Oxidative and Flavor Stability of Oil from Lipoxygenase-Free Soybeans

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ABSTRACT: Soybeans that lack or contain three lipoxygenase (LOX) isozymes, LOX-1, LOX-2, and LOX-3, were evaluated for oxidative and flavor stability at 60°C in the dark and at 35°C in the light. Although the two types of soybeans had a similar genetic background, there were significant differences ($P \leq 0.01$) in fatty acid percentages between the lipoxygenase-free and normal oils before and after storage at both temperatures. The linolenic acid content of oil from LOX-free germplasm before storage averaged 7.2%, while normal lines averaged 6.6%. The linoleic acid content after storage averaged 6.9% for LOX-free and 6.6% for normal oils. LOX-free oil was not significantly different from normal oil in flavor, as judged by a sensory panel, or in concentrations of volatiles during storage at either storage condition. LOX-free oil had less hexanal than normal oil before storage, but had significantly greater ($P \leq 0.05$) levels after storage for two weeks at 35°C. Peroxide values of oil from LOX-free soybeans were significantly greater ($P \leq 0.01$) than oil from the normal soybean after storage at 60 and 35°C. LOX-free oil had significantly greater ($P \leq 0.01$) levels of α-, β-, and γ-tocopherols. In general, oil from LOX-free soybeans did not have improved flavor or oxidative stability. Differences between the two oil types in peroxide value and in production of a few volatiles were probably a result of the differences in initial fatty acid composition.

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

The activity of lipoxygenase (LOX) enzymes plays a role in the development of grassy, beany off-flavors in soybean products through oxidation of polyunsaturated fatty acids. The grassy, beany off-flavor comes from hexanal and other six-carbon aldehydes (1). Three LOX isozymes, LOX-1, LOX-2, and LOX-3, are found in soybeans (2). LOX isozymes require a substrate that contains a *cis*, *cis*-1,4 pentadiene system, such as linoleic acid (18:2) and linolenic acid (18:3), in oxidation reactions. In soybean oil, the polyunsaturated fatty acids account for approximately 60% of the fatty acids (3).

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The effects of genetic removal of one or more of the LOX isozymes on the flavor and oxidative stability of soybean homogenates, soybean oil, and soy food were studied. LOX-2 seemed to be the main isozyme responsible for the formation of hexanal in aqueous soybean homogenates (4). In homogenates from soybeans lacking both LOX-1 and 3, hexanal levels increased from 0.23 nmol/mg protein before storage to 0.6 nmol/mg protein after storage at 25°C for 60 min at a pH of 6.5 to 7.0. Homogenates lacking only LOX-2 had the lowest hexanal levels: 0.10 nmol/mg protein or less (4). Another study showed that samples lacking both LOX-2 and 3 had lower levels of hexanal and lower thiobarbituric acid (TBA) values than homogenates and flours from soybeans lacking only LOX-2 (5). The presence of LOX-3 decreased the production of hexanal, presumably by converting 13-hydroperoxy-9,11-octadecadienoic acid into forms that could not be broken down into hexanal (6).

After storage at 4°C for 60 min near pH 7.0, the 1,3-diethyl-2-thiobarbituric acid values of soybean homogenates decreased in the order of those containing LOX-1,2,3 > LOX-2 > LOX-3 > LOX-1 > LOX-free (no lipoxygenase isozymes) (7). Similar results were found for flours, except that the LOX-2 and LOX-3 samples were in reverse order. Hexanal levels were lowest in the flour from soybeans lacking the three LOX isozymes after storage for 60 min at 4°C, but highest in the flour from soybeans with only LOX-2 present (7).

Soymilk made from soybeans lacking LOX-2 had lower beany, rancid, and oily flavors than soymilk from soybeans lacking LOX-1, LOX-3, both LOX-2 and 3, or both LOX-1 and 3 (8). The concentrations of several volatiles in the headspace of soymilk from soybeans lacking the three LOX enzymes were significantly lower ($P \leq 0.01$) than those found in soymilk from normal soybeans or those lacking both LOX-2 and 3 (9). The level of 1-octen-3-ol was not significantly different between the samples.

