# Flavor and Oxidative Stability of Oil Processed From Null Lipoxygenase-1 Soybeans

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**The role played by lipoxygenase in the flavor quality of soybean oil was investigated by comparing the oil processed from special soybeans lacking lipoxygenase-1 (Forrest x P.I. 408251) with the oil from normal (Century) beans. Quality assessment was based on sensory evaluations and on capillary gas chromatographic (GC) analyses of volatiles of the extracted crude, partially processed, and refined, bleached and deodorized oils. In direct comparisons of oil products from the two types of beans, no significant differences were found in either flavor quality or in flavor stability based on total volatiles, and in analyses for 2,4-decadienal. Although thermal tempering did not significantly affect the initial flavor scores of crude and degummed oils from Century and low L-1 soybeans, the initial scores of refined and bleached oils from Century soybeans were significantly improved by this treatment. Similarly, thermal tempering was just as important in producing good quality flour from the special beans lacking lipoxygenase-1 as the flour from normal beans. Therefore, factors other than lipoxygenase-1 appear to affect the food quality of soybean oils and meals.**

Lipoxygenase has been regarded as an important factor in the formation of undesirable flavor compounds in soybean products (1-4). However, the role that this enzyme system plays in the flavor quality of the extracted and processed soybean oil is not yet clear. Thermal inactivation of lipoxygenase improves the flavor quality of soybean protein products, but decreases their protein solubility (4-6). Heat treatment of soybeans prior to extraction produces oils of high quality, but the meal contains higher levels of residual oil and the crude oil retains a higher level of phospholipids (7).

Recent research has focused attention on genetically altering the enzyme content to improve the quality of soybeans. Hildebrand and Hymowitz (8) discovered two soybean genotypes lacking lipoxygenase-1 (L-1). Kitamura et al. (9) reported two cultivars lacking lipoxygenase-3 (L-3). Researchers have investigated soybeans that naturally lack one or more of the lipoxygenase isozymes, but no studies have been reported on the quality or stability of the final oil products.

Our objective in this paper was to process soybeans lacking L-1 and to evaluate the flavor quality of the oil and meal by sensory techniques and by gas chromatographic (GC) analyses of the extracted, partially and completely processed oils.

## EXPERIMENTAL

*Materials.* Control soybeans (Century, 1984) were obtained locally for direct comparisons with the null L-1 soybeans (Forrest x P.I. 408251) provided by Dr. T. Hymowitz, University of Illinois.

*Processing.* All soybeans were cracked and dehulled. One-half of each type was conditioned in 250 g batches with steam at 100 C for 2 min. All soybeans were flaked and extracted with hexane for 5 hr in a large side-arm Soxhlet (10). Residual hexane was removed from the micella with a rotating evaporator under vacuum (ca. 1 mm) at 50 C. For sensory evaluations the flakes were ground into flour. Similar batches of crude oils were combined and degummed (2% water), refined (10% NaOH), bleached (1% Superfiltrol), and deodorized (3 hr, 220 C with 100 ppm citric acid added on the cooling side of deodorization) as described previously (11). All samples were stored under nitrogen atmosphere between processing steps.

*Enzyme assay.* Enzyme activity was determined spectrophotometrically on full-fat meal extracts by methods (12) developed on the basis of procedures reported by Ben-Aziz et al. (13), using substrates, pH and wavelengths described by Axelrod et al. (14). L-1 activity was determined by measuring conjugated diene absorption at 234 nm with linoleic acid as substrate at pH 9.0. Lipoxygenase-2 (L-2) activity was determined by measuring absorption at 238 nm with arachidonic acid as subtrate at pH 6.1. L-3 activity was determined at 234 nm and 280 nm (keto dienes) with linoleic acid as substrate at pH 6.5. Some overlap of the three isoenzyme activities would be expected in the crude soybean systems used in this study, especially L-2 and L-3 activities.

