Oxidative Stabilities of Soybean Oils That Lack Lipoxygenases

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ABSTRACT: Lipoxygenase (LOX)-null soybean lines that lack LOX 2, or LOX 2 and 3, and contain normal (8.0–8.6%) or low (2.0–2.8%) linolenate (18:3) amounts were evaluated for their oil qualities and storage stabilities. Soybean oils of six genotypes were extracted by both laboratory-scale and pilot-plant systems 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. Under storage conditions of 55–60°C in the dark, soybean oils with low 18:3 contents were significantly ($P \leq 0.05$) more stable as measured by peroxide values than were oils with normal 18:3 contents, regardless of the LOX content of the beans. The volatile analysis showed few differences between oils with low and high 18:3 contents or among oils from beans that lack different LOX enzymes. After 16 d of storage, the amount of 1-octen-3-ol was significantly greater in oils with low 18:3 content, and soybean oils from beans with normal LOX content had a significantly ($P \leq 0.05$) lower amount of 1-octen-3-ol than did the oils that lacked LOX enzymes. Storage at 35°C under light showed no differences in volatile amounts or sensory evaluations after 14 d of storage. During storage, peroxide values tended to be lower in oils from beans with normal 18:3 content and in oils from beans with normal LOX content. Generally, the absence of LOX 2 or LOX 2 and 3, although having a small effect on lipid oxidation, was not as important to oil quality as was the 18:3 content.

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KEY WORDS: Autoxidation, fatty acids, hydroperoxide, lipoxygenase, peroxide value, sensory evaluation, soybean oil, volatile compounds.

The flavor instability of soybean oil has been a challenge to the food industry. The relatively high linolenic acid (18:3) content (8%) is believed to be the major contributor to off-flavor development. Recently, mutation breeding has been used to change the fatty acid composition of soybean oil and to increase its stability. Previous research showed that soybean oil with low 18:3 (3%) was more stable than soybean oil with normal 18:3 (8%) contents (1,2). Soybean oil with low 18:3 content is now commercially available (3).

In addition to the fatty acid composition, lipoxygenase (LOX) enzymes are also thought to contribute to flavor instability of soybean oils (4–8). LOX includes a group of enzymes that catalyze the hydroperoxidation of polyunsaturated fatty acids or esters that contain a *cis, cis*-1,4 pentadiene system (9,10). The abundant amount of polyunsaturated fatty acids in soybean oil, such as linoleic acid (18:2) and 18:3, provides substrate for this enzyme. LOX has three isozymes—LOX 1, LOX 2, and LOX 3. The main differences in the activities of these isozymes include pH optimum, heat stability, and substrate specificity (11).

Genetic removal of the LOX 2 isozyme reduced off-flavors in soy foods, such as soy milk (6), thus improving acceptability of soy foods as off-flavor is the major factor restricting the use of soy foods (12). Frankel *et al.* (7) found that the absence of LOX 1 did not affect the oxidative stability of soybean oil. Endo *et al.* (13) 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 (100 lux). Sensory evaluations and peroxide values, measured on days 1, 3, 5, and 7, did not show any influence of these combinations of missing LOX on oxidative stability. Soybean oils from beans that lack additional combinations of the LOX isozymes may have better oxidative stability than the LOX-null soybean oils already tested and from beans with normal LOX enzyme content.

The objectives of this study were to compare the oxidative stabilities of soybean oils with normal and reduced LOX contents, in combination with normal and reduced 18:3, to determine their influences on oil stability. The influence of LOX 1 was not studied because beans lacking this isozyme were not available.

MATERIALS AND METHODS

Materials. Commercial soybeans (Century 84), a low linolenic (18:3) soybean genotype (A89-269043), and four experimental soybean genotypes were grown near Ames, Iowa in 1992. After harvest, the soybean seeds were grouped according to 18:3 and LOX contents, as described later. Cen-

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tury 84 and A89-269043 were grown as pure genotypes with known LOX and 18:3 contents.

Experimental soybean genotypes that lack one or two LOX enzymes were developed in the Department of Agronomy, Iowa State University (Ames, IA). The plant cross method is described briefly: The first filial generation seeds were obtained from the cross AX7788 (L2L3-2-4 × A89) in Ames in 1990. L2L3-2-4, a genotype lacking LOX 2 and 3, was obtained from Purdue University (LaFayette, IN), and A89 is a 2% 18:3 genotype. The plants of the second filial generation were grown and harvested individually. Individual plants were screened for LOX by using a rapid spot test designed by Hammond *et al.* (14). Plants of the second filial generation that missed LOX 2 and LOX 2 and 3 were analyzed for fatty acid composition according to a method described by Hammond (15), in which the triacylglycerides are converted into fatty acid methyl esters (FAME) and injected onto the gas chromatograph (GC). Theoretical response factors for quantitating the fatty acids were applied to the results as proposed by Ackman (16). Selected plants were pooled and planted. Individual third filial-generation plants were analyzed for fatty acid contents and LOX contents, and again were pooled as LOX 2 or LOX 2 and 3. All tested soybean seeds were pooled into six categories according to 18:3 and LOX contents (Table 1).

Screening for lipoxygenase. The beans were analyzed for LOX content to indicate the presence or absence of LOX 2 and LOX 3 or both (14). Soybean seeds were crushed in a hydraulic press to allow the LOX to react with added pure soybean oil. Due to the presence of LOX hydroperoxides formed during this step reacted with ferrous sulfate and ammonium thocyanate to form color. A second LOX screening method (17) was used simultaneously with the method of Hammond *et al.* (14) to test LOX activity of the flakes during the processing steps. The second method became available during the course of the study, so it was used to compare LOX results with the first test.