There were no significant differences in flavor scores between oils from normal soybeans and soybeans lacking LOX-1 after storage at 60°C for 8 d, but the peroxide value (PV) of the oil from untempered soybeans lacking LOX-1 was slightly higher (7 meq/kg) than that of normal soybean oil (4 meq/kg) (10). Oils from soybeans lacking LOX-2 or LOX-2 and 3 had less oxidative stability, as measured by PV, than

normal soybean oil during storage at 35°C in the light for 14 d. At 60°C storage in the dark, there were no differences between PV of oils from soybeans lacking LOX-2 or LOX-2 and 3 (11).

The effect of genetic removal of all three LOX isozymes on the flavor and oxidative stability of soybean oil has not been reported. The objective of this study was to compare the oxidative and flavor stability of oil from soybeans that lack the three LOX isozymes with oil from soybeans that are normal in LOX content.

MATERIALS AND METHODS

Materials. A LOX-free soybean genotype, produced by gamma irradiation, was obtained from K. Kitamura (12). The genotype was used as the donor parent to backcross the null alleles for absence of LOX into IA2020 stock, which has good agronomic traits in Iowa. Seed of LOX-free and LOX-normal lines, obtained from the first backcross to IA2020, was produced in the same field near Ames, Iowa in 1996.

LOX screening. The harvested seed of each line of soybeans was screened for LOX activity using the method of Suda *et al*. (13). Twenty-five random seeds of each line were tested individually. Each seed was broken into pieces, and separate pieces of the same seed were used to test for each of the three LOX isozymes. To provide an adequate amount of soybeans for this study, seven lines containing the three LOX isozymes were combined, and a separate set of seven LOX-free lines were combined. Bags containing the two separate types of mixed seed were double-wrapped in moisture-proof plastic bags and stored at 4°C until used.

Oil extraction. Samples (400 g) of the two types were evaluated for moisture, protein, oil, and fiber by near-infrared spectroscopy (0.4 kg each) (14). The two types were similar to each other with an average of 10.6% moisture, 38.9% protein, 17.6% oil, and 4.9% fiber. For oil extraction, the seeds were dehulled, cracked, heated, and flaked to about 0.25 mm in thickness before extraction by the pilot-plant procedure of Shen *et al*. (11). Three random bags (replications) each of LOX-free and normal seeds were extracted at 65.5°C, with a ratio of solvent to flakes of 1.75:1 per stage in a batch-advance solvent extractor (French Oil Mill Machinery Co., Piqua, OH). The flakes were extracted in five stages with 10 min of extraction during each stage and 4 min of draining between stages. The miscella was flash-desolventized at 104.4°C under atmospheric pressure, then stripped with sparge steam at 104.4°C, under vacuum at 64.8 cm for 3 min. The crude oils were purged with nitrogen and stored at −14°C until further processing.

Refining, bleaching, and deodorizing. Duplicates of each of the three LOX-free and normal soybeans were refined, bleached, and deodorized (RBD) to give a total of 12 samples. Two sets of 12 oil samples were prepared (RBD), one for each storage condition.

Free fatty acid percentages of the oils were determined by the American Oil Chemists' Society (AOCS) method Aa-6-38 (15). The oils were refined with 9.5% NaOH according to the official method of the AOCS, Ca-9b-52 (15). The oils were stirred for 1.5 h at 25°C, followed by heating at 60–65°C for 20 min with stirring. The oils were held at 60–65°C without stirring for 1 h and left overnight for a minimum of 12 h at room temperature without stirring.

The oils were vacuum-filtered with a buchner funnel through Whatman #1 filter paper to remove soapstock. Official natural bleaching earth (AOCS) was added at 4% of the weight of the filtered, refined oil, and the oil was bleached using the official AOCS method Cc-8a-52 (15). The temperature of the oil mixture was raised rapidly to 120°C, and the mixture was stirred at 120°C for 5 min. The mixture was vacuum-filtered immediately through Whatman #1 filter paper in a heated Büchner funnel.

The bleached oils were deodorized at 230°C for 2 h under vacuum (<0.5 Torr). The apparatus used was described by Shen *et al.* (11), and a steam distillation method of Stone and Hammond (16), modified by Moulton (17), was used. One hundred parts per million of citric acid was added to each oil during the cool-down phase of deodorization. The deodorized samples were placed in amber glass bottles, purged with nitrogen gas, and sealed with teflon-coated caps. The oils were stored at −14°C until used for the storage study.

