High-Temperature Stabilities of Oils from Soybeans That Lack Lipoxygenases

N. Shen[a], W. Fehr[b], and P. White[a,*]

[a]Department of Food Science and Human Nutrition, and Center for Crops Utilization Research, and [b]Department of Agronomy, Iowa State University, Ames, Iowa 50011

ABSTRACT: Oils from normal or low-linolenic acid (18:3) soybeans that lack lipoxygenase (LOX) 2 or LOX 2 plus LOX 3 activities were evaluated for their stability during frying and for oxidative stability in bread cubes stored after frying. Soybean oils were extracted by a pilot-plant system and were refined, bleached, and deodorized in the laboratory. Citric acid was added to oils during the cool-down stage of deodorization. Two replications, separated at the point of conditioning, were evaluated for each genotype. Each sample (250 g) was heated to 180 ± 5°C in a minifryer. Bread cubes were fried at the beginning of heating and after 20 h of heating. Heating of the oils was continued for 10 h each day for three consecutive days. Soybean oils with low 18:3 contents were significantly ($P \leq 0.05$) more stable, as measured by conjugated dienoic acids and polymer values, than were oils with normal 18:3 contents. Low-LOX 2 or low-LOX 2 + 3 activity had no effect on peroxide values of soybean oils extracted from bread cubes. Sensory evaluation did not differentiate between oils that contained low or high 18:3 amounts or among oils from beans that lacked different LOX enzymes.

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KEY WORDS: Conjugated dienoic acids, fatty acids, oxidative stability, polymerization, soybean oils.

The flavor instability of soybean oil and the tendency of soybean oil to polymerize at high temperatures limit its usefulness for frying in food preparation. This problem is associated with the relatively high linolenate (18:3) concentration (*ca*. 8% of total fatty acids) in soybean oil. Other vegetable oils, such as corn, sunflower, and cottonseed, have less than 1.0% 18:3 and are adequate for cooking or frying use (1). A common way to reduce the 18:3 content in soybean oil is by partial hydrogenation. Keijbets *et al*. (2) reported that hydrogenated soybean oil with reduced 18:3 content was more stable than unhydrogenated oil in prefrying of deep-frozen products.

Mensink and Katan (3) reported that *trans* fatty acids (*t*FA) formed during hydrogenation increased low-density lipoprotein cholesterol and lower high-density lipoprotein in human plasma. Hydrogenated soybean oil is claimed to be the main source of *t*FA in the U.S. diet (4). Mag (5) suggested that margarine consumption in Canada has declined at least partly because consumers are reducing their consumption of *t*FA.

To avoid *t*FA and to improve the stability of soybean oil, the 18:3 content of soybeans has been reduced genetically to 3% (6). Soybean oil with reduced 18:3 (<3%) is more stable at frying temperatures than that with normal 18:3 (8%) (7,8). Mounts *et al*. (9) noted that low-18:3 soybean oils could be alternative frying oils. Warner *et al*. (10) reported that the best flavor stability of potato chips was obtained from a frying oil with 68% oleic acid, 20% linoleic acid, and 3% 18:3. Recently, Kraft Food Ingredients began to market a low-18:3 (3%) soybean oil, named SOY•LL. The soybean cultivars with reduced 18:3 used for SOY•LL were developed at Iowa State University and grown by Pioneer Hi-Bred International Inc. (11).

Lipoxygenase (LOX) enzymes are believed to contribute to flavor instability (12–15). Genetic removal of LOX 2 reduced off-flavors in soy foods such as soy milk (16); however, Frankel *et al*. (13) found that the absence of LOX 1 did not affect the oxidative stability of soybean oil. Endo *et al*. (17) studied the oxidative stability of soybean oil from LOX 1- and 3-null and LOX 2- and 3-null beans. The oils were stored at 30°C under light. Sensory evaluation and peroxide values were not affected by combinations of LOX.