Oil extraction. The moisture content of each replication of soybeans as measured by using a Gac2000 Grain Analysis Computer (Dickey-John Corporation, Auburn, IL). To equalize moisture content for all beans, moisture was adjusted to 11% by spraying the appropriate amount of water over each group of beans, thoroughly mixing them, and storing them in moisture-proof plastic bags at 5°C. The storage time was overnight (12 h) for laboratory-extracted beans and two days for the greater quantity of beans used in pilot-plant extraction. Random moisture determinations of small amounts of beans after storage indicated homogeneity of the moisture distribution.

Soybean seeds were cracked, heated, and flaked for each type of extraction. Soybean seeds were cracked into six to eight pieces in a corrugated roller mill (Ferrell-Ross Co., Oklahoma City, OK). For laboratory extraction, the cracked seed was heated within 20 min to 70°C in a 10-gallon-steam kettle (Lee Metal Products Co. Inc., Philipsburg, PA). For pilot-plant extraction, the greater quantity of cracked soybean seeds was heated to 70°C in a three-tray seed conditioner (French Oil Mill Machinery Co., Piqua, OH). Heated and cracked soybean seeds then were flaked in a smooth roller mill (Roskamp Manufacturing Inc., Waterloo, IA) to about 0.25 mm in thickness. For each extraction method, samples from each genotype were divided into two lots before moistening to give two replications for each of the six soybean genotypes. Soybean flakes were extracted by two methods, laboratory-scale and pilot-plant-scale extraction, to examine the possible influence of extraction methods on oil stability.

Laboratory-scale extraction. Soybean flakes from each genotype were extracted in duplicate, to give twelve total sample extractions. The extraction method was described in an accompanying paper (18).

The miscella was desolventized in a rotary evaporator (Wheaton, Heidolph, Germany) at 60°C at 80 rpm. For each 0.70 kg of miscella, 17 min was needed to desolventize. After desolventizing, the crude oils were stored at −14°C under nitrogen until needed.

Pilot-plant extraction. Soybean seeds (11.4 kg) from each genotype were extracted on a pilot-plant scale in duplicate, to give twelve total samples. Soybean flakes (9.1 kg per replicate) were extracted at 60°C, with a ratio of solvent to flakes of 1.3:1 (per stage). During each extraction, the flakes were extracted in a batch-advance solvent-extractor (French Oil Mill Machinery Co.) in five stages with 10 min of extraction, plus 3 min of draining between stages.

The miscella was desolventized, and the meal was toasted in a three-tray desolventizer toaster (French Oil Mill Machinery Co.). The miscella was heated to 104.4°C at 330.2 mm of

TABLE 1
Lipoxygenase (LOX) and Approximate Linolenic Acid Contents of Soybean Genotypes

Name[a]	Sample designation	LOX content	Approximate linolenic acid (18:3)
Century 84	#1	Normal LOX	>8.0% 18:3
L2-3	#2	No LOX 2	>8.0% 18:3
L2L3-2-4	#3	No LOX 2 and 3	>8.0% 18:3
A89-269043	#4	Normal LOX	<3.0% 18:3
L2/3%18:3	#5	No LOX 2	<3.0% 18:3
L2L3/3%18:3	#6	No LOX 2 and 3	<3.0% 18:3

[a]Beans grown near Ames, Iowa, in 1992.

Hg vacuum and maintained for 5 min. The resulting crude oils were stored at −14°C under nitrogen.

Refining, bleaching, and deodorizing. Crude oils from each replicate from both types of extraction were refined, bleached, and deodorized before being used in storage stability tests.

The free fatty acid contents of the crude oils were determined according to AOCS official method Ca-5a-40 (19). An amount of NaOH was added to each oil according to AOCS official method Ca-9d-52 (19) and stirred for 90 min at 25°C, followed by heating to 65°C for 20 min with stirring and then holding for 1 h at 65°C. Finally, the oil was allowed to sit for a minimum of 12 h at room temperature. Soapstock was removed by filtration.

The alkali-refined oils were bleached by using AOCS official method Cc-8b-52 (19). Official natural bleaching earth (3%) was added to the oil, the mixture was heated to 120°C for 5 min and filtered immediately by using Fleaker® filtration units (Spectrum, Houston, TX) with Whatman No. 1 filter paper.

The bleached oils were steam-deodorized at high vacuum (<0.5 Torr) and high temperature (230–240°C for 2 h). The steam-distillation method was described by Stone and Hammond (20) and modified according to Moulton (21). When the oil was cooled to 100°C, 100 ppm citric acid was added. Duplicate sets of each oil were refined, bleached, and deodorized separately. The oil was stored under nitrogen at −14°C until needed.

Experimental design of oxidative stability tests. Oils were evaluated for oxidative stabilty in three separate studies. The tocopherol contents for each replicate of soybean oils extracted in the pilot plant were measured in duplicate according to Dove and Ewan (22).

Study 1: Storage of laboratory-extracted oils at 60°C in the dark. Twelve replicate oil samples (40 g each) were stored in 100-mL beakers without covers in the dark at 60°C for 12 d. An aliquot of soybean oil was removed after mixing for analysis every third day. Oils were tested in duplicate; each duplicate was measured two times, for peroxide value (PV) and the results were averaged.

PV was measured according to the Stamm method as modified by Hamm *et al.* (23). Fatty acid compositions of the oils were measured before and after storage by the procedure described previously. The oxidizability was calculated for each oil according to a formula proposed by Fatemi and Hammond (24): Oxidizability = [oleate% + 10.3 (linoleate%) + 21.6 (linolenate%)]/100.