Storage studies. Oils were tested at 60°C in the dark and at 35°C in the light. For each study, six 300-g samples each of RBD LOX-free and normal oils (12 total samples) were placed in 600-mL beakers, covered with plastic wrap, and stored in a constant-temperature oven. The samples were rotated daily. For the study at 35°C in the light, a 100-W circular fluorescent light bulb was placed at the bottom of the storage oven. The light intensity, measured with a light-level meter (Weston Instruments, Newark, NJ), was 560 lx. Samples were stored for 15 d at 60°C and for 14 d at 35°C.

Peroxide values (PV) were measured before and every 2 d during storage (18). Each oil sample was measured in duplicate, and the results were averaged. The initial rates of oxidation were determined from the slopes of the regression lines of the linear portion of the plots of PV as a function of time. Induction times were extrapolated from the intersecting PV point of the regression lines for the initial and final stages of oxidation (19). Volatile compounds were analyzed by gas chromatography (GC) with a flame ionization detector (FID) at zero time, after 8 and 15 d of storage at 60°C, and before and after storage for 14 d at 35°C (20). Fatty acid percentages were determined by GC with a FID on the oils before and at the end of storage at each temperature (21). The oxidizabilities of the oils were calculated using the formula: oxidizability = [% oleic acid + 10.3 (% linoleic acid) + 21.6 (% linolenic acid)]/100 (22). The tocopherol contents of replicate crude and refined oil samples were measured by the method of Dove and Ewan (23).

Sensory evaluations of oil samples were accomplished by using a modified AOCS method, Cg 2-83 (15). Thirteen panelists were trained in 6 sessions for familiarity in identifying flavors in fresh and oxidized oils. Fresh oils and oils that had

been oxidized for 8 and 15 d were used as examples. Before sensory evaluation, samples were placed in glass vials with caps and equilibrated to 25°C in the dark. For oil evaluations, panelists were asked to swirl the oil in the vial before opening the cap, taste the oil in order of increasing off-odor, and rinse their mouths several times with distilled, deionized water between samples. Panelists judged the off-flavor of the samples on a scale of 1 to 10 with a score of 10 being bland and a score of 1 being extreme off-flavor (15).

Statistical analysis. A split-plot repeated measures design with variety in the main plot and time as the subplot was used for this experiment. The general linear models procedure of the Statistical Analysis System (SAS) was used for the analysis of variance (24).

RESULTS AND DISCUSSION

Fatty acid analysis. The fatty acid contents of the oils in this study were similar to that commonly reported for soybean oil (Table 1) (3). The unsaturated fatty acids made up approximately 86% of the total fatty acids in the LOX-free and normal oils. There were significantly higher ($P \leq 0.01$) mean percentages of palmitic, linoleic, and linolenic acid and significantly lower ($P \leq 0.01$) mean percentages of stearic and oleic acid in the LOX-free oil than in the normal oil stored at 60°C (Table 1). The calculated average oxidizability for the normal oil was 7.04 and for the LOX-free oil was 7.24, which indicates that the LOX-free oil may oxidize slightly faster. There was a significant change ($P \leq 0.05$) with time in all fatty acid percentages for oil stored at 60°C (Table 1). Levels of linoleic acid and linolenic acid decreased, and levels of the other fatty acids increased. The variety × time interaction was not significant.

There was a significant change ($P \leq 0.01$) with time in percentage of stearic acid for oil stored at 35°C (Table 1). The variety × time interaction was significant for stearic acid ($P \leq 0.05$) and linoleic acid ($P \leq 0.05$) levels during storage at 35°C. Over time, the percentage of linoleic acid remained the same for the normal oil and decreased by 0.5% for LOX-free oil. The percentage of stearic acid in the normal oil decreased by 0.16% and in LOX-free oil by 0.02% over time (Table 1).

Sensory evaluation. There were no significant differences in flavor between the LOX-free and normal oils during storage at 60 or 35°C for 2 wk (Table 2). The flavor of the normal and LOX-free oils stored at 35°C in the light deteriorated more quickly than the flavor of the oils stored at 60°C in the dark. The average flavor scores for the LOX-free oil stored at 35°C in the light changed from 9.0 before storage to 3.9 after 7 d and to 3.5 after 14 d. The average flavor scores for LOX-free oil stored at 60°C in the dark were 4.6 after 8 d and 4.2 after 15 d of storage. Singlet oxygen, which requires light for its formation, can react 1400 times faster than triplet oxygen in lipid oxidation (25). This may explain why the flavor of oil stored in the light at 35°C deteriorated more quickly than the flavor of oil stored in the dark at 60°C.