The objective of this study was to evaluate oil from soybeans with normal and reduced LOX contents in combination with normal and reduced 18:3 for their high-temperature stabilities and oxidative stabilities of bread cubes stored after frying. These soybean lines, developed by breeders at Iowa State University, were only available and studied in our laboratory for our objectives.

MATERIALS AND METHODS

Materials. Soybean (*Glycine max* L. Merr) genotypes Century 84, L2-3, and L2L3-2-4 were developed by USDA–ARS and Purdue University; A89-269043, A89-269043-L2, and A89-269043-L2L3 were developed at Iowa State University in 1992 from the cross L2L3-2-4 × A89-269043. The F3-derived lines that contained <3% 18:3 and that lacked LOX 2 were bulked to form A89-269043-L2. The F3-derived lines

*To whom correspondence should be addressed at 2312 Food Sciences Building, Iowa State University, Ames, IA 50011.

that contained <3% 18:3 and that lacked LOX 2 + 3 were bulked to form A89-269043-L2L3.

Methods. Soybean seeds of six genotypes (Table 1) were processed into refined, bleached, and deodorized oils and analyzed as described by Shen *et al*. (18). The tocopherol contents for each replicate of the oils were measured in duplicate according to Dove and Ewan (19). Two replicate samples (250 g of each oil) were used for deep-fat frying. Each replicate was placed into a Teflon-coated electric minifryer with a total capacity of 473 mL (Presto Fry Baby Electric Fryer; National Presto Industries, Inc., Eau Claire, WI) and heated to 180 ± 5°C within 10 min. One batch (40 g) of 1.0-inch^3 crust-free bread cubes was fried for 1.5 min and drained. Part of the fried bread cubes were loosely covered and stored at 60°C in the dark for 4 d. The rest of the cubes were extracted with hexane, and the resulting oil was analyzed for peroxide value. Soybean oils were heated 10 h for three consecutive days with cooling to room temperature overnight. At the end of each day of heating, a 20-g oil sample was removed and stored under nitrogen at −14°C until analysis for fatty acid composition, conjugated dienoic acid value, and polymer value. The oil was not replenished or filtered. After 20 h of heating, additional bread cubes (40 g) were fried for 1.5 min and stored for sensory evaluation and peroxide value (PV) determination. Bread cubes were loosely covered and stored at 60°C in the dark for 4 d.

Additional bread cubes were fried in fresh Wesson® vegetable oil (100% soybean oil) purchased from a local grocery. These cubes were stored at −14°C in freezer bags with the air pressed out by hand and were used as references during sensory training and evaluation.

PV of oil from fried bread cubes. The PV of the oils extracted from the cubes were determined by the Stamm test as modified by Hamm *et al*. (20). The oil was extracted from bread cubes (3 g) with 25 mL hexane three times for 10 min each. The solvent was removed by rotary evaporation (7).

Sensory evaluation. Eleven trained panelists evaluated the fried bread cubes at room temperature for intensity of oxidized flavor according to the AOCS official method Cg 2-83 (21). On the flavor intensity scale, 10 is bland and 1 is an extremely intense flavor. The panelists were trained by judging fresh and stored bread cubes fried in Wesson vegetable oil. Three training sessions were conducted to develop agreement on oxidized flavor scores. The fried cubes were presented at room temperature and in two sets based on 18:3 contents. Set 1 included soybean oils with normal 18:3 contents, and set 2 with low 18:3 contents. In each set, there were three samples: One had normal LOX content (#1 or #4), one was from seeds that lacked LOX 2 (#2 or #5), and one was from beans that lacked LOX 2 + 3 (#3 or #6).

The panelists were instructed first to smell the cubes and then to taste them in an approximate order of increasing odor intensity. This procedure reduced the possibility of a strongly oxidized sample overwhelming a panelist's ability to evaluate less-oxidized samples before all samples were judged. Panelists were told, however, to make final evaluations after samples had been placed in their mouths. Panelists were instructed to expectorate after chewing and evaluating each sample. Distilled water and unsalted crackers were provided to rinse the oxidized flavor from their mouths. The thawed fried cubes that had been fried in Wesson vegetable oil and stored at −14°C were provided as a reference in judging the samples (8).