Study 2: Storage of pilot plant-extracted oils at 50°C in the dark. Twelve replicate oil samples (200 g each) were stored in 250-mL beakers without covers at 50°C in the dark for 22 d. An aliquot of soybean oil was removed for analysis of PV, FAME, and volatile compounds every other day. Volatile compounds were analyzed according to a method proposed by Lee *et al.* (25). Volatile compounds were measured in duplicate on day 0 and day 16 for each oil sample.

Study 3: Storage of pilot plant-extracted oils at 35°C under light. Twelve replicate soybean oils (40 g) were stored in 100-mL beakers without covers at 35°C under fluorescent light for 14 d. Light intensity, measured by a light-level meter (Weston Illumination Meter, Weston Instruments, Division of Daystrom, Inc., Newark, NJ), was 160 foot-candles. PV was analyzed every other day. For each replicate, volatile compounds and FAME were measured in duplicate on days 0 and 14. Sensory evaluations were performed at the beginning and at the end of storage. Panelists were trained during four practice sessions with both fresh and oxidized soybean oils to familiarize panelists with samples similar to those they would be evaluating and with the evaluation methods. Sensory evaluations were performed by using the emulsion method described by Dixon and Hammond (26). The emulsion was a mixture of 10 g of oil (1%), 6.5 g of gum acacia (0.65%), and 1 kg of water. A line-scale questionnaire was used in which the scale was a line 15-cm long, without marks ("anchors") at both ends. The left end of the scale referred to the weakest off-flavor, whereas the right end referred to the strongest off-flavor. Panelists used the line scale by placing a perpendicular mark on the scale according to their perceptions of off-flavor intensities. The marks from the line scales were converted to numbers by measuring the distance of each mark from the left end of the scale. Each replicate oil sample was evaluated two times by the sensory panel on day 0 and again on day 14.

Statistical analysis. A randomized 2×2 factorial design was used for this experiment. Data from all treatments in each storage test were analyzed by analysis of variance and least SD for statistical significance on SAS (27). Significance was accepted at a level of $P \leq 0.05$. The main plot was represented by six treatments (2×3), plus two replicates.

RESULTS AND DISCUSSION

To analyze both the influence of 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 and 3). Statistical analyses were performed based on the data in these two groups.

The fatty acid compositions of soybean oil types studied are shown in Table 2. Century 84 (#1) and A89-269043 (#4) served as controls but had less 18:3 and more 18:1 than did the other oils in their respective groups. The calculated oxidizabilities of these two oils were lower than the others within the group. These fatty acid differences may reduce the impact of eliminating the LOX isozymes. The soybean oils used in all studies were from the same seeds; however, different extraction methods were used for Study 1 and Studies 2 and 3, and likely resulted in slightly different fatty acid compositions.

To determine the influence of the processing steps on activities of the LOX enzymes, LOX was tested after the soybean seeds were flaked. Methods of both Hammond *et al.* (14) and of Suda *et al.* (17) were used. Both sets of results indicated that the processing did not totally denature LOX. The

TABLE 2
Fatty Acid Composition (%) and Calculated Oxidizability of Soybean Oils with Different Lipoxygenase and Fatty Acid Contents Before and After Storage[a]

Fatty acid	Status	Soybean oil genotypes					
		#1	#2	#3	#4	#5	#6
Study 1							
16:0	Start	12.3	13.7	13.3	11.9	12.8	12.4
	End[b]	12.7	13.0	12.9	12.1	13.0	13.0
18:0	Start	3.7	3.7	4.1	4.2	3.9	4.1
	End	4.1	4.1	4.2	4.4	4.1	4.2
18:1	Start	22.3	19.6	19.9	30.2	22.8	23.5
	End	23.7	21.1	20.5	31.0	23.6	24.0
18:2	Start	52.5	53.9	53.7	50.9	57.1	56.5
	End	51.6	53.3	53.5	49.8	56.2	55.5
18:3	Start	7.8	8.4	8.4	2.2	2.5	2.5
	End	7.1	7.7	8.1	2.0	2.4	2.4
Oxidizability		7.3	7.6	7.5	6.0	6.6	6.6
Studies 2 and 3							
16:0	Start	9.9	10.6	10.7	10.1	11.0	11.2
	Study 2 end[c]	9.8	10.6	10.8	10.1	11.1	11.6
	Study 3 end[d]	9.8	10.6	10.7	10.2	11.2	11.11
18:0	Start	4.0	3.8	4.4	4.2	3.9	4.0
	Study 2 end	3.9	3.5	4.2	4.2	3.9	3.7
	Study 3 end	4.5	4.5	4.5	4.8	4.3	4.5
18:1	Start	23.1	21.0	21.2	30.9	23.6	23.9
	Study 2 end	23.5	21.5	20.9	31.1	23.6	24.1
	Study 3 end	23.7	21.1	21.3	31.3	23.6	24.2
18:2	Start	54.0	55.2	54.7	51.5	57.9	57.3
	Study 2 end	54.1	55.2	55.0	51.3	57.8	57.0
	Study 3 end	53.3	54.7	54.5	50.6	57.4	56.7
18:3	Start	8.2	8.6	8.2	2.4	2.8	2.7
	Study 2 end	7.9	8.3	8.3	2.4	2.8	2.7
	Study 3 end	7.9	8.3	8.3	2.4	2.7	2.7
Oxidizability		7.6	7.8	7.6	6.1	6.8	6.7

[a]See Table 1 for abbreviations.
[b]Oils were stored for 12 d.
[c]Oils were stored for 22 d.
[d]Oils were stored for 14 d.

latter test was somewhat more sensitive than the former test, in that a reduction in LOX activity was noted when it was used on the heated flakes. The method of Hammond *et al.* (14) was either positive or negative, but the method of Suda *et al.* (17) could use the rate of color change to indicate the relative activities of the enzymes. Neither method was quantitative.