*Sensory evaluation.* All finished and partially processed oils were evaluated for flavor by a 15-member panel experienced in oil tasting. Crude and partially processed oils were tasted diluted in a ratio of 5:95 with good quality freshly deodorized soybean oil (15). The oil taste panelists were screened for their ability to discriminate between crude oils from thermally tempered and untempered soybeans, and to distinguish between types of grassy flavors (eg. grassy-hay or grassy-green). Soybean meal products also were tasted as 2% dispersions in carbon-filtered water (16) by a 14-member panel trained and experienced in soybean-cereal evaluations. All sensory analyses were based on a 10-point intensity scoring scale with 10 being bland and 1 strong. Panelists also described predominant flavors present in the samples.

*Gas chromatographic analyses.* Volatiles in oils were determined with a gas chromatograph (Perkin-Elmer, model 3920, Oak Brook, Illinois) using a Durabond DB-5 fused silica capillary column (30 m × 0.32 mm, film thickness 1 micron, J&W Scientific Co., Rancho Cordova, California), by a direct injection technique (17).

*Other analyses.* Degummed oils were analyzed for free fatty acids (Ca 5a-40); crude and degummed oils

were analyzed for phosphorus (Ca 12-55); all oils were analyzed for peroxide value (Cd 8-53) at each processing step, by standard AOCS methods (18).

## RESULTS AND DISCUSSION

Special soybeans bred at the University of Illinois to remove L-1 were compared with normal soybeans to assess the role played by lipoxygenase on the flavor quality and stability of the oils. The effect of thermal tempering was also evaluated to establish if this treatment can be omitted in the processing of the soybeans free of L-1. The extracted oils and meals were processed in the laboratory to evaluate for flavor and oxidative stability by sensory techniques, peroxide values and GC analyses for volatiles.

Preliminary experiments indicated that tempering of the soybean cracked meats with steam for 2 min gave flour and crude oil of higher scores than tempering for 4 min, which produced "over-heated" or "overcooked" responses by the taste panel. The effectiveness of thermal tempering for 2 min was also evaluated by measuring lipoxygenase activity in the tempered and untempered full-fat soybean flakes (Table 1). Analyses of all heat-treated soybeans showed no detectable lipoxygenase activity. The L-1 activity in the unheated Century soybeans was 3.9, compared to 0.19 in the 1984 soybeans with low L-1 activity. A sample of 1983 null L-1 soybeans showed no L-1 activity, but it was too small for further processing. Both samples of null L-1 soybeans showed different levels of L-2 and L-3 activities. L-2 activity was 4.28 for null L-1 soybeans and 2.88 for Century soybeans. However, L-2 and L-3 activities would be expected to overlap in the crude soybean preparations used in this study.

Thermally tempered and untempered flakes from 1984 Century soybeans were compared with the corresponding flakes from low L-1 soybeans. Steam treatment of the flakes for two min improved the quality of the resulting flours from both types of soybeans. Table 2 shows that the flavor scores of defatted flours from heat-tempered flakes of both Century soybeans and low L-1 soybeans were significantly higher than the flavor scores of corresponding untempered flakes. Thermal tempering was just as important to produce good quality flour from the soybeans lacking L-1 as the flour from Century soybeans. This result indicates that heat treatment is necessary even in the absence of lipoxygenase-1. The phosphorus content of the crude oils was much higher after tempering in the null L-1 soybeans (1074 vs 377 ppm) than in the Century soybeans (784 vs 742 ppm). After degumming, the phosphorus level was relatively higher (64 ppm) in the oil from untempered Century flakes than in the other oils (8-9 ppm). Therefore, tempering has a significant effect in the flakes from normal soybeans, by producing an oil which is more readily hydratable. This effect of tempering was not observed in the oil from special L-1 soybeans. Free fatty acid values were slightly lower in the oils obtained from heat-tempered soybeans. The oil yields were also lower from heat-tempered flakes than untempered flakes in both samples of soybeans. These results on the effects of heat treatment on the phosphorus content and yields of normal soybeans are in agreement with previously published results (7). Lovibond color and fatty acid composition were similar in all four samples.