Fatty acid compositions. Fatty acid compositions were measured at 0, 10, 20, and 30 h of heating. Fatty acid methyl esters (FAME) of the frying oils were prepared by transesterifying the oils with sodium methoxide in methanol and injecting the esters into a gas chromatograph (GC), as described by Hammond (22). The theoretical response factors for each fatty acid were applied as calculated by Ackman (23).

Conjugated dienoic acid (CDA). The CDA were measured to estimate shifting of double bonds during oxidation according to AOCS official method Ti 1a-64 (21). Soybean oils (0.100 g) were dissolved in 25 mL isooctane and diluted; the absorbency was measured at 233 nm. Dilution was made so that the systematic peaks could be obtained even for the most oxidized samples.

High-performance size-exclusion chromatography (HPSEC). HPSEC was used to measure polymerized high-molecular weight compounds formed during heating (24). The HPSEC system was composed of a Beckman 110A pump, a 20-μL injector loop, a Beckman 210 sample injector (Beckman Instruments, Inc., Fullerton, CA), a Hitachi 100-10 variable wavelength ultraviolet/visible (UV/VIS) detector (Hitachi, Ltd., Tokyo, Japan), a Beckman 10-inch strip-chart recorder, and two [500 Å (0.8 × 30 cm) and 1000 Å (0.77 × 30 cm)] μ-Spherogel columns (Altex Scientific, Inc., Berkeley, CA). A column inlet filter between the injector and the column was used to prevent blockage of the column inlet frit. High-performance liquid chromatography (HPLC)-grade methylene chloride (Fisher Scientific, Pittsburgh, PA) was used as the mobile phase, with a flow rate of 1 mL/min. The sample preparation involved dissolving 0.600 ± 0.005 g of a heated oil in 4.5 mL of HPLC-grade methylene chloride. The UV wavelength of 254 nm was used for measurement. Polystyrene standards (Supelco, Inc., Bellefonte, PA) of various molecular weights (794, 2,000, 2,500, 5,000, 9,000, 17,500, 30,000, and 50,000 daltons) were used as external standards to determine the approximate molecular weight separation on the columns.

TABLE 1
Lipoxygenase and Approximate Linolenic Acid Contents of Soybean Genotypes Used for the Experiment

Name	Abbreviation	Lipoxygenase (LOX) content	Approximate 18:3 content
Century 84	#1	Normal LOX	>8.0%
L2-3	#2	Lacking LOX 2	>8.0%
L2L3-2-4	#3	Lacking LOX 2 and 3	>8.0%
A89-269043	#4	Normal LOX	<3.0%
A89-269043-L2	#5	Lacking LOX 2	<3.0%
A89-269043-L2L3	#6	Lacking LOX 2 and 3	<3.0%

Statistical analysis. A randomized 2 × 3 factorial design was used for this experiment. To analyze the influence of both fatty acid composition and LOX content, all data (except those in Tables 1 and 2) were grouped according to 18:3 content (low or normal) and LOX content (normal, lacking LOX 2, or lacking LOX 2 + 3). Data from all treatments in each test were analyzed based on data in these two groups by using analysis of variance and least squares difference for statistical significance (25). Significance was accepted at a level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Frying oils. Concentrations of polyunsaturated fatty acids, especially 18:3, decreased during heating (Table 2). After 30 h of heating, the 18:3 concentration of #4 declined from 2.4 to 1.0%, which was only 42% of the original concentration. Oil #6 retained about 55% of its original 18:3 concentration. Other soybean oils retained about 50% of their 18:3 concentrations. When 18:3 concentration was reduced, the relative percentage of saturated fatty acids increased (by over 50% for 16:0 and 18:0, data not shown). This pattern also was observed in other studies (7,8). The calculated oxidizability (28) of the oils (Table 2) suggests a greater speed of oxidation for oils #1, #2, and #3, than for oils #4, #5, and #6, with oil #4 having the greatest stability among all oils. The percentage loss of 18:3 among the oils, then, was not entirely consistent with the predictions.