The tocopherol contents of soybean oils extracted in the pilot plant are shown in Table 3. There were no significant differences in tocopherol contents between the two 18:3 groups, except for β-tocopherol. Considering the low portion of total β-tocopherol present, and its minimal antioxidant ability compared with α- and γ-tocopherols, this difference was likely not important. Among the LOX groups, all soybean oils had similar amounts of β- and δ-tocopherols. Soybean oils from beans that lacked LOX 2 and 3 (M2-3) had significantly greater amounts of α- and γ-tocopherols. These differences could potentially influence the oxidative stabilities of the oils, and the impact is discussed at the conclusion of this paper.

Study 1: Storage of laboratory-extracted oils at 60°C in the dark. PV values of all soybean oil types during storage for 12 d are shown in Table 4. PV were significantly different between 18:3 groups for all days, except for days 3 and 12. There were no significant differences in PV among the LOX groups. Values for individual soybean oil groups suggested the same trends. These data indicate that a low 18:3 content is important to oxidative stability of soybean oil, but that removal of LOX 2 or of LOX 2 and 3 had no significant effect. Other researchers have shown similar effects on stability of soybean oils with a reduction of 18:3 (2,28).

The fatty acid profiles were determined before and after oxidation (Table 2). Generally, the contents of polyunsaturated fatty acids tended to decrease because of oxidation. Oxidizability was calculated from the fatty acid composition, as proposed by Fatemi and Hammond (24). The PV of oils with the lowest calculated oxidizability (#4, #5, and #6) were significantly lower than oils with the highest calculated oxidizability (#1, #2, and #3) (Table 4). Differences in calculated oxidizability among oils with similar 18:3 contents, but from beans with different isozyme contents, were minimal, except for #4 (value of 6.0), which may have had a slight influence on oil stability.

TABLE 3
Tocopherol Contents (mg/kg) of Pilot Plant–Extracted Soybean Oils[a]

Tocopherol	Soybean oil genotypes (#)						18:3 group[b]		LOX group[c]		
isomer	#1	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
α	32.6	31.3	53.0	28.7	32.8	52.0	38.9[d]	37.8[d]	30.6[d]	32.0[d]	52.5[e]
β	5.7	5.4	5.6	3.6	4.1	5.2	5.5[e]	4.6[d]	5.2[d]	4.7[d]	5.4[d]
γ	263.3	255.5	313.3	264.6	301.0	357.7	277.4[d]	307.8[d]	263.9[d]	278.3[d]	335.5[e]
δ	124.2	112.8	114.7	109.3	117.3	119.4	117.2[d]	117.0[d]	119.2[d]	115.0[d]	117.0[d]
Total	425.8	405.0	486.6	407.2	455.2	534.3	439.0	467.2	418.9	430.0	510.4

[a]See Table 1 for abbreviations.
[b]Within each 18:3 group, M1-1 = means of tocopherol isomers in soybean oils with normal 18:3 and M1-2 = means of tocopherol isomers in soybean oils with low 18:3 content.
[c]Within each LOX group, M2-1 = means of tocopherol isomers in soybean oils with normal LOX, M2-2 = means of tocopherol isomers in soybean oils from beans without LOX 2, and M2-3 = means of tocopherol isomers in soybean oils from beans without LOX 2 and 3.
[d,e]Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

TABLE 4
Peroxide Values (meq/kg) of Laboratory-Extracted Soybean Oils During Storage at 60°C in the Dark (Study 1)[a]

	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Day	#1	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0	0.40	0.15	0.14	0.15	0.14	0.15	0.23[e]	0.15[d]	0.27[d]	0.14[d]	0.14[d]
3	2.24	0.68	0.74	0.68	0.79	0.70	1.22[d]	0.72[d]	1.46[d]	0.73[d]	0.72[d]
6	22.1	17.8	13.0	4.1	5.7	3.2	17.6[e]	4.33[d]	13.1[d]	11.8[d]	8.1[d]
9	70.5	75.4	69.2	46.4	51.6	40.1	71.7[e]	46.0[d]	58.4[d]	63.4[d]	54.6[d]
12	100.2	98.2	106.0	93.3	100.2	106.4	101.5[d]	99.9[d]	96.7[d]	99.4[d]	106.6[d]

[a]See Table 1 for abbreviations.
[b]Within each 18:3 group, M1-1 = means of peroxide values (PV) of soybean oils with normal 18:3 and M1-2 = means of PV of soybean oils with low 18:3.
[c]Within each LOX group, M2-1 = means of PV of soybean oils with normal LOX, M2-2 = means of PV of soybean oils from beans without LOX 2, and M2-3 = means of PV of soybean oils from beans without LOX 2 and 3.
[d,e]Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

Study 2: Storage of pilot plant–extracted oils at 50°C in the dark. The stabilities of soybean oils from pilot plant extractions were evaluated at 50°C, to be consistent with the temperature used to collect the volatile compounds. PV confirmed that soybean oils with low 18:3 (#4, #5, and #6) were more stable than those with normal 18:3 content (#1, #2, and #3) (Table 5). These differences were significant after six days of storage. Because larger samples (200 g instead of 40 g) and lower storage temperature (50°C instead of 60°C) were employed in Study 2 than in Study 1, the development of oxidation was much slower in Study 2 than in Study 1. From day 2 to day 12 of storage, there was a tendency for soybean oils from beans without LOX 2 isozyme (#2 and #5) to have the highest PV, whereas the oils from beans without LOX 2 and 3 (#3 and #6) had the lowest PV. This tendency was significant on days 2, 8, and 10. From day 16 to 22, soybean oils from beans of normal LOX content (#1 and #4) tended to have the lowest PV. On days 20 and 22, this difference was significant. After 14 d of storage, soybean oils without LOX 2 and 3 (#3 and #6) tended to have the highest PV. These differences in LOX group were not observed in Study 1. The calculated oxidizabilities suggested that #3 and #6 (and even #2 and #4) might be less oxidatively stable than #1 and #5 (Table 2), and data in Table 5 confirms, this trend.