Shen *et al*. (11) also found no flavor differences among oils from normal soybeans, those lacking LOX-2 alone, and LOX-2 and 3, before and after storage at 35°C for 14 d. No differences in flavor were observed between oils from normal soybeans and those lacking LOX-1 (10). Significantly lower ($P < 0.01$) beany, rancid, and oily flavor was found in soymilk from soybeans lacking LOX-2 than in soymilk from normal soybeans and from those lacking LOX-1, LOX-3, LOX-1 and 3, or LOX-2 and 3 (8). There seemed to be a difference between oils and soymilk in the ability of panelists to distinguish an effect of LOX content on the flavor. This effect could be due to the protein of the soymilk that can bind flavor volatiles. The various proteins and LOX isozymes may have different binding coefficients for the various flavor volatiles (26,27).

Volatiles analysis. The volatiles identified, shown in Table 3, were quantified by the percentage of the total GC

TABLE 1
Fatty Acid Composition of Oils Stored at 60°C in the Dark and 35°C in the Light

			Fatty acid percentage[a,b]				
Temperature (°C)	Time (d)	Type	Palmitic[c,d] (16:0)	Stearice (18:0)	Oleic[c] (18:1)	Linoleic[c,e] (18:2)	Linolenic[c] (18:3)
60	0	Normal	9.8	4.5	26.8	52.2	6.8
		LOX-free	10.0	4.3	25.5	53.0	7.3
60	15	Normal	10.0	4.6	27.1	51.8	6.6
		LOX-free	10.1	4.4	25.9	52.6	7.0
35	0	Normal	10.0	4.8	27.3	51.4	6.4
		LOX-free	9.9	4.6	25.9	52.5	7.0
35	14	Normal	10.1	4.7	27.3	51.4	6.5
		LOX-free	10.3	4.6	26.2	52.0	6.8

[a]Fatty acid percentage changed significantly with time unless otherwise noted ($P \leq 0.05$).
[b]Significant differences between varieties unless otherwise noted ($P \leq 0.05$).
[c]No significant change with time after 35°C storage for 14 d.
[d]No significant difference between varieties after 35°C storage for 14 d.
[e]Significant difference between variety for variety × time interaction ($P \leq 0.05$) after 35°C storage for 14 d. LOX, lipoxygenase.

TABLE 2
Sensory Evaluation Scores of Oils Stored at 60°C in the Dark or 35°C in the Light

Temperature (°C)	Time (d)	Score[a]	
		Normal	LOX-free
60	0	8.5	9.0
	8	5.6	4.6
	15	4.1	4.2
35	0	8.9	9.0
	7	4.1	3.9
	14	3.2	3.5

[a]Significant change with time ($P \leq 0.01$). A score of 1 = extreme off-flavor, 10 = bland. No significant differences between varieties. See Table 1 for abbreviation.

peak area. The peak area for each volatile in GC units can be calculated from the data in Table 3. There were no significant differences between the LOX-free and normal oils in amounts of volatiles (peak area) during storage at 60°C in the dark, but the hexanal level in the LOX-free oil was significantly higher ($P \leq 0.02$) than that in the oil from normal soybeans after 14 d of storage at 35°C (Table 3). The level of hexanal before storage generally was lower in LOX-free oil than in normal oil. After storage at either set of conditions, hexanal levels tended to be higher in the LOX-free oil, a fact which may be related to the greater content of linoleic acid (a precursor of hexanal) in LOX-free oil than in the normal oil (28). There were significant changes with time during storage at 60°C for 15 d in the dark in the amounts of *t*-2-pentenal ($P \leq 0.01$), hexanal ($P \leq 0.01$), *t*-2-hexenal ($P \leq 0.01$), heptanal ($P \leq 0.01$), *t*-2-heptenal ($P \leq 0.01$), 1-octen-3-ol ($P \leq 0.05$), and *t,t*-2,4-heptadienal ($P \leq 0.01$), but not in the levels of *t*-2-octenal, nonanal, and *t,t*-2,4-decadienal.

The percentage of 1-octen-3-ol, based on the total peak area of known volatiles, decreased greatly during storage at 60°C in the dark and at 35°C in the light, and decreased more rapidly for LOX-free oil than for normal oil (Table 3). Storage at 35°C in the light for 14 d resulted in significant increases in percentages of hexanal ($P \leq 0.01$) and *t*-2-heptenal ($P \leq 0.05$) with time, with a greater increase in percentage of the total peak area of known volatiles for the LOX-free oils than the normal oils (Table 3).