There was a tendency for low-18:3 oils (#4, #5, and #6) to have lower CDA values than normal-18:3 oils (#1, #2, and #3) throughout the heating period (Table 3). At 0 and 30 h of heating, the differences were significant. Other researchers also noted less CDA formation in low-18:3 soybean oils (7,8). Soybean oils with normal LOX contents tended to have lower CDA values than did the other two oils in the LOX group (i.e., that were lacking LOX 2 or LOX 2 + 3). This finding was significant at 30 h of heating. During heating, autoxidation of polyunsaturated fatty acids causes a shift in the double bonds to produce a conjugated diene, which can be measured by UV absorption at 233 nm (26,27). Absorption at this wavelength increases proportionally with oxidation in the early stages but plateaus during frying because of the establishment of an equilibrium between the rate of formation of conjugated dienes and the rate of polymerization (27).

HPSEC of the oils showed differences in formation of high-molecular weight (MW) compounds during frying. Previous work (24) showed that four peaks formed during the heating of soybean oil. In this study, only three peaks were measurable, which were identified as triacylglycerides and fatty acid trimers with MW of 1,000 daltons, dimeric triglycerides with MW of 2,000 daltons, and tetrameric triacylglycerides with MW of >4,000 daltons.

The high-MW compounds, measured as dimeric and polymeric triglycerides, formed in the greatest quantities during heating (Table 4). When the oils were heated to 180°C, soybean oils with normal 18:3 concentrations (#1, #2, and #3) tended to have greater amounts of high-MW compounds than did oils with low 18:3 concentrations (#4, #5, and #6). After 30 h of heating, the difference was significant. The data from HPSEC had a similar pattern to that of CDA values. In both instances, soybean oils with normal 18:3 contents were not as stable as soybean oils with low 18:3 content.

There were no significant differences and no notable trends in formation of polymerized compounds within the LOX group (#1 and #4, #2 and #5, or #3 and #6) throughout the study. One exception was at 0 h of heating, when soybean oils with normal LOX (#1 and #4) had a significantly greater amount of dimeric triacylglycerides than did the other two groups of oils within the LOX group (#2 and #5; and #3 and #6), but this difference is probably not important because the total amount is so small (Table 4).

Bread cubes. The PV of soybean oils extracted from bread cubes fried at 0 h without storage were similar (Table 5).

TABLE 2
Mean Unsaturated Fatty Acid Composition (%) and Calculated Oxidizability of Soybean Oils at Different Heating Times

Fatty acid	Heating (h)	Soybean oil genotypes[a] #1	#2	#3	#4	#5	#6
18:1	0	22.7	20.6	19.7	30.1	22.6	23.0
	10	23.4	20.6	20.2	30.7	23.3	23.9
	20	25.9	23.2	22.9	33.4	25.1	26.0
	30	29.7	27.6	27.1	37.2	29.9	29.1
18:2	0	54.0	54.9	55.5	51.7	58.3	57.7
	10	52.6	53.8	53.8	50.0	56.6	56.1
	20	50.1	51.3	51.5	47.1	53.6	53.3
	30	43.8	43.9	44.1	39.7	45.3	45.9
18:3	0	8.2	8.5	8.8	2.4	2.9	2.9
	10	7.7	8.2	8.1	2.4	2.7	2.7
	20	6.3	6.6	6.6	1.9	2.4	2.2
	30	4.3	4.1	4.0	1.0	1.5	1.6
Oxidizability[b]		7.6	7.7	7.8	6.1	6.9	6.8

[a]See Table 1 for description of genotype number.
[b]Calculated oxidizability = [oleate% + 10.3 (linoleate%) + 21.6 (linolenate%)]/100 (Ref. 28).