The fatty acid compositions before and after storage were measured (Table 2). Because the oils were not oxidized severely, the ending values were not substantially different from the beginning values. This observation also was noted by White and Miller (28). When PV were less than 30 meq/kg, the ending values of 18:3 were close to the beginning values.

Volatile compounds were analyzed by GC on days 0 and 16 (Table 6). Nine volatile compounds were chosen for measurement based on previous work (25). Many of these volatiles were often selected by other researchers to measure oil oxidation (29–31). Among these volatile compounds, both *t,c*-2,4-heptadienal and *t,t*-2,4-heptadienal are generally believed to be from the oxidation of 18:3 and both 1-octen-3-ol and 2-octenal from 18:2 (32).

On day 0, there were no significant differences in amounts of the volatile compounds between 18:3 content groups. Among LOX groups, the only significant differences were in hexenal and 1-octen-3-ol contents. Oils from beans without LOX 2 (#2 and #5) had significantly more 1-octen-3-ol and significantly more hexenal, a major off-flavor contributor in oxidizing soybean oils, than did oil from normal beans. Soybean oils with normal 18:3 (#1, #2, and #3) tended to have more *t,t*-2,4-heptadienal than did the soybean oils with low 18:3 content (#4, #5, and #6), because this volatile compound

TABLE 5
Peroxide Values (meq/kg) of Pilot Plant–Extracted Soybean Oils During Storage at 50°C in the Dark (Study 2)

	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Day	#1	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0	0.12	0.20	0.13	0.13	0.20	0.12	0.15[d]	0.16[d]	0.13[d]	0.20[d]	0.13[d]
2	0.29	0.37	0.26	0.23	0.30	0.19	0.31[d]	0.25[d]	0.27[de]	0.33[e]	0.23[d]
4	0.43	0.55	0.39	0.35	0.44	0.31	0.47[d]	0.38[d]	0.39[d]	0.49[d]	0.35[d]
6	0.54	0.66	0.47	0.47	0.50	0.40	0.57[d]	0.46[d]	0.50[d]	0.58[d]	0.44[d]
8	9.71	0.92	0.63	0.57	0.62	0.51	0.78[e]	0.58[d]	0.64[de]	0.77[e]	0.57[d]
10	0.82	1.04	0.75	0.65	0.70	0.57	0.89[e]	0.65[d]	0.73[de]	0.87[e]	0.66[d]
12	1.02	1.29	0.99	0.75	0.84	0.75	1.10[e]	0.78[d]	0.87[d]	1.06[d]	0.87[d]
14	2.15	3.4	3.3	1.07	1.30	1.31	2.89[e]	1.21[d]	1.61[d]	2.35[d]	2.33[d]
16	5.6	8.3	9.3	2.20	2.76	4.1	7.41[e]	2.81[d]	3.89[d]	5.52[d]	6.72[d]
18	9.6	12.6	15.1	6.0	5.7	9.0	11.9[e]	5.9[d]	7.0[d]	9.1[d]	12.1[d]
20	15.1	16.8	23.6	7.6	9.6	14.8	17.5[e]	9.8[d]	11.3[d]	13.2[de]	19.2[e]
22	23.8	23.9	33.2	11.8	15.1	21.6	25.7[e]	15.1[d]	17.8[d]	19.5[de]	27.4[e]

[a]See Tables 1 and 4 for abbreviations.
[b]Within each 18:3 group, M1-1 = means of PV of soybean oils with normal 18:3 and M1-2 = means of PV of soybean oils with low 18:3.
[c]Within each LOX group, M2-1 = means of PV of soybean oils with normal LOX, M2-2 = means of PV of soybean oils from beans without LOX 2, and M2-3 = means of PV of soybean oils from beans without LOX 2 and 3.
[d,e]Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

TABLE 6
Peak Areas (10^{-5}) of Volatile Compounds From Pilot Plant–Extracted Soybean Oils Stored at 50°C in the Dark (Study 2)[a]

