Technical

Effect of Selected Oat Sterols on the Deterioration of Heated Soybean Oil

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Two sterol fractions of different purity, each containing both Δ^5-avenasterol and β-sitosterol, were separated from oat oil, and their antioxidant effects studied in soybean oil at 180 C. Oil samples with added pure β-sitosterol and control samples (no added sterol) also were studied.

Fatty acid changes, conjugated diene formation and polymerization were monitored in all samples. All heated oils with added oat-sterol fractions containing Δ^5-avenasterol deteriorated more slowly than did the controls. Oil with added pure β-sitosterol was altered at a rate similar to that of the controls.

Soybean oil oxidizes rapidly at frying temperatures because of its high percentage of unsaturated fatty acids, especially linolenic acid. This results in a darkened color, increased viscosity, increased foaming and a reduction in fatty acids in the oil. In general, a loss in both nutritional and sensory quality occurs.

The need to protect frying oils from oxidation has resulted in wide use of methyl polysiloxanes (1). Certain sterols also have been reported to reduce frying oil deterioration (2–4). The unsaponifiables from olive, corn, wheat germ and *Vernonia anthelmintica* oils were found to protect safflower oil from oxidative polymerization during heating at frying temperature (2). Specifically, vernosterol, Δ^7-avenasterol and citrostadienol were the effective agents. In a similar study (3), Δ^5-avenasterol and olive oil sterol mixtures containing Δ^5-avenasterol reduced oxidation in cottonseed oil at frying temperatures. Controls containing β-sitosterol showed this sterol was ineffective initially, and it became slightly prooxidant after prolonged heating. Δ^5-Avenasterol and fucosterol were effective as antioxidants at 180 C in a triglyceride mixture similar in composition to olive oil (4).

All the sterols effective at preventing oxidation at frying temperatures have an ethylidene group in their side-chain (2). Gordon and Magos (4) hypothesized that lipid free-radicals react rapidly with sterols that have unhindered allylic carbon atoms such as in the ethylidene group. Isomerization then produces a stable allylic tertiary free-radical and interrupts the oxidation chain.

The purpose of the present study was to examine the high-temperature antioxidant effects of sterol fractions containing Δ^5-avenasterol. The fractions were isolated from oat oil and added to soybean oil for the oxidation experiments. Changes were monitored by measuring increased conjugated dienoic acids, changes in fatty acids, and formation of high molecular weight (HMW) compounds.

EXPERIMENTAL PROCEDURES

Materials. Bleached, deodorized soybean oil, containing no additives, was obtained from Anderson-Clayton Company (Richardson, Texas) and was stored below 0 C until needed. Several varieties of oats (Iowa State University Agronomy Department) were screened for sterol content. Multiline E77 (5), which had the largest amount of sterol, was used for sterol isolations.

Stigmasterol, cholesterol, 7,22-cholestadien-24b-ethyl-3,5α-diol, 7,(5α)-cholesten-3-β-ol, and 5-cholesten-24b-ethyl-3-β-ol (β-sitosterol) were purchased from Steraloids, Inc., Wilton, New Hampshire, for use as standards. Heptadecanoic acid methyl ester (Sigma Chemical Co., St. Louis, Missouri) was used as the internal standard for fatty acid methyl ester determinations.

Separation of the sterol fractions. To remove the oil, oats (500 g) were boiled in 1.5 l of water to inactivate the lipase (6), filtered to remove excess water, dried in a 105 C oven and ground in a grist-mill (Magic Mill II, Salt Lake City, Utah) by using the coarsest setting. The oat flour was stirred for 24–48 hr with 3 l of methylene chloride: methanol (2:1), filtered, and the residue washed with an additional 4.5 l methylene chloride:methanol. The solvent was removed by using rotary evaporation followed by evaporation under nitrogen in a 40 C water bath. The resulting oil was then fractionated by chromatography by two procedures to obtain the sterol concentrates which we designated as sterol fractions A and B.

To obtain sterol fraction A the crude oil was first fractionated by column chromatography (CC) on Silica gel. Fifteen g oat oil was passed through a 60 × 4-cm column packed with 100 g Silica gel (Davisil 62, 60/200 mesh, Davison Chemical Company, Baltimore, Maryland) and 240 ml distilled hexane. The column was washed with 3 l ethyl ether:hexane (15:85), and the eluate collected in 100-ml fractions. The sterol-rich fractions (1.2 through 2.0 l) were identified by comparison with the previously mentioned sterol standards on thin layer chromatography (TLC) (0.25 mm Silica gel G, 70/230 mesh ASTM, E. Merck) with ethyl ether:hexane (60:40) as the developing solvent. The sterol-rich fractions were pooled, the solvent was evaporated, and the residue was saponified following AOCS Official Method Ca 6a-40 (7).