TABLE 3
Mean Conjugated Dienoic Acid Values (%) of Soybean Oils Heated at 180°C

Heating (h)	18:3 concentration[a] Normal	Low	LOX group[b] Normal	LOX 2	LOX 2 + 3
0	0.36[d]	0.24[c]	0.30[c]	0.30[c]	0.30[c]
10	1.68[c]	1.45[c]	1.51[c]	1.57[c]	1.61[c]
20	3.24[c]	2.74[c]	2.92[c]	3.03[c]	3.01[c]
30	4.16[d]	3.88[c]	3.83[c]	4.11[d]	4.11[d]

[a]Within each 18:3 group, normal = mean values of soybean oils from beans with normal 18:3 concentration, and low = mean values of soybean oils from beans with low 18:3 concentration.
[b]Within each LOX group, normal = mean values of soybean oils from beans with normal LOX content; LOX 2 = mean values of soybean oils from beans missing LOX 2; and LOX 2 + 3 = mean values of soybean oils from beans missing LOX 2 and 3. See Table 1 for abbreviation.
[c,d]Values in the same row within each category with different superscripts were significantly different ($P \leq 0.05$).

TABLE 4
Mean Peak Areas (cm^2) from High-Performance Size Exclusion Chromatography Analysis of Soybean Oils Heated to 180°C

		18:3 concentration[b]		LOX group[b]		
Time	Peak[a]	Normal	Low	Normal	LOX 2	LOX 2 + 3
0	TG	4.43[d]	3.08[c]	3.89[c]	3.80[c]	3.58[c]
	DTG	0.40[d]	0.24[c]	0.40[d]	0.31[c]	0.25[c]
10	TG	7.6[d]	6.2[c]	7.0[c]	7.0[c]	6.8[c]
	DTG	10.1[c]	9.3[c]	9.6[c]	9.7[c]	9.8[c]
20	TG	12.0[c]	10.3[c]	10.7[c]	11.7[c]	11.1[c]
	DTG	23.0[c]	20.3[c]	21.4[c]	21.7[c]	21.8[c]
	PTG	1.9[c]	1.3[c]	1.8[c]	1.7[c]	1.4[c]
30	TG	12.0[c]	11.7[c]	11.7[c]	11.9[c]	11.9[c]
	DTG	20.6[c]	25.3[c]	22.1[c]	22.7[c]	23.9[c]
	PTG	18.0[d]	8.7[c]	13.3[c]	13.9[c]	12.8[c]

[a]Peaks were measured as TG = triacylglycerides, DTG = dimeric triacylglycerides, and PTG = polymeric triacylglycerides.
[b]See Table 1 for abbreviation. See Table 3 for definition of 18:3 groups and LOX groups.
[c,d]Values in the same row within each category with different superscripts were significantly different ($P \leq 0.05$).

After storage for 4 d, PV of oils from bread cubes fried in low-18:3 oils tended to be lower than from oils with normal 18:3 concentration. The PV of soybean oils from beans that lacked LOX 2 + 3 (#3 and #6) tended to be the highest among the LOX group, but the differences were not significant. For oils from bread cubes fried after 20 h of heating, PV from normal-18:3 oils tended to be higher than those from low-18:3 oils, regardless of the time of frying. After 4 d of storage, PV of oils from bread cubes fried in soybean oils from beans lacking LOX 2 tended to be the greatest among the LOX group, whereas PV of soybean oils from beans with normal LOX content tended to be the lowest. These differences might be related to calculated oxidizability (Table 2), which suggested greater stability (within each 18:3 grouping) for oils from beans with normal LOX content. Calculated oxidizabilities of oils from beans lacking LOX 2 or LOX 2 + 3 were quite similar, however, so the PV of the stored cubes should have been similar to each other.

