-d_2, and F22 were enriched by urea fractionation and were finally determined by mass chromatography of the protonated molecular ions, obtained at *m/z* 337 (F20), *m/z* 339 (F20-d_2) and *m/z* 365 (F22) after chemical ionization.

Fatty acid composition. The methyl esters of the fatty acids from each SBO were prepared by sodium methoxide-catalyzed transesterification (11). Gas chromatography (GC 5160; Fisons, Mainz-Kastel, Germany) of the methyl esters was performed on a fused-silica capillary column (30 m × 0.32 mm i.d.) coated with DB-FFAP (free fatty acid phase; column coating nitroterephthalic acid esters; Fisons). The temperature of the oven was programmed as follows: 35°C for 1 min; 40°C/min ramp rate to 100°C, 1 min hold; 8°C/min ramp rate to 250°C; and 10 min final hold. Helium was the carrier gas. The fatty acids were quantitated by electronic integration.

Fractionation of SBO sample A1 (12). SBO (1 g) in hexane (5 mL) was applied onto a water-cooled column (30 × 2 cm) packed with silica gel. Stepwise elution was performed with hexane (300 mL; fraction I), hexane/diethyl ether (97:3, vol/vol, 300 mL; fraction II), hexane/diethyl ether (90:10, vol/vol, 300 mL; fraction III), diethyl ether (300 mL; fraction IV), and finally methanol (300 mL; fraction V). Each fraction was collected, then evaporated to dryness and weighed.

Distribution of F20 and F22 in the triacylglycerol fraction (Refs. 13,14). Fraction III (0.95 g) was suspended in a mixture that consisted of an NH_4Cl/NH_4OH-buffer (pH 8.5; 10 mL), hexane (25 mL), an aqueous solution of $CaCl_2 \cdot 6H_2O$ (22%, wt/vol; 2 mL), and pancreatic lipase (100 mg). During stirring for 2 h at 37.5°C, the pH of the suspension was controlled and maintained at 8.5 by the addition of aqueous NH_4OH (15%, w/w). After incubation, the reaction mixture was acidified with HCl (4 mol/L) to pH 1. Water (10 mL) was added, and the mixture was extracted with diethyl ether (2 × 50 mL). After evaporation of the extract to dryness, the residue was dissolved in dichloromethane (10 mL) and applied onto a water-cooled column (30 × 2 cm) packed with a slurry of silica gel 60 in pentane. Stepwise elution was performed with toluene/diethyl ether/acetic acid (90:10:1, vol/vol/vol, 250 mL) and diethyl ether (200 mL; fraction IIIa). Fraction IIIa, which contained the 2-monoacylglycerols, was evaporated to dryness and weighed.

Filtration of SBO through silica gel. SBO (50 g; sample A1), stored for 3 d in daylight (4), was dissolved in pentane/diethyl ether (90:10, vol/vol, 50 mL) and then filtered through a thick-walled column (50 × 3 cm), which was packed with silica gel 60 (see Materials). The column was washed with the same solvent mixture (200 mL) at a pressure of 50 kPa (flash chromatography system; Machery-Nagel, Düren, Germany). The whole effluent was collected, and the solvent mixture was removed from the oil by evaporation under vacuum (10 Pa, 35°C).

Bleaching and deodorizing. Kaolin (5 g) was added to SBO (100 g; sample A2). The suspension was stirred for 1 h at 23°C under high vacuum (3 mPa). Kaolin was removed by centrifugation and filtration. For deodorizing, the bleached SBO was placed into a round flask (1 L), which was connected to a steam distillation apparatus. The oil was heated to 100°C, and the volatiles were distilled off under vacuum (3 Pa) for 1 h. The bleached and deodorized SBO was cooled to 21°C.

Daylight exposure procedure. SBO (50 g or 400 g) was placed into a 2-L glass culture flask (19 cm diameter). The flask was sealed by a cotton plug and then stored at 21–23°C at a window facing north (4) for 2 d or 30 d. Light intensity ranged from 2000 to 3000 lux during daylight.

RESULTS AND DISCUSSION

SBO sample A1 was separated by silica gel chromatography into five fractions. The furanoid fatty acids F20 and F22 were only detected in fraction III, which consisted of the triacylglycerols (Table 2). This fraction was hydrolyzed with pancreatic lipase, and the 2-monoacylglycerols formed (150 mg, fraction IIIa) were separated from the diacylglycerols. The concentrations of F20 (8 mg/kg) and F22 (11 mg/kg) in fraction IIIa were low (Table 2). It was calculated from the weight difference of fraction III and fraction IIIa (Table 2) that 192 mg/kg of F20 and 164 mg/kg of F22 occurred in the 1- and 3-positions of the triacylglycerols. This result suggests that, in

TABLE 2
Distribution of F20[a] and F22[b] on the Primary and Secondary OH-Groups of the Triacylglycerol Molecules Occurring in SBO (Sample A1)[c]

SBO/Fraction	F20 (mg/kg)	F22 (mg/kg)
SBO	204	180
Fraction I, II, IV, V	<0.5	<0.5
Triacylglycerols (fraction III)	200	175
2-Monoacylglycerols (fraction IIIa)	8	11

[a]F20, 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid.
[b]F22, 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid.
[c]Data are means of two assays, maximum SD: ± 10%. See Table 1 for other abbreviation.

TABLE 3
Effect of Hydrogenation on the Concentrations of F20 and F22 in SBO Sample B[a]

Batch	Processing of SBO	F20 (mg/kg)	F22 (mg/kg)
B1	Neutralized, bleached	104	64
B2	Partially hydrogenated	72	48
B3	Fully hydrogenated	55	38

[a]Data are means of two assays, maximum SD: ± 10%. See Tables 1 and 2 for abbreviations.