Shen *et al.* (11) found significantly lower ($P \leq 0.05$) hexenal levels in oils from soybeans lacking LOX-2 and 3 than in soybeans lacking LOX-2 before storage at 35°C, but no differences after storage for 14 d. There was no difference in hexanal level between the normal and LOX-null types. After storage at 50°C in the dark for 14 d, the oil from soybeans lacking LOX-2 and 3 had significantly greater amounts of 2-heptenal, *t,c*-2,4-heptadienal, and 2-octenal than oils from normal soybeans or those lacking LOX-2 (11). In other work, hexanal levels were 40–82% lower in the filtrate from homogenates of soybeans lacking LOX-3 or LOX-2 and 3 (5). In the current study, there were fewer differences in amounts of volatiles produced from the two oil types. During storage at 60°C in the dark, no differences in volatile concentrations were found between normal and LOX-free oils. These varied results between studies may be due to the different concentrations of each unsaturated fatty acid in the oils from the soybeans used. Each unsaturated fatty acid is believed to be associated with the production of certain volatiles (28).

Hexanal levels in soymilk from soybeans lacking the three LOX isozymes were significantly lower than hexanal levels in soymilk from normal soybeans (9). Other researchers showed that initial hexanal levels of full-fat soybean flour homogenates from soybeans lacking the three LOX isozymes were the same as hexanal levels of full-fat normal soybean flour homogenates (7). After storage at 4°C for 60 min, the hexanal accumulation for full-fat soybean flour homogenates lacking the three LOX isozymes was 100 nmol/g seed,

TABLE 3
Volatiles in Oils Stored at 60°C in the Dark and 35°C in the Light

	Total[a] GC peak area for 60°C (%)						Total[a] GC peak area for 35°C (%)			
	Normal			LOX-free			Normal		LOX-free	
Volatile	0	8	15	0	8	15	0	14	0	14
t-2-Pentenal[b]	3.5	11.4	19.8	0.0	19.6	15.0	0.7	15.8	1.0	15.6
Hexanal[b,c,d]	1.7	18.9	29.3	1.1	26.5	32.3	20.4	54.7	3.0	61.3
t-2-Hexenal[b]	0.5	1.2	2.2	0.2	1.4	2.7	2.1	0.9	0.9	1.2
Heptanal[b]	0.9	1.5	0.6	0.4	1.0	0.6	6.9	1.4	6.0	1.5
t-2-Heptenal[b,c]	1.1	7.4	27.0	0.5	14.2	27.2	2.9	8.8	2.2	10.5
1-Octen-3-ol[b]	90.6	45.3	10.7	95.8	21.1	13.4	48.0	13.6	79.8	3.3
t,t-2,4-Heptadienal[b]	0.0	11.2	8.5	0.0	9.4	6.7	4.0	2.2	0.6	4.2
t-2-Octenal	0.8	1.3	1.4	0.7	3.6	1.7	4.1	1.4	2.8	1.4
Nonanal	0.6	1.4	0.3	0.5	2.6	0.3	8.2	1.0	2.0	0.9
t,t-2,4-Decadienal	0.3	0.4	0.2	0.8	0.5	0.1	2.6	0.0	1.6	0
Total area	8635144	4734716	29583557	10008599	3805289	40329978	2295107	7775385	4835220	10113134

[a]Total peak area of known volatiles only; time unit in days.
[b]Significant change in gas chromatography (GC) units of area with time ($P \leq 0.05$) for 60°C storage.
[c]Significant change in GC units of area with time ($P \leq 0.05$) for 35°C storage.
[d]Peak areas were significantly different ($P \leq 0.05$) between normal and LOX-free oil for 35°C storage. See Table 1 for other abbreviation.