TABLE 5
Mean Peroxide Values (meq/kg) of Soybean Oils Extracted from Fried Bread Cubes

Storage[a]	18:3 concentration[b]		LOX group[b]		
(d)	Normal	Low	Normal	LOX 2	LOX 2 + 3
Bread cubes fried at 0 h of heating and stored					
0 day	1.3[c]	1.4[c]	1.3[c]	1.4[c]	1.3[c]
4 days	30.2[c]	12.0[c]	18.6[c]	18.7[c]	26.1[c]
Bread cubes fried after 20 h of heating and stored					
0 day	6.1[c]	4.1[c]	4.8[c]	4.7[c]	5.7[c]
4 days	422.4[c]	284.1[c]	253.2[c]	454.9[c]	351.6[c]

[a]Storage conditions: fried bread cubes held at 60°C in the dark.
[b]See Table 3 for definition of 18:3 groups and LOX groups. See Table 1 for abbreviation.
[c]Values in the same row within each category with different superscripts were significantly different ($P \leq 0.05$).

Sensory evaluations were done on the stored bread cubes (Table 6). When bread cubes had been fried at 0 h and stored in the dark at 60°C for 4 d, cubes fried in soybean oils with normal 18:3 concentration tended to have better sensory scores (less off-flavors). When oils that had been heated for 20 h were used to fry bread cubes subsequently stored for 4 d, those fried in soybean oils with normal 18:3 concentration tended to have slightly more off-flavor than cubes fried in soybean oils with low-18:3 concentration. Bread cubes fried in soybean oils with normal LOX contents tended to have the best scores (the least off-flavors) among the LOX group for both types of stored bread cubes. The sensory data for bread cubes fried at 20 h had the same tendency as did PV. The calculated oxidizability (Table 2) also showed that soybean oils with low-18:3 concentration were more stable than those with normal 18:3 concentration.

This study showed that soybean oils with low 18:3 concentrations had significantly better frying stabilities than did oils with normal 18:3 concentrations. The results are similar to those from a previous study that evaluated the accelerated room-temperature storage of these same oils (18). In general, removal of LOX did not affect the frying stability of soybean oils. Because the activity of LOX 1 in soybean mutant lines was not known, its influence on the frying stability of soybean oils could not be determined. Furthermore, the time between flaking and extracting could be critical when determining the influence of LOX. If the time was too short, there would be no influence of LOX at all. If the time was too long, however, the differences in influences of LOX would be overlooked because oxidation would have reached the maximum rate. The oil extraction procedure used in this research mimicked that of commercial oil extractions, which may have influenced the effects of LOX on frying stability of soybean oils. At least, the conclusion obtained could be used to predict the influence of LOX on the frying stability of soybean oil in the soybean oil industry.

The tocopherol contents of the soybean oils are listed in a previous paper (18). Although there were a few minor significant differences in tocopherol contents of the oils, these differences were probably not important to the outcome of the results presented in this paper. In general, oils with the great-

TABLE 6
Mean Flavor Intensity Scores[a] of Bread Cubes Fried in Soybean Oils[b]

	18:3 concentration[c]		LOX group[c]		
Heating (h)	Normal	Low	Normal	LOX 2	LOX 2 + 3
0 h	7.3[d]	6.6[d]	7.4[d]	6.9[d]	6.6[d]
20 h	5.1[d]	5.4[d]	5.9[d]	4.8[d]	5.0[d]

[a]A score of 1 = extreme and 10 = bland off-flavor.
[b]Experimental conditions: frying temperature, 180°C. Heating periods for oils prior to frying: 0 or 20 h. Storage conditions for fried bread cubes prior to analysis: 60°C, held for 4 d in darkness.
[c]See Table 3 for definition of 18:3 groups and LOX groups. See Table 1 for abbreviation.
[d]Values in the same row within each category with different superscripts were significantly different ($P \leq 0.05$).

est amounts of tocopherols (oils lacking LOX 2 + 3) performed the same as, or slightly worse than, the other oils.

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