SBO, the two furanoid acids are preferentially esterified with the primary OH groups of the glycerol molecules.

The data summarized in Table 3 indicate that hydrogenation reduced the concentration of the two furanoid fatty acids in SBO only somewhat, because sample B3, in which the unsaturated C_{18} fatty acids were completely hydrogenated (Table 1), contained 53% and 59%, respectively, of the initial concentrations of F20 and F22 (Table 3).

Exposure of SBO (sample A1) to daylight led to a nearly complete degradation of F20 and F22 within two days (Table 4). This was in contrast to linoleic acid and linolenic acid, which were not affected by this treatment. The small concentration changes of these unsaturated fatty acids, indicated by comparing A1 and A2 in Table 1, lay within the limit of error of the analytical method. This result suggests that F20 and F22, which as furan derivatives are effective scavengers of singlet oxygen (15, 16), have protected the two unsaturated C_{18} fatty acids from photooxidation. In addition, it has been reported (17) that the tetraalkyl-substituted furan fatty acids F20 and F22 are radical-trapping antioxidants, which suppress the oxidation of linoleic acid.

β-Carotene, which is known to inhibit photosensitized oxidation (18–20) at a concentration of 20 mg/kg, improved the flavor stability of light-exposed SBO (21). In the following experiment, this quantity of β-carotene was added to SBO sample A1. As summarized in Table 4, the degradation of the furanoid fatty acids was delayed, because 25% and 12% of F20 and F22, respectively, were still detectable after light exposure of the SBO for 48 h, whereas a nearly complete degradation of both acids was observed in the control without addition of β-carotene.

The amounts of MND and hexanal, the latter of which is formed by peroxidation of linoleic acid (22), were determined after storage for 30 d in light. The results in Table 5 indicate an increase of MND from less than 0.5 up to 204 μg/kg and of hexanal from 35 up to 1270 μg/kg. β-Carotene retarded the production of both carbonyl compounds, but the effect of MND (80% inhibition) was somewhat greater than on hexanal (66%).

The two tocopherols added to SBO at the relatively high amount of 2 g/kg protected differentially the furanoid fatty acids (Table 4). α-Tocopherol was as effective as β-carotene, whereas γ-tocopherol did not prevent a decrease of F20 and F22 down to 16% and 7%, respectively. The production of MND during storage of SBO was inhibited only to 49% by both tocopherols (Table 5). α-Tocopherol acted as prooxidant because more hexanal (1740 μg/kg) was formed in its presence than in the control (1270 μg/kg). In contrast, γ-tocopherol inhibited the formation of hexanal to 35% and was not so effective as β-carotene.

In the next experiment, the furanoid fatty acids were destroyed in SBO sample A1 by light exposure, as reported in Table 4. Then the oil was bleached and deodorized to yield sample A3 (Table 6). After storage for 30 d in daylight, the amount of MND formed in sample A3 was 39% of the control (Table 6). The generation of MND during storage of SBO in daylight was reduced further in sample A4, which was additionally filtered through silica gel before bleaching and deodorizing (Table 6). This result suggests that breakdown products of F20 and F22, which act as precursors of MND, are removed

TABLE 4
Effect of β-Carotene and of α- and γ-Tocopherols on the Stability of F20 and F22 in SBO Sample A During Light Exposure[a]

Storage conditions[b]			Concentration (μg/kg)[c]	
Time (days)	Additive	(mg/kg)	F20	F22
0	None	0	204	180
2	None	0	5	5
2	β-Carotene	20	53	23
2	α-Tocopherol	2000	50	22
2	γ-Tocopherol	2000	32	12

[a]See Tables 1 and 2 for abbreviations.
[b]The SBO sample (400 g) in a glass Fernbach culture flask was stored at a window facing north (3). Over a period of 2 d, the sample was exposed 30 h to daylight at 2000–3000 lux and 18 h to darkness.
[c]Data are means of two assays, maximum SD: ± 10%.

TABLE 5
Effect of α-, γ-Tocopherols and β-Carotene on the Formation of MND and Hexanal in SBO Sample A1 During Light Exposure[a]

Storage conditions[b]			Concentration (μg/kg)[c]	
Time (days)	Additive	(mg/kg)	MND	Hexanal
0	None	0	<0.5	35
30	None	0	204	1270
30	β-Carotene	20	40	430
30	α-Tocopherol	2000	103	1740
30	γ-Tocopherol	2000	105	830

[a]MND, 3-methylnonane-2,4-dione. For other abbreviation see Table 1.
[b]Storage conditions same as those in Footnote *b* of Table 4.
[c]Data are means of two assays, maximum SD: ± 5%.

TABLE 6
Formation of MND as Affected by Different Treatments of SBO Sample A1[a]

SBO sample	Treatment	MND (μg/kg) after storage for 30 d[b,c]
A1	None	204
A3	Exposure to daylight (48 h)[d], followed by bleaching and deodorizing	80
A4	Exposure to daylight (48 h)[d], followed by filtration through silica gel bleaching and deodorizing	5

[a]For abbreviations see Tables 1 and 5.
[b]The SBO sample was stored at a window facing north.
[c]The data are means of two assays, maximum SD: ± 5%. MND in fresh samples A1, A3, and A4 amounted to less than 0.5 μg/kg.
[d]Storage conditions same as those in Footnote *b* of Table 4.

from SBO by treatment with silica gel. The results in Table 6 suggest that it might be interesting to investigate by sensory evaluations whether the flavor stability of SBO is improved when the furanoid fatty acids are destroyed by exposing the oil to light before refining it.

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