The crude sterol fraction A was purified further by TLC using procedures based on the work of Knights (8); however, several modifications were made. To separate Δ^5- and Δ^7-sterols, 100 mg of the sterol fraction was plated on .75-mm plates by using petroleum ether:ethyl acetate (80:20) as the developing solvent. For zone identification, a 2-cm strip along the edge of the plate was sprayed with 2% 2,7-dichlorofluorescein in ethanol and observed under UV light. Only one band at the Δ^5 position was observed. The unsprayed zone containing the Δ^5-sterols was then scraped off the plate, and the sterols were eluted with ethyl acetate. To separate mono- and di-unsaturated sterols, the Δ^5-sterols from the previous plate were streaked on .75-mm plates of 25% silver nitrate in Silica gel. Petroleum ether:ethyl acetate (70:30) served as the developing solvent. Zone identification and elution of the sterol was as described. β-sitosterol was purified for use

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in test 2 by TLC on Silica gel G with ethyl ether:hexane (60:40) as the developing solvent.

To obtain the sterol isolate we designated as sterol fraction B, the CC procedure described and the second TLC plate (25% silver nitrate in Silica gel) were eliminated. Otherwise, the isolation procedures for fractions A and B were identical.

Gas liquid chromatography (GC). A Varian Aerograph series 3700 GC equipped with a hydrogen flame detector was used. The free sterols were separated on a fused Silica capillary column (Alltech Associates, Deerfield, Illinois) of 30 m × 25 mm i.d. coated with SE-30. The column was programmed at 5 C/min from 150–300 C with nitrogen as the carrier gas. Injector and detector temperatures were set at 280 C (9).

The method of Metcalfe et al. (10) was used for preparation of fatty acid methyl esters. The GC contained a stainless steel packed column (WAW support with CS-10 coating; Alltech Associates) of 6.0 ft × .085 in. Peak areas were measured by width times height at half height and the actual area related to the internal standard, methyl heptadecanoate, to standardize the area. This method of measurement was suggested by Waltking and Zmachinski (11) to be the preferred method in determining total polyunsaturated fatty acids.

Gas chromatography-mass spectrometry (GC-MS). A Finnigan Model 400 GC-MS aided in the identification of the sterol fractions. The ionizing voltage was 70 eV.

Heating. Soybean oil samples (60 g) were heated in 100-ml Pyrex beakers at 180 C ± 5 C for 7 hr each day for 4 or 5 days. Samples were cooled to room temperature the remainder of the time. Aliquots were removed every 2 to 3 hr (while at 180 C) in test 1 and daily in tests 2 and 3 and stored under nitrogen at −18 C until analyzed.

For test 1, 0.05% (30 mg) of sterol fraction A was added to one oil sample, whereas in tests 2 and 3, 0.17% (100 mg) and 0.25% (150 mg), respectively, of sterol fraction B was used. An oil sample with only pure β-sitosterol added (0.05% or 30 mg) was included in test 2, and a control sample without additives was included in each experiment.

Analysis of heated oils. All test results are the average of duplicate samples unless noted otherwise. The data for fatty acid methyl esters reported in the current study list the change in percentage remaining for each fatty acid over the heating time, based on the amount at time 0.

Conjugated dienoic acids were measured by using AOCS Official Method Ti 1a-64 (7), and peroxide values were determined by using AOCS Official Method cd 8-53 (7).

Polymer formation was analyzed with a high performance size-exclusion chromatography (HPSEC) system that included a Beckman Model 110A pump, 20-μl injector loop, Beckman Model 210 sample injector, and a Hitachi 100-10 variable-wavelength UV/Vis detector. Two μ-Spherogel columns (500 Å and 1000 Å, Altex, Berkeley, California) were used following the procedure of White and Wang (12). The accumulation of HMW compounds was measured from the size of a peak (peak 4) representing compounds ranging in MW from 4000 to 6000 Daltons.