TABLE 4
Peroxide Values (meq/kg) of Oils Stored at 60°C in the Dark and 35°C in the Light[a]

Temperature (°C)	Type	Peroxide value: Time (d) 0	2	4	6	8	10	12	14	15
60	Normal	0.13[b]	0.70[b]	0.88[b]	2.50[b]	12.6[b]	26.6[b]	40.7[b]	65.0[b]	63.1[b]
	LOX-free	0.13[b]	0.79[b]	0.97[b]	7.85[b]	21.4[c]	36.4[c]	50.7[c]	72.0[c]	63.8[b]
35	Normal	0.21[d]	1.21[d]	2.07[d]	3.23[d]	4.54[d]	7.27[d]	12.5[d]	21.2[d]	35.7[d]
	LOX-free	0.21[d]	1.27[d]	2.64[d]	7.56[e]	11.9[e]	16.5[e]	20.5[e]	29.2[e]	46.2[e]

[a]Significant change over time ($P <\leq 0.01$). Means within the same column with different superscript letters (b,c) are significantly different ($P \leq 0.05$) for oils stored at 60°C. Means within the same column with different superscript letters (d,e) are significantly different ($P \leq 0.01$) for oils stored at 35°C. See Table 1 for abbreviation.

whereas the hexanal levels of normal full-fat soybean flour homogenates were 500 nmol/g seed (7). Kobayashi *et al*. (9) found similar results for 1-octen-3-ol in soybean milk. As concluded from sensory analysis, these differing results among soymilk, homogenates, and oils may be due to the effect of flavor volatile binding by proteins (26,27).

PV analysis. The PV of oils from LOX-free soybeans were significantly greater ($P \leq 0.01$) than oils from normal soybeans after storage for 8 d at 60°C in the dark and for 6 d at 35°C in the light (Table 4). The PV of both oils at both temperatures changed significantly ($P <\leq 0.01$) with time. The initial rates of oxidation during storage at 60°C were 0.21 meq/kg/d for the LOX-free oil and 0.19 meq/kg/d for the normal oil. The initial rates of oxidation during storage at 35°C were 0.61 meq/kg/d for LOX-free oil and 0.50 meq/kg/d for normal oil. The induction time for the LOX-free oil was 5.6 d, and for normal oil it was 6.4 d when stored at 60°C in the dark, whereas the induction time at 35°C in the light was 5.7 d for LOX-free oil and 7.2 d for normal oil. There were significant differences between the two types from day 8 to day 12 for the oils stored at 60°C ($P \leq 0.02$) and from day 6 to day 14 for the oils stored at 35°C ($P \leq 0.01$). Shen *et al*. (11) found that oils from soybeans lacking LOX-2 or LOX 2 and 3 oxidized more quickly than oils from normal soybeans during storage at 35°C in the light, but not during storage at 60°C in the dark. A significantly greater ($P \leq 0.05$) PV was found for samples that lacked LOX-2 and 3 after 8 d of storage, and after 10 d for samples lacking LOX-2 under storage at 35°C (11). In the current study, a significant difference ($P \leq 0.01$) between the two types was found after only 6 d of storage at 35°C. Other studies utilized different normal soybean varieties as controls, so different levels of minor constituents, such as chlorophyll, phospholipids, and tocopherols, may have affected the oxidative stability of the oils. Shen *et al*. (11) observed a slightly greater calculated oxidizability for oils from soybeans lacking LOX-2 (7.6) or LOX-2 and 3 (7.5) than for normal soybean oil (7.3). These oxidizability values were greater than those found in this study and were due to greater concentrations of linolenic acid found in the normal oil (7.8%) and LOX variants (8.4%) of Shen *et al*. (11) than in the normal (6.8%) and LOX-free oils (7.3%) in the current study.

Tocopherol analysis. The amount of each tocopherol isomer was greater in the crude oils from the LOX-free soybeans than in normal soybeans (Table 5). During RBD processing of the oils, approximately 24–44% of each tocopherol isomer was removed. There were significant differences ($P \leq 0.01$) between oils in the levels of α-, β-, and γ-tocopherols, but not in δ-tocopherol in the RBD oils. The average levels of α-tocopherol in the RBD oils from the normal soybeans was 28.9 μg/g and from the LOX-free soybeans was 42.7 μg/g. The levels of β- and γ-tocopherols were also greater in the RBD oil from LOX-free soybeans than from the normal soybeans.

Shen *et al*. (11) found significantly higher ($P \leq 0.05$) levels of α- and γ-tocopherols in RBD oils from soybeans lacking LOX-2 and 3 than from normal oil or from soybeans lacking LOX-2, but found no significant differences in β- and δ-tocopherol levels. Shmulovich (29) observed a tendency for increased tocopherol content with increased unsaturated fatty acid content in soybean oil, a factor which may also have existed in the current study.