Volatile	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Compound	#1	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
Day 0											
Pentanal	0.31	0.11	0.60	0.03	0.50	0.20	0.39[d]	0.44[d]	0.17[d]	0.34[d]	0.74[d]
2-Pentenal	0.10	0.25	1.41	0.09	0.14	0.00	0.58[d]	0.10[d]	0.09[d]	0.19[d]	0.74[d]
Hexenal	0.39	2.10	1.00	1.03	3.1	1.68	1.42[d]	1.93[d]	0.71[d]	2.60[e]	
1.72[de]											
2-Heptenal	0.08	1.38	1.28	0.13	4.1	0.49	0.91[d]	1.78[d]	0.10[d]	3.04[d]	0.88[d]
1-Octen-3-ol	0.09	0.33	0.28	0.00	1.02	0.20	0.23[d]	0.41[d]	0.04[d]	0.67[e]	
0.24[de]											
t,c-2,4-Heptadienal	0.00	0.45	0.73	0.15	0.53	0.54	0.39[d]	0.40[d]	0.07[d]	0.48[d]	0.63[d]
t,t-2,4-Heptadienal	0.29	1.16	2.60	0.42	2.01	0.55	1.37[d]	0.99[d]	0.35[d]	1.58[d]	1.61[d]
2-Octenal	0.33	1.22	0.92	0.30	1.80	0.85	0.82[d]	1.12[d]	0.53[d]	1.51[d]	0.88[d]
Nonanal	0.90	1.80	2.86	0.93	2.08	2.01	1.81[d]	1.66[d]	0.84[d]	1.94[d]	2.43[d]
Day 16											
Pentanal	0.26	0.20	0.35	0.22	0.31	0.38	0.30[d]	0.30[d]	0.24[d]	0.29[d]	0.36[d]
2-Pentenal	1.20	0.81	3.0	0.63	1.11	2.00	1.85[d]	1.27[d]	1.17[de]	0.96[d]	2.54[e]
Hexenal	0.80	0.85	16.1	8.2	22.0	19.2	10.6[d]	16.7[d]	8.0[d]	15.3[d]	17.6[d]
2-Heptenal	16.1	0.58	4.0	18.0	38.4	50.9	23.8[d]	35.8[d]	17.0[d]	23.0[d]	49.3[e]
1-Octen-3-ol	5.0	3.1	9.7	5.7	23.9	35.4	5.9[d]	21.7[e]	5.3[d]	13.5[e]	22.6[f]
t,c-2,4-Heptadienal	5.0	1.15	34.0	0.63	4.8	6.9	13.6[d]	6.5[d]	6.3[d]	3.0[d]	20.8[e]
t,t-2,4-Heptadienal	4.4	1.45	14.0	5.1	8.4	6.1	6.6[d]	6.5[d]	4.8[d]	4.9[d]	10.0[d]
2-Octenal	3.0	1.01	8.4	5.2	5.1	6.8	4.1[d]	5.7[d]	4.1[d]	3.0[d]	7.6[e]
Nonanal	1.84	0.60	2.41	2.5	2.9	1.00	1.67[d]	2.20[d]	2.17[d]	1.82[d]	1.81[d]

[a]See Table 1 for abbreviations.
[b]Within each 18:3 group, M1-1 = means of volatile compounds of soybean oils with normal 18:3 and M1-2 = means of volatile compounds of soybean oils with low 18:3 content.
[c]Within each LOX group, M2-1 = means of volatile compounds of soybean oils with normal LOX content, M2-2 = means of volatile compounds of soybean oils from beans without LOX 2, and M2-3 = means of volatile compounds of soybean oils from beans without LOX 2 and 3.
[d,f]Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

came from the oxidation of 18:3. Soybean oils with normal LOX (#1 and #4) tended to have less *t,c*-2,4-heptadienal and *t,t*-2,4-heptadienal than did the other two soybean oils. Soybean oils with normal 18:3 content (#1, #2, and #3) tended to have less 1-octen-3-ol and 2-octenal than did the other oils, because the precursor to these compounds (18:2) (32) was lower in these oils than in oils with low 18:3 content (#4, #5, and #6).

On day 16, there were no significant differences between the 18:3 content groups, except for 1-octen-3-ol. The amount of 1-octen-3-ol from the low 18:3 content group (#4, #5, and #6) was significantly greater than that from the normal 18:3 content group (#1, #2, and #3). This observation was likely caused by the higher content of 18:2 in oils #4, #5, and #6. Soybean oils with normal 18:3 content

tended to have more *t,c*-2,4-heptadienal than did oils with low 18:3.

Among LOX groups, soybean oils from beans without LOX 2 and 3 (#3 and #6) had significantly greater amounts of many of the volatiles than did oils from the other two types. Oils from beans without LOX 2 and 3 (#3 and #6) had significantly more 2-pentenal than did oils from beans lacking LOX 2 (#2 and #4). The amounts of 2-heptenal, *t,c*-2,4-heptadienal, and 2-octenal released from soybean oils from seeds without LOX 2 and 3 (#3 and #6) were greater than those from the other two types. Soybean oils from beans without LOX 2 and 3 (#3 and #6) released the greatest amount of 1-octen-3-ol, whereas soybean oils with normal LOX contents (#1 and #4) had the least. In general, the volatile analyses showed that soybean oils from beans without LOX 2 and 3 (#3 and #6) released more volatile compounds than did the other two groups. On day 16, PV confirmed that oils from seeds without LOX 2 and 3 (#3 and #6) showed greater oxidation than did the other two groups. The calculated oxidizability predicted the same trend as did Study 1. The data in Study 2 reflected the results of Study 1; soybean oils with low 18:3 contents were significantly more stable than were oils with normal 18:3 contents. LOX did not play an important role in oxidative stability.

Study 3: Storage of pilot plant–extracted oils at 35°C under light. Storage of the samples in an oven at 35°C under fluorescent light caused much faster oxidation than at 50 or 60°C in the dark (Table 7 vs. Tables 4 and 5). In general, there was a tendency for soybean oils with normal 18:3 contents (#1, #2, and #3) to have the lowest PV. On days 8 and 14, this tendency was significant. Among LOX groups, the PV of soybean oils from beans without LOX 2 and 3 (#3 and #6) were significantly greater than PV from oils with normal LOX contents from days 8 to 14. The calculated oxidizability correctly predicted greater oxidation of #3 and #6 than that of #1 and #4 (Table 2). The oils from beans without LOX 2 (#2 and #5) had the greatest overall oxidizability scores, yet tended to be intermediate in PV. Because this study was conducted under light at 35°C versus in the dark at 50–60°C (Studies 1 and 2), the oxidation pathway might have been different.