RESULTS AND DISCUSSION

The CC step used in the separation of sterol fraction A was time consuming and resulted in low sterol yields; therefore, after verification of the sterols present in this fraction, CC was omitted for isolation of sterol fraction B. In addition, TLC separations of sterol fraction A on 25% silver nitrate in Silica gel did not appear to result in further separation of the Δ^5-sterol into mono- and diunsaturated sterols as indicated by Knights. Thus, it was omitted from the procedure for isolation of sterol fraction B.

Mass spectral data for sterol fraction A used in test 1 gave principal fragmentations that were essentially similar to those for two sterols, β-sitosterol and Δ^5-avenasterol (Table 1 and Fig. 1). The presence of about 5% Δ^5-avenasterol was estimated from the size of the characteristic fragmentation ions at m/e 296 [m − (C_7H_{14} + H_2O)], 281 [M − (CH_{14} + CH_3 + H_2O)], 271 [M − (side-chain + 2H)], and 314 [M − part of side-chain (C_7H_{14})] (13,14). The ions at 296 and 314, in particular, indicate the presence of the ethylidene side-chain present in the Δ^5-avenasterol. The molecular ion at m/e 414 corresponded to that of the β-sitosterol. The molecular ion for Δ^5-avenasterol (m/e 412) was too weak to observe. Direct GC analysis of the same fraction revealed one major peak whose retention time matched that of the β-sitosterol standard. A smaller peak with a slightly longer

TABLE 1

Mass Spectral Data for Sterol Fraction A Used in Test 1

Characteristic fragments m/e (relative abundance)
107(100), 92(83), 69(65), 81(60), 67(55), 79(48), 109(46), 95(43), 91(38), 119(38), 314 (3.4), 281(2.5), 186(2.3), 414(1.3), 296(1.2), 205(1.2), 241(1.2), 271(.94), 267(.23)

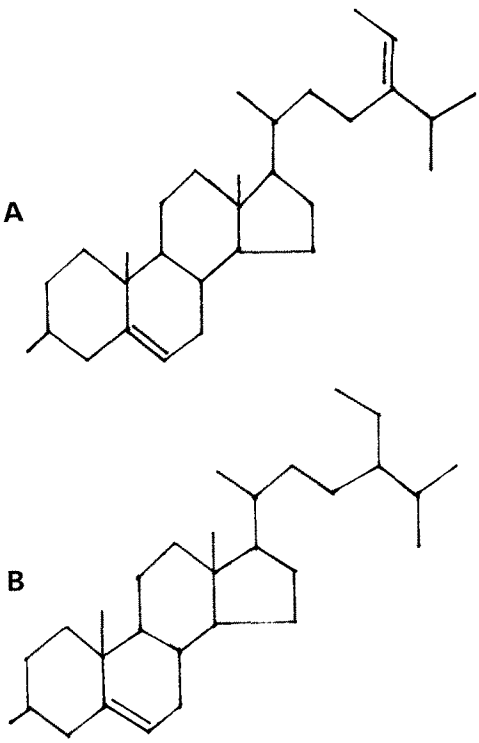

FIG. 1. A, Δ^5-avenasterol; B, β-sitosterol.

retention possibly represented Δ^5-avenasterol. Previous research (8) reported that β-sitosterol is the principal sterol in oats, with Δ^5-avenasterol representing 32% of the total sterol. Little difference in these values was obtained from different varieties of oats.

Gas chromatography also was used to verify the purity of the purchased (pure) β-sitosterol used in test 2 and to confirm that sterol fraction B used in tests 2 and 3 also contained β-sitosterol and Δ^5-avenasterol. Chromatograms from sterol fraction B gave several minor peaks in addition to those from the two sterols found in fraction A, likely because of the omission of the second thin layer plate (25% silver nitrate in Silica gel) during the isolation procedure. Other Δ^5-sterols such as cholesterol, stigmasterol and campesterol might have accounted for these peaks. The presence of these Δ^5-sterols in oats has been reported previously (8). Thin layer plates also were run with α-tocopherol and confirmed that the sterol fractions did not contain any of this natural antioxidant.