In this research, the genetic removal of the three LOX enzymes from soybeans resulted in oil that was less oxidatively stable, as measured by PV, than oil from normal soybeans. Differences in flavor stability could not be distinguished by volatiles or sensory analysis. The differences in peroxide formation may have been due to the slightly greater percentages of linoleic and linolenic acids in the LOX-free oil, which resulted in greater calculated oxidizabilities of the LOX-free oil

TABLE 5
Tocopherol Contents (μg/g) of Oils from Normal and LOX-Free Soybeans[a]

Variety	Tocopherol isomer: α	β	γ	δ	Total
Normal (RBD)	28.94[a]	3.38[a]	195.92[a]	61.17	289.41[a]
LOX-free (RBD)	42.74[b]	4.41[b]	220.44[b]	62.52	320.11[b]
Normal (crude)	55.70[c]	4.66	270.82[c]	100.10	431.28
LOX-free (crude)	76.88[d]	5.96	303.12[d]	111.29	497.25

[a]Means within the same column with different superscript letters (a,b) are significantly different ($P \leq 0.01$). Means within the same column with different superscript letters (c,d) are significantly different ($P \leq 0.01$). RBD, refined bleached deodorized. See Table 1 for other abbreviation.

than of the normal oil. The greater level of tocopherols found in the LOX-free oil may not have been adequate to retard or prevent oxidation. Also present in the oils may be other minor constituents, such as chlorophyll, which is a sensitizer of singlet oxygen, or phospholipids, which also can contribute to oxidative deterioration of the oils. Further research is necessary to determine whether minor components may have contributed to differences in flavor and oxidative stability of the oils from LOX-free and normal soybeans. Also, despite the optimal conditions of the current study, no improvement in oxidative stability of LOX-free oil over that of normal oil occurred. Different results may be obtained if the soybeans are stressed during shipment or processing. In damaged soybeans, the lipoxygenase enzymes would have time to act on the *cis,cis*-1,4 pentadiene substrate of the polyunsaturated fatty acids, thus having a greater impact on off-flavor development. Future studies should address the implications of nonoptimal storage and/or processing conditions on the quality of normal and LOX-free soybean oils.