The fatty acid compositions of the oils used in Study 3 before and after oxidation are listed in Table 2. Few differences in beginning and ending fatty acid data were noted.

The same volatile compounds as in Study 2 were measured by using the same method (Table 8). On day 0, there were no significant differences in amounts of volatiles between 18:3 content groups and among LOX groups, except for hexenal. Oils from beans without LOX 2 and 3 (#3 and #6) had significantly less hexenal than did oils from beans without LOX 2 (#2 and #5). Seeds containing all LOX (#1 and #4) were intermediate in amount of hexenal. Soybean oils with normal 18:3 content tended to have more *t,c*-2,4-heptadienal and *t,t*-2,4-heptadienal, as expected.

On day 14, there were no significant differences between 18:3 content groups or among LOX groups, and few tendencies were noted. Again, soybean oils with normal 18:3 content tended to have more of both *t,c*-2,4-heptadienal and *t,t*-2,4-heptadienal. Because of different storage conditions, the volatile compounds developed under light were different from those produced in the dark (33,34).

Sensory evaluations were conducted to determine the sensory quality of fresh and stored samples. Among the oils judged by the sensory panel (Table 9), the oils from the low 18:3 group (#4, #5, and #6) tended to have a lower sensory score (weakest off-flavor) on day 0. By day 14, this tendency was not evident. Among LOX groups, sensory scores tended to be lowest for oils from beans without LOX 2 and 3 (#3 and #6) on day 0 and highest for oils from beans without LOX 2 (#2 and #5). On day 14, oils with normal LOX (#1 and #4) and oils from beans without LOX 2 and 3 (#3 and #6) were similar, and oils from beans without LOX 2 (#2 and #5) tended to have the strongest off-flavor. The calculated oxidizability scores were consistent with the high sensory scores for samples #2 and #5. PV and volatiles at day 14 did not suggest similar tendencies. Perhaps differences in PV were not great enough for sensory panelists to detect differences in the oils.

TABLE 7
Peroxide Values (meq/kg) of Pilot Plant–Extracted Soybean Oils During Storage at 35°C Under Light (Study 3)[a]

	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Day	#1	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0	0.26	0.34	0.34	0.30	0.36	0.28	0.31[d]	0.31[d]	0.28[d]	0.35[d]	0.31[d]
2	1.53	1.71	1.99	1.54	1.69	1.65	1.74[d]	1.63[d]	1.54[d]	1.70[d]	1.8[d]
4	2.45	2.73	4.8	3.5	3.4	5.7	3.22[d]	4.2[d]	2.98[d]	3.1[d]	5.1[d]
6	4.2	5.1	8.2	7.2	7.3	12.6	5.8[d]	9.0[d]	5.7[d]	6.2[d]	10.4[d]
8	6.3	9.8	15.8	12.7	14.8	21.9	10.6[d]	16.4[e]	9.5[d]	12.3[d]	18.8[e]
10	14.3	17.4	21.8	19.2	22.7	29.6	17.8[d]	23.8[d]	16.7[d]	20.0[de]	25.7[e]
12	19.6	22.1	30.1	26.1	27.5	35.5	23.9[d]	29.7[d]	22.8[d]	24.8[de]	32.8[e]
14	25.2	28.8	34.5	31.1	34.4	41.2	29.5[d]	35.5[e]	28.1[d]	31.6[de]	37.8[e]

[a]See Tables 1 and 4 for abbreviations.

[b]Within each 18:3 group, M1-1 = means of PV of soybean oils with normal 18:3 and M1-2 = means of PV of soybean oils with low 18:3.

[c]Within each LOX group, M2-1 = means of PV of soybean oils with normal LOX, M2-2 = means of PV of soybean oils from beans without LOX 2, and M2-3 = means of PV of soybean oils from beans without LOX 2 and 3.

[d,e]Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

TABLE 8
Peak Areas (10^{-5}) of Volatile Compounds of Pilot Plant–Extracted Soybean Oils Stored at 35°C Under Light (Study 3)[a]