Test results are shown in Tables 2, 3 and 4. It is clear that sterol fraction A in test 1 (0.05%) and sterol fraction B in test 3 (0.25%) had a considerable effect in retarding oxidation as measured by the increase in conjugated dienes, the percentage retention of individual fatty acids, and the accumulation of HMW compounds. The amount of sterol fraction B (0.17%) used in test 2 had only a slight antioxidant effect that was detected by the HMW compound accumulation and conjugated diene results. The percentage retention of the fatty acids over the heating time differed little from controls containing

TABLE 2

Percentage Retention of Individual Fatty Acids for Tests 1, 2 and 3

		% Retention						
		Hours of heating						
Sample	Fatty acid	0	3	7	14	21	28	35
Test 1								
0.05%	16:0	100	NA	108.7[a]	102.7	99.2	77.0	NA
sterol	18:0	100	NA	115.7[a]	98.4	106.9	90.9	NA
fraction	18:1	100	NA	114.6[a]	91.9	98.0	75.2	NA
A	18:2	100	NA	91.2[a]	90.1	85.8	54.9	NA
	18:3	100	NA	118.6[a]	92.8	87.4	43.9	NA
Control	16:0	100	NA	95.7	79.4	53.4	40.4	NA
	18:0	100	NA	95.7	96.8	63.4	52.3	NA
	18:1	100	NA	94.5	78.8	49.2	32.4	NA
	18:2	100	NA	87.8	64.6	34.6	15.5	NA
	18:3	100	NA	68.8	55.5	23.4	0.0	NA
Test 2								
0.17%	16:0	100	87.3	80.2[a]	80.7	68.9	66.0[a]	55.8
sterol	18:0	100	91.8	78.3[a]	72.1	67.1	59.6[a]	52.5
fraction	18:1	100	88.5	79.6[a]	74.7	62.4	58.7[a]	47.7
B	18:2	100	88.3	75.7[a]	69.1	52.9	44.4[a]	31.7
	18:3	100	96.1	73.0[a]	58.7	42.2	30.6[a]	25.1
0.05%	16:0	100	78.0	84.1	77.7	70.8	67.1[a]	54.4
β-sitosterol	18:0	100	80.2	72.9	68.7	64.7	62.0[a]	49.9
	18:1	100	80.1	77.8	71.7	63.7	60.5[a]	45.6
	18:2	100	78.0	77.4	64.8	53.1	44.3[a]	29.2
	18:3	100	71.6	64.7	44.7	41.6	28.2[a]	21.6
Control	16:0	100	88.6	81.6[a]	78.7	69.2	63.2	48.1
	18:0	100	91.9	77.0[a]	68.4	63.3	58.2	45.9
	18:1	100	88.9	76.8[a]	71.4	62.0	55.4	40.7
	18:2	100	86.1	74.6[a]	64.0	50.7	40.5	26.1
	18:3	100	79.7	71.7[a]	48.6	48.5	30.0	17.7
Test 3								
0.25%	16:0	100	98.6	100.9	99.0[a]	100.3	94.4	89.2
sterol	18:0	100	99.0	102.9	99.2[a]	100.8	100.1	91.4
fraction	18:1	100	98.4	100.4	98.6[a]	98.2	94.1	87.2
B	18:2	100	98.6	99.6	96.4[a]	93.3	85.3	72.4
	18:3	100	93.6	94.2	90.3[a]	92.2	77.2	78.8
Control	16:0	100	97.9	95.8	89.5	79.5[a]	74.9	59.8
	18:0	100	96.2	98.5	96.4	84.1[a]	85.7	60.3
	18:1	100	95.7	93.1	85.5	74.3[a]	69.1	52.4
	18:2	100	93.0	86.0	71.4	54.9[a]	47.0	30.2
	18:3	100	89.0	73.1	58.5	30.3[a]	15.3	11.4

[a]One sample only.

TABLE 3

Percentages of Conjugated Dienoic Acid for Tests 1, 2 and 3

	Conjugated dienoic acid (%)						
	Hours of heating						
Sample	0	3	7	14	21	28	35
Test 1							
0.05% sterol fraction A	0.41	0.55	0.90	1.19	1.89	3.29	NA[a]
Control	0.41	0.96	1.93	2.75	3.94	3.95	NA
Test 2							
0.17% sterol fraction B	0.44	0.65	1.40	2.37	2.98	3.52	3.69
0.05% β-sitosterol	0.44	0.96	1.73	2.66	3.27	3.85	3.83
Control	0.44	0.96	1.73	2.69	3.28	3.76	3.92
Test 3							
0.25% sterol fraction B	0.42	0.56	0.72	1.10	1.45	1.97	2.64
Control	0.42	1.10	1.85	2.76	3.45	3.83	4.08

[a]Not analyzed.