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REFERENCES

1. Hatanaka, A., The Fresh Green Odor Emitted by Plants, *Food Rev. Int. 12*:303–350 (1996).
2. Axelrod, B., T.M. Cheesborough, and S. Laasko, Lipoxygenase from Soybean, *Methods Enzym. 71*:441–451 (1981).
3. Pryde, E.H., Composition of Soybean Oil, in *Handbook of Soy Oil Processing and Utilization*, edited by D.R. Erickson, E.H. Pryde, O.L. Brekke, T.L. Mounts, and R.A. Falb, American Soybean Association, St. Louis, Missouri and American Oil Chemists' Society, Champaign, 1980, pp. 13–31.
4. Matoba, T., H. Hidaka, H. Narita, K. Kitamura, N. Kaizuma, and M. Kito, Lipoxygenase-2 Isozyme Is Responsible for Generation of n-Hexanal in Soybean Homogenate, *J. Agric. Food Chem. 33*:852–855 (1985).
5. Moreira, M.A., S.R. Tavares, V. Ramos, and E.G. de Barros, Hexanal Production and TBA Number Are Reduced in Soybean [*Glycine max* (L.) Merr.] Seeds Lacking Lipoxygenase Isozymes 2 and 3, *Ibid. 41*:103–106 (1993).
6. Hildebrand, D.F., T.R. Hamilton-Kemp, J.H. Loughrin, K. Ali, and R.A. Andersen, Lipoxygenase-3 Reduces Hexanal Production from Soybean Seed Homogenates, *Ibid. 38*:1934–1936 (1990).
7. Nishiba, Y., S. Furuta, M. Hajika, K. Igita, and I. Suda, Hexanal Accumulation and DEBTA Value in Homogenate of Soybean Seeds Lacking Two or Three Lipoxygenase Isozymes, *Ibid. 43*:738–741 (1995).
8. Davies, C.S., S.S. Nielsen, and N.C. Nielsen, Flavor Improvement of Soybean Preparations by Genetic Removal of Lipoxygenase-2, *J. Am. Oil Chem. Soc. 64*:1428–1433 (1987).
9. Kobayashi, A., Y. Tsuda, N. Hirata, K. Kubota, and K. Kitamura, Aroma Constituents of Soybean [*Glycine max* (L.) Merril] Milk Lacking Lipoxygenase Isozymes, *J. Agric. Food Chem. 43*:2449–2452 (1995).
10. Frankel, E.N., K. Warner, and B.P. Klein, Flavor and Oxidative Stability of Oil Processed from Null Lipoxygenase-1 Soybeans, *J. Am. Oil Chem. Soc. 65*:147–150 (1988).
11. Shen, N., W. Fehr, L. Johnson, and P. White, Oxidative Stabilities of Soybean Oils That Lack Lipoxygenases, *Ibid. 73*:1327–1336 (1996).
12. Kitamura, K., Genetic Improvement of Nutritional and Food Processing Quality in Soybean, *J. Agron. Res. Qual. 29*:1–8 (1995).
13. Suda, I., M. Hajika, Y. Nishiba, S. Furuta, and K. Igita, Simple and Rapid Method for the Detection of Individual Lipoxygenase Isozymes in Soybean Seeds, *J. Agric. Food Chem. 43*:742–747 (1995).
14. Rippke, G.R., C.L. Hardy, C.R. Hurburgh, Jr., and T.J. Brumm, Calibration and Field Standardization of Tecator Infratec Analyzers for Corn and Soybeans, Paper presented at the International Conference of Infrared Spectroscopy, Montreal, Canada (1995).
15. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by D. Firestone, 4th edn., American Oil Chemists' Society, Champaign, 1989.
16. Stone, R.R., and E.G. Hammond, An Emulsion Method for the Sensory Evaluation of Edible Oils, *J. Am. Oil Chem. Soc. 60*:1277–1281 (1983).
17. Moulton, K.J. Sr., Laboratory Deodorization of Vegetable Oil, *Ibid. 66*:302–308 (1989).
18. Hamm, D.L., E.G. Hammond, V. Parvanah, and H.E. Snyder, The Determination of Peroxides by the Stamm Method, *Ibid. 42*:920–922 (1965).
19. Warner, K., and E.N. Frankel, Flavor Stability of Soybean Oil Based on Induction Periods for the Formation of Volatile Compounds by Gas Chromatography, *Ibid. 62*:100–103 (1985).
20. Lee, I., S.H. Fatemi, E.G. Hammond, and P.J. White, Quantitation of Flavor Volatiles in Oxidized Soybean Oil by Dynamic Headspace Analysis, *Ibid. 72*:539–546 (1995).
21. Hammond, E.G., Organization of Rapid Analysis of Lipids in Many Individual Plants, in Modern Methods of Plant Analysis, New Series, Vol. 12, *Essential Oils and Waxes,* edited by H.F. Linskens and J.F. Jackson, Springer-Verlag, New York, 1991, pp. 321–330.
22. Fatemi, S.H. and E.G. Hammond, Analysis of Oleate, Linoleate and Linolenate Hydroperoxides in Oxidized Ester Mixtures, *Lipids 15*:379–385 (1980).
23. Dove, C.R., and R.C. Ewan, Effect of Trace Minerals on the Stability of Vitamin E in Swine Grower Diets, *J. Anim. Sci. 69*:1994–2000 (1991).
24. *SAS User's Guide: Basic*, 5th edn., SAS Institute, Inc., Cary, 1985.
25. Rawls, H.R., and P.J. van Santen, A Possible Role for Singlet Oxygen in the Initiation of Fatty Acid Autoxidation, *J. Am. Oil Chem. Soc. 47*:121–125 (1970).
26. O'Keefe, S.F., L.A. Wilson, A.P. Resurreccion, and P.A. Murphy, Determination of the Binding of Hexanal to Soy Glycinin and Beta-Conglycinin in an Aqueous Model System Using a Headspace Technique, *J. Agric. and Food Chem. 39*:1022–1028 (1991).
27. O'Keefe, S.F., A.P. Resurreccion, L.A. Wilson, and P.A. Murphy, Temperature Effect on Binding Volatile Flavor Compounds to Soy Protein in Aqueous Model Systems, *J. Food Sci. 56*:802–806 (1991).
28. Frankel, E.N., Chemistry of Autoxidation: Mechanism, Products and Flavor Significance, in *Flavor Chemistry of Fats and Oils*, edited by D.B. Min and T.H. Smouse, American Oil Chemists' Society, Champaign, IL, 1985, pp. 1–38.
29. Shmulovich, V.G., Interrelation of Contents of Unsaturated Fatty Acids and Vitamin E in Food Products Lipids, *Appl. Biochem. Micro. 30*:547–551 (1994).

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