♣ Oxidative Stabilities of Low-Linolenate, High-Stearate and Common Soybean Oils

Pamela J. White* and **Lynne A. Miller**
Food and Nutrition Department, 111 MacKay Hall, Iowa State University, Ames, IA 50011

It is generally agreed that the high linolenate (18:3) content of soybean oil (SBO) contributes to its flavor instability. In this study, the oxidative stability of five SBO of various fatty acid (FA) compositions was compared by using peroxide values, conjugated dienoic acid values and sensory panel scores. Three of the oils were from common commercial varieties representing the range of 18:3 content normally found in SBO. The other two oils were from seed developed in a mutation breeding program. One of these oils from the line A5 had an 18:3 content of 3.5%, and the other from the line A6 had a stearate (18:0) content of 24%. Seed from the five soybean varieties was cold pressed, refined and deodorized without additives under laboratory conditions. Two oxidation experiments were conducted. In the first, the oils were stored at 28 C for 67 days. In the second, the oils were stored at 60 C for eight days. Sensory comparisons were done by using the AOCS Flavor Intensity Scale. The A5 and A6 oils were more stable than the commercial varieties as measured by chemical tests, but the sensory data were inconclusive. Oils with similar 18:3 contents did not have similar rates of oxidation. The differences between the oils were not as distinct in the 60 C test as in the 28 C test.

Soybean oil (SBO) oxidizes rapidly to form off-flavors which are caused by the release of volatile compounds during the breakdown of fatty acids (FA). When oxygen reacts with the unsaturated FA, hydroperoxides are formed. Hydroperoxides are flavorless but unstable, resulting in rapid transformation to secondary products which do contribute to off-flavors. A wide range of end products is possible, including aldehydes, alcohols, ketones, acids, hydrocarbons, esters and lactones (1, 2).

The rate of FA breakdown has been related to the number of double bonds in the carbon chain of the molecule. As the number of double bonds increases, the rate of oxidation increases. The ratios of the rates of oxidation of oleate (18:1) to linoleate (18:2) to linolenate (18:3) have been reported to be 1:10:20 (3,4,5). Because 18:3 oxidized faster than the other FA, it has been implicated as a major cause of off-flavor development in soybean oil (SBO), even though it accounts for only 7–10% of the total FA in SBO.

Durkee (6) first suggested 18:3 breakdown as the major cause of off-flavors in SBO. Dutton et al., Schwab et al. and Sanders (7–9) confirmed Durkee's observations by using various methods. Frankel summarized support for this theory in a review (1) and identified compounds that are believed to come from the oxidation of 18:3. These include acetaldehyde, propanal, 2-pentenal, 3-(cis or trans)-hexenal, 2,4-(trans,trans or trans,cis)-heptadienal and 2-(cis or trans)-1-pentenyl furans.

Because of the proposed relationship between 18:3 and off-flavor development in SBO, most oils that are sold commercially have been partially hydrogenated to reduce the 18:3 content from an original value of around 7–10% to 3% (10–12). Also, much research effort has gone into breeding a soybean with an 18:3 content below 3%. Researchers at Iowa State University have developed a soybean line (A5) that produces oil with approximately 3.5% 18:3 (13). In the process of developing this oil, another line (A6) with approximately six times the normal stearic acid content and an 18:3 content of approximately 6 to 7% was developed (13).

The purpose of this study was to determine whether the different FA compositions of A5 and A6 oils lower the rate of oxidation and development of off-flavors in SBO.

EXPERIMENTAL PROCEDURES

Extraction, refining and deodorization. Soybean seed from five genotypes was grown at ISU. The oil was removed from the seed by cold-pressing with a Hander Screw Press (model H54, Osaka, Japan). For the 28 C storage study, 16 kg of seed from the 1984 crop of A5, A6, Pella and BSR 101 was pressed. For the 60 C storage study, 23 kg of seed from the 1985 crop of A5, A6, Hardin and BSR 101 was pressed. Oils from each genotype and year were pressed separately. The commercial varieties, Pella, Hardin and BSR 101, were selected to represent the normal range of 18:3 found in soybean oil.

Free FA contents were determined by using AOCS method Ca-5a-40 (14) and then were removed according to AOCS method Ca-9d-52 (14). Because refined A6 oil solidifies at refrigerator temperature, the procedure for coconut oil was used in the final step. In addition, a hotplate and water bath with a large magnetic stirrer set at slow speed were used to simulate the paddle and hot water bath described in the method. Finally, a 20-min centrifugation at 8,000 rpm was required at the end of the refining procedure to separate the soapstock (free FA, phospholipids and contaminating nonoil compounds) from the oil. The alkali-refined oil was steam deodorized according to a method by Stone and Hammond (15). Immediately after deodorization, all oils were stored under nitrogen and held at −10 C until storage tests began. The oils were not bleached, and citric acid or other additives were not included.

Storage tests. Two storage tests were conducted. In test 1, oils from the 1984 crop were stored at 28 C. One hundred g of each oil (A5, A6, Pella and BSR 101) were stored in 150-ml beakers that were loosely covered. Aliquots were removed periodically and stored under nitrogen at −10 C until analyzed.

In test 2, duplicate lots of each oil from the 1985 crop (A5, A6, Hardin and BSR 101) were deodorized separately and stored at 60 C. Ninety g of each oil were stored in 100-ml beakers that were loosely covered. Aliquots were removed periodically and stored under nitrogen at −10 C until analyzed.

Sensory evaluation. Panelists were selected by using triangle tests to determine their ability to distinguish oxidized flavors. Three additional training sessions were conducted by using emulsions prepared from fresh and oxidized SBO to develop agreement on oxidized flavor and sample scores. Eight trained panelists evaluated the samples stored at 28 C, and 10–12 trained panelists evaluated the oils stored at 60 C. The oils were tasted at room temperature in the form of oil-in-water emulsions according to a method by Dixon and Hammond (16). This method has the advantage of allowing examination of multiple samples without the usual carryover that occurs when oils are tasted directly. This feature is particularly important when sampling strong-flavored oils, as was done in the present study. An additional reason for sampling the oils in an emulsion form was that one of the oils (A6) was cloudy, making it appear different from the other oils. In an emulsion form, all oils looked alike. The emulsions were scored on the AOCS Flavor Intensity Scale in which 10 is bland and 1 is extremely intense flavor (17). Panelists were instructed to judge only on intensity of oxidized flavor. The samples were presented in random order, and the panelists were instructed to first smell the oils and then to taste them in approximate order of increasing odor intensity. This reduced the possibility of a strongly oxidized oil overwhelming a panelist's ability to discriminate less oxidized oils before all emulsions were sampled. Blank samples and duplicate samples were included at random to check the panel's performance.

Chemical analyses. Methyl esters of the FA were prepared according to a method of Hammond and Fehr (18). FA compositions were determined by gas-liquid chromatography (GLC) of the methyl esters on a 3.2-mm by 2-m column packed with a moderately polar cyano-silicone phase (10% SP 2330, Supelco Inc., Bellefonte, Pennsylvania) on 100/120 Chromosorb W. A Beckman GC5 gas chromatograph (Fullerton, California) was used.

Peroxide values (PV) of the oils were determined by using a method of Hamm et al. (19), and conjugated dienoic acids (CD) were measured by using AOCS method Ti la-64 (14).

Statistical analysis. The data were analyzed by Analysis of Variance (ANOVA). Least significant differences were calculated by using the statistical analysis system (20).

RESULTS AND DISCUSSION

Test 1, 28 C storage. The FA compositions of the oils are presented in Table 1. The 18:3 content of the oils increased in the following order: A5 <A6 <Pella <BSR 101, with the 18:3 content of A6 oil being very close to that found in Pella. When compared with the commercial varieties, the A6 oil contained a greater amount of 18:0 as well as a smaller amount of all the FA, but particularly of 18:2.

The A5 oil contained less 18:2 and more 18:1 compared with oils from Pella and BSR 101. This resulted in fewer double bonds in A5.

Typically, oxidation causes a decrease in the percentage of 18:3 and an increase in the percentage of saturated FA. The end FA values for Pella and BSR 101 oils reflected this. The A5 and A6 oils showed a

TABLE 1

Fatty Acid Composition[a] of Oils
Before and