TABLE 4

Changes in Peak 4 (High Molecular Weight Compounds) by HPSEC Analyses for Tests 1, 2 and 3

	Peak areas[a] (cm^2)						
	Hours of heating						
Sample	0	3	7	14	21	28	35
Test 1							
0.05% sterol fraction A	0	0	0	0	20	32	NA[b]
Control	0	0	0	54	97	126	NA
Test 2							
0.17% sterol fraction B	0	0	0	31	51	80	88
0.05% β-sitosterol	0	0	0	42	54	87	98
Control	0	0	0	42	66	85	101
Test 3							
0.25% sterol fraction B	0	0	0	0	13	31	42
Control	0	0	0	35	67	88	94

[a]Peak areas are the average of two measurements and are calculated for 10 μg of injected oil. Sensitivity, 0.02; chart speed, 1 cm/min; uv detector 234 nm. 1 cm^2 of peak area is equivalent to approximately 0.63 μg of polystyrene standard.
[b]Not analyzed.

no added sterols except for values reported at 35 hr. The addition of pure β-sitosterol (0.05%) in test 2 had essentially no effect on oil oxidation, thus confirming that the effective portion of sterol fraction A was Δ^5-avenasterol. Other researchers have also reported that β-sitosterol has no antioxidant effect (2) or even a prooxidant effect (3).

The magnitude of the antioxidant properties of the sterol fraction, presumably from Δ^5-avenasterol, can be quantified by considering the effect on the polyunsaturated fatty acid content (Table 2). The approximate time at 180 C for a 50% reduction in linoleic acid content in the absence of sterols was around 21 hr for tests 2 and 3 and between 14 and 21 hr for test 1. The addition of 0.05% of sterol fraction A in test 1 increased that time to slightly more than 28 hr, while in test 3, 72.4% linoleic acid still remained after 35 hr of heating with the addition of 0.25% sterol fraction B. According to Gordon and Magos (4), the effectiveness of Δ^5-avenasterol as an antioxidant increases with concentration in the range of 0.01 to 0.1%. The saturated fatty acids generally are considered stable; however, even these fatty acids decreased with heating in all oil samples except the one with sterol in test 3 (0.25% sterol fraction B). The decreases were greatest in the control oils.

The controls from each test varied in their measured values of oxidative abuse, which makes it difficult to com-

pare the effectiveness of the sterol fractions to each other. In addition, aliquots were removed from the oils in test 1 (0.05% fraction A and control) every 2–3 hr rather than every 7 hr, as in tests 2 (0.17% fraction B, 0.05% β-sitosterol and control) and 3 (0.25% fraction B and control). This practice increased the surface:volume ratio of the oils as the test progressed, and thus the rate of deterioration. The results from the additional samples collected during test 1 are not reported because they did not add significantly to our conclusions. Although comparisons among tests are difficult, it does seem that the different amounts of sterol fractions used in tests 1 (0.05% fraction A) and 3 (0.25% fraction B) had similar effects when compared with their controls, whereas the amount used in test 2 (0.17% fraction B) was only slightly effective. More sterol fraction B was needed to produce an antioxidative response similar to that of fraction A, most likely because the former was diluted by other Δ^5-sterols as mentioned previously. These other Δ^5-sterols, possibly cholesterol, stigmasterol and campesterol, do not contain the ethylidene side-chain and theoretically would not be effective as antioxidants. In fact, cholesterol and stigmasterol have been shown by other researchers to be ineffective or even prooxidant at high temperatures (2).

Beginning and ending peroxide values (PV) for all test oils were measured to monitor the quality of the oils. Fritsch et al. (15) have reported that peroxides are very unstable during the heat abuse of oils, so values were expected to increase little. All oils had beginning PV of around 0.40 and, for tests 2 (0.17% fraction B, 0.05% β-sitosterol and control) and 3 (0.25% fraction B and control), ending PV of about 1.5 to 2.0. Ending PV for test 1 (0.05% fraction A and control), however, was about 5.5, probably because of the increased surface:volume ratio in these oils. The addition of the sterols had virtually no effect on the ending PV. During room temperature storage of oils, PV's are known to increase up to a certain point and decrease thereafter due to breakdown of peroxides to secondary oxidation products. Perhaps if PV's had been determined at more frequent intervals there would have been greater differences due to the effects of the additives. However, in preliminary studies on the effects of these sterol additions to heated oil more frequent PV measurements were taken and little accumulation of PV's was found in any samples.

Further work is needed to better separate the Δ^5-avenasterol from other sterols in the unsaponifiable fraction of oats. In addition, a comparison of the rates of deterioration of individual measures, such as fatty acid changes, with the concentration of Δ^5-avenasterol would be helpful.

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