Oils from thermally tempered and untempered soybeans were evaluated for flavor and peroxide development at each processing step and after deodorization. Crude and partially processed oils were

**TABLE 1.**

**Isoenzyme Activities of Full-Fat Century and Null Lipoxygenase-1 Soybeans**

| Soybeans | Steam tempering (min)[b] | Isoenzymes[a] L-1 (234 nm) | L-2 (238 nm) | L-3 (234 nm) | L-3 (280 nm) |
|---|---|---|---|---|---|
| 1984 Century | none | 3.90 | 4.28 | 2.92 | 0.432 |
| 1984 Century | 1 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1984 Century | 2 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1984 Century | 4 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1983 L-1 Null[c] | none | 0.0 | 2.47 | 1.96 | 0.29 |
| 1984 L-1 Null[c] | none | 0.19 | 2.88 | 2.11 | 0.29 |
| 1984 L-1 Null[c] | 4 | 0.0 | 0.0 | 0.0 | 0.0 |

[a]Activity units: absorbance/min/mg soybeans; see Experimental section for assay conditions.
[b]On dehulled, cracked soybeans at 100 C.
[c]From University of Illinois.

**TABLE 2.**

**Analyses of Oil and Defatted Flour from Century and Null Lipoxygenase-1 Soybeans**

| Analyses | 1984 Century | | 1984 Null L-1 | |
|---|---|---|---|---|
| | Heat-temp[a] | Un-tempered | Heat-temp[a] | Un-tempered |
| Defatted flour | | | | |
| Flavor score[b] | 6.3 | 5.2 | 6.5 | 4.7 |
| Oil | | | | |
| Yield, %[c] | 17.9 | 22.3 | 18.1 | 20.2 |
| Phosphorus, ppm | | | | |
| Crude oil | 784 | 742 | 1074 | 377 |
| Degummed oil | 8 | 64 | 9 | 8 |
| Free fatty acids, % | | | | |
| Degummed oil | 0.16 | 0.21 | 0.20 | 0.31 |
| Fatty acid compn., % | | | | |
| Crude oil | | | | |
| 16:0 | — | 10.8 | — | 11.5 |
| 18:0 | — | 3.9 | — | 3.3 |
| 18:1 | — | 22.6 | — | 23.1 |
| 18:2 | — | 54.8 | — | 55.3 |
| 18:3 | — | 7.9 | — | 6.8 |
| Lovibond color[d] | | | | |
| Crude oil | 70Y 15R | 70Y 10R | 70Y 10R | 70Y 10R |
| Deodorized oil | 2Y | 2Y | 3Y | 2Y |

[a]On dehulled, cracked soybeans with steam for 2 min.
[b]Tested as 2% dispersion in water. Scale: 1-10, 10 is bland, 1 is strong.
[c]Soxhlet extraction with hexane for 5 hr.
[d]With Lovibond colorimeter, Y, yellow; R, red.

tasted as 95:5 dilutions with good quality refined, bleached and deodorized (RBD) soybean oil (15). The undiluted RBD oils were tasted and analyzed for peroxide values. Thermal tempering did not significantly affect the initial flavor scores of crude and degummed oils from Century and low L-1 soybeans (Table 3). However, thermal tempering significantly improved the initial scores of refined and bleached oils from Century soybeans. After deodorization, no significant differences were detected in the oils from either soybean variety, whether or not the flakes were tempered. Typical descriptions given to crude and partially processed oils were grassy-green when the soybeans were untempered, and grassy-hay and cooked or burnt when the soybeans were thermally tempered. Cooked and burnt flavor descriptions were noted particularly when the soybeans were steam-tempered for 4 min instead of 2 min.

To evaluate flavor stability, oil samples were stored at 60 C for varying times according to the stage of processing. Crude oils were the most stable and, after 24 days of storage, the flavor scores decreased in the oils from both Century and null L-1 untempered soybeans (Table 3). No significant differences were noted in the oils from corresponding steam-tempered soybeans. Crude oils from low L-1 soybeans showed a much greater increase in peroxide valve (PVs 19 and 40) than the oils from Century soybeans (PVs 11 and 8). Partially processed oils were less stable than the crude oil, based on peroxide values. After eight days of storage, all degummed oils displayed no significant change in flavor scores, but showed greater increases in peroxide values in samples from untempered (PVs 17 and 21) than in samples from heat-tempered soybeans (PVs 11 and 18). After four days of storage, the refined oils from heat-tempered soybeans were less stable than the corresponding oils from untempered 1984 Century soybeans, as shown by a decrease in flavor score (1.0) and increase in peroxide value (PV 15). After two days of storage, no effect was observed on the flavor scores of bleached oils, but the peroxide value was greater in the oil from heat-tempered (PV 11) than untempered (PV 4) Century soybeans. No difference was observed in the corresponding oils from low L-1 soybeans. The RBD oils showed much greater effects on the flavor scores than peroxide values after storage at 60 C. Essentially no differences in flavor stability were observed in the RBD oils from both soybeans whether or not they were heat-tempered. After eight days of storage at 60 C, decreases in flavor scores were 1.9-2.3 in the oils from Century soybeans and 2.2-2.5 in the oils from low L-1 soybeans. A greater peroxide increase was noted in the oil from untempered low L-1 soybeans (PV 7) than in the oil from untempered Century soybeans (PV 4).

The oxidative and flavor stabilities of oils were further evaluated by determining 2,4-decadienal and induction periods based on total volatiles by capillary GC analyses. Increases in 2,4-decadienal levels with storage at 60 C were generally greater in the oils from untempered than the oils from heat-tempered soybeans (Table 4). Except in the degummed oils, increases in 2,4-decadienal were greater in the oils from untempered low L-1 soybeans than the corresponding oils from Century soybeans.

Measurements of induction periods based on total volatiles showed stabilities decreasing in the order: crude, degummed, deodorized, refined, and bleached oils (Table 5). No significant differences in induction periods were evident between the oils from either sets of soybeans or from either heat-tempered or untempered soybeans. Previous work indicated that induction periods measured on the basis of volatile

**TABLE 3.**

**Flavor Scores and (Peroxide Values) of Oils from Century and Null Lipoxygenase-1 Soybeans[a]**

| Oil samples[b] | Storage 60 C | 1984 Century | | 1984 Null L-1 | |
|---|---|---|---|---|---|
| | | Heat-temp[c] | Un-tempered | Heat-temp[c] | Un-tempered |
| Crude | 0-time | 6.4 (0) | 6.6 (0) | 6.1 (0) | 6.4 (0) |
| | 24 days | 6.5 (11) | 5.9 (8) | 6.1 (19) | 5.8 (40) |
| Degummed | 0-time | 6.5 (0) | 6.7 (0) | 6.4 (0) | 7.1 (0) |
| | 8 days | 6.4 (11) | 6.5 (17) | 6.9 (18) | 7.0 (21) |
| Refined | 0-time | 7.0 (0) | 6.2 (0) | 6.3 (0) | 6.8 (0) |
| | 4 days | 6.0 (15) | 6.4 (10) | 5.8 (15) | 6.5 (11) |
| Bleached | 0-time | 6.6 (0) | 5.1 (0) | 5.3 (0) | 6.1 (0) |
| | 2 days | 6.3 (11) | 5.6 (4) | 5.1 (9) | 6.0 (10) |
| Deodorized | 0-time | 8.1 (0) | 8.5 (0) | 8.6 (0) | 8.5 (0) |
| | 8 days | 6.2 (4) | 6.2 (4) | 6.1 (3) | 6.3 (7) |

[a]Flavor scale: 1-10; 10, bland; 1, strong; least significant differences for flavor scores, 0.8; peroxide values in parentheses, me/kg.
[b]Crude and partially processed oils were tasted diluted 95:5 with good quality deodorized oil (15); deodorized oils were tasted undiluted.
[c]On dehulled, cracked soybens with steam for 2 min.

**TABLE 4.**

**Capillary Gas Chromatographic Analyses of 2,4-Decadienal[a] in Oils from Century and Null Lipoxygenase-1 Soybeans**

| Oil samples | Storage 60 C | 1984 Century | | 1984 Null L-1 | |
|---|---|---|---|---|---|
| | | Heat-temp[b] | Un-tempered | Heat-temp[b] | Un-tempered |
| Crude | 0-time | 20 | 20 | 10 | 30 |
| | 24 days | 190 | 270 | 210 | 770 |
| Degummed | 0-time | 20 | 20 | 20 | 30 |
| | 8 days | 210 | 410 | 380 | 415 |
| Refined | 0-time | 80 | 60 | 100 | 30 |
| | 4 days | 620 | 430 | 600 | 500 |
| Bleached | 0-time | 30 | 30 | 60 | 30 |
| | 2 days | 140 | 150 | 160 | 230 |
| Deodorized | 0-time | 6 | 8 | 6 | 7 |
| | 8 days | 200 | 250 | 250 | 330 |

[a]GC integration counts $\times 10^3$.
[b]On dehulled, cracked soybeans with steam for 2 min.

**TABLE 5.**

**Induction Periods[a] for Total Volatiles by Gas Chromatographic Analyses of Oils from Century and Null Lipoxygenase-1 Soybeans**

| Oil samples | 1984 Century | | 1984 Null L-1 | |
|---|---|---|---|---|
| | Heat-temp[b] | Un-tempered | Heat-temp[b] | Un-tempered |
| Crude | 16.0 | 16.0 | 16.0 | 16.3 |
| Degummed | 7.2 | 4.9 | 5.9 | 4.1 |
| Refined | 3.4 | 2.0 | 4.0 | 4.0 |
| Bleached | 1.6 | 2.0 | 1.7 | 1.0 |
| Deodorized | 4.0 | 4.0 | 4.0 | 4.0 |

[a]Days at 60 C required to observe a sudden increase in total volatiles (17).
[b]On dehulled, cracked soybeans with steam for 2 min.

analyses by GC (17) are more sensitive and reliable measures of stability than induction periods based on peroxide values. The results in Table 5 confirm the flavor evaluation data in Table 3 in establishing no significant differences in either the flavor or oxidative stabilities of the oils from Century soybeans and from low L-1 soybeans. Therefore, we conclude that factors other than L-1 appear to affect the flavor and oxidative stability of soybean oils. Our results on the role played by lipoxygenase are limited to L-1 isoenzyme activity, because the null L-1 soybeans evaluated in this study contained significant L-2 and L-3 activities (Table 1). Further work on the importance of lipoxygenase L-2 and L-3 activity is needed by evaluating soybeans bred to remove these isoenzymes (9).

Much progress has been made in the development of new varieties of soybeans by breeding with the goal of producing oil of improved quality (1,8,9,19). However, these programs usually are directed at changing one characteristic at a time, such as, for example, low linolenic acid (19) and null lipoxygenase-1 (8). Because the characteristic changes in seeds may also be accompanied by adverse changes in oil stability, it is important to establish the effect of each genetic manipulation on the ultimate flavor and oxidative quality of the extracted oil. The sensory and GC volatile techniques used in this study are sufficiently sensitive and reliable that they should be useful to breeders in their screening programs aimed at producing oils of enhanced flavor and oxidative quality.

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