Volatile	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Compound	#1	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
Day 0											
Pentanal	1.45	0.68	5.85	0.30	0.38	0.20	2.66[d]	0.29[d]	0.88[d]	0.53[d]	3.03[d]
2-Pentenal	0.50	0.25	0.63	0.10	0.55	0.13	0.46[d]	0.26[d]	0.30[d]	0.40[d]	0.38[d]
Hexenal	2.65	2.25	2.23	1.43	5.15	1.13	2.38[d]	2.57[d]	2.04[de]	3.70[e]	1.68[d]
2-Heptenal	0.65	0.80	1.30	0.20	1.50	0.48	0.91[d]	0.81[d]	0.43[d]	1.26[d]	0.89[d]
1-Octen-3-ol	0.45	0.93	0.90	4.9	8.7	3.60	5.4[d]	5.7[d]	6.2[d]	4.8[d]	5.8[d]
t,c-2,4-Heptadienal	1.83	1.10	1.20	0.48	2.38	0.33	1.38[d]	1.06[d]	1.15[d]	1.74[d]	0.76[d]
t,t-2,4-Heptadienal	1.13	1.50	1.83	0.98	0.83	0.95	1.48[d]	0.92[d]	1.05[d]	1.16[d]	1.39[d]
2-Octenal	3.25	1.48	3.68	2.40	1.95	1.63	2.80[d]	1.99[d]	2.83[d]	1.71[d]	2.65[d]
Nonanal	2.23	1.58	2.28	0.00	1.50	0.60	2.03[d]	0.93[d]	1.46[d]	1.54[d]	1.44[d]
Day 14											
Pentanal	2.13	2.13	1.60	1.30	2.43	1.33	1.95[d]	1.68[d]	1.71[d]	2.28[d]	1.46[d]
2-Pentenal	3.20	5.5	11.9	5.2	6.4	11.0	6.9[d]	7.7[d]	4.2[d]	6.0[d]	11.8[d]
Hexenal	52.1	61.9	5.2	4.8	62.0	6.5	57.0[d]	68.1[d]	63.5[d]	61.9[d]	62.3[d]
2-Heptenal	53.5	59.0	51.3	66.9	59.0	50.6	54.6[d]	59.1[d]	60.2[d]	59.3[d]	51.0[d]
1-Octen-3-ol	38.1	2.40	20.5	43.4	38.0	30.4	28.6[d]	37.5[d]	40.7[d]	33.0[d]	25.4[d]
t,c-2,4-Heptadienal	2.15	3.3	3.8	1.65	2.18	1.88	3.11[d]	1.90[d]	1.90[d]	2.75[d]	2.86[d]
t,t-2,4-Heptadienal	6.5	2.80	3.8	0.50	2.60	1.00	4.4[d]	1.68[d]	3.60[d]	2.69[d]	2.76[d]
2-Octenal	2.33	2.38	3.2	3.2	3.3	4.0	2.63[d]	3.49[d]	2.78[d]	2.84[d]	3.56[d]
Nonanal	3.2	1.25	1.50	0.80	0.65	1.30	2.05[d]	0.82[d]	1.96[d]	0.95[d]	1.39[d]

[a]See Table 1 for abbreviations.
[b]Within each 18:3 group, M1-1 = means of volatile compounds of soybean oils with normal 18:3 and M1-2 = means of volatile compounds of soybean oils with low 18:3 content.
[c]Within each LOX group, M2-1 = means of volatile compounds of soybean oils with normal LOX content, M 2-2 = means of volatile compounds of soybean oils from beans without LOX 2, and M2-3 = means of volatile compounds of soybean oils from beans without LOX 2 and 3.
[d,e]Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

TABLE 9
Sensory Evaluation Scores (cm)[a] of Pilot Plant–Extracted Soybean Oils Stored at 35°C Under Light (Study 3)[b]

	Soybean oil genotypes						18:3 group[c]		LOX group[d]		
Day	#1	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0	5.0	4.5	3.8	3.0	5.9	3.0	4.4[e]	3.6[e]	4.0[e]	5.0[e]	3.4[e]
14	8.1	8.6	8.3	7.9	10.7	7.8	8.3[e]	8.4[e]	8.0[e]	9.3[e]	8.0[e]

[a]A score of 1 = weakest and 15 = strongest off-flavor.
[b]See Table 1 for abbreviation.
[c]Within each 18:3 group, M1-1 = means of sensory scores of soybean oils with normal 18:3 and M1-2 = means of sensory scores of soybean oils with low 18:3 content.
[d]Within each LOX group, M2-1 = means of sensory scores of soybean oils with normal LOX, M2-2 = means of sensory scores of soybean oils from beans without LOX 2, and M2-3 = means of sensory scores of soybean oils from beans without LOX 2 and 3.
[e]Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

The amount of 18:3 is believed to be the main factor contributing to off-flavor development (35). In the current project, the importance of the amount of 18:3 was shown in Studies 1 and 2 to be the major factor affecting oxidative stability. This phenomenon was not observed during Study 3, where there were no significant differences between 18:3 groups or among LOX groups. Differences in oxidation conditions of light vs. dark and of 35°C vs. 50 to 60°C might have accounted for the different results. The influence of 18:3 content on the oxidative stabilities of soybean oils has not always been observed. Mounts *et al.* (36) conducted a study with four soybean genotypes, having 3.3, 4.2, 4.8, or 7.7% 18:3 content, plus a hydrogenated soybean oil with 3% 18:3 content. After accelerated storage at 60°C for 4 d, they found no significant differences among the oils in peroxide development during storage.

In this research, soybean oils with low 18:3 contents generally were significantly more stable than were those with normal 18:3 contents, regardless of the LOX content of the beans. PV on day 0 in each test indicated the qualities of soybean oils extracted from both laboratory and pilot-plant were adequate for these tests. Because storage conditions in Study 3 were different, the oxidation rate was fast, perhaps minimizing differences among the oils. Flavor and oxidative stability of soybean oils may vary, depending on the oxidation

conditions, such as light vs. dark (33,34). It is difficult to attribute differences in oxidative stabilities of the oils to differences in tocopherol contents because the oils that contained significantly greater amounts of tocopherols (those lacking LOX 2 and 3) generally had the poorest stabilities.

In general, it seemed that removal of LOX 2 or of LOX 2 and 3 from soybean seeds tended to result in lower oxidative and flavor stability in the oils; however, these differences may have been a result of overall differences in fatty acid compositions, as was also noted from the calculated oxidizability values. All soybean seeds contained at least one LOX. Under this condition, the activities of LOX in the different mutant lines are not known. Furthermore, the time between flaking and extracting could be critical in 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 might be minimized because oxidation would have reached the maximum rate. The conditions used in this research were designed to mimic the conditions used by the soybean oil industry. Thus, the conclusions obtained could be used to predict the influence of eliminating LOX 2 or LOX 2 and 3 on soybean oil quality of commercially produced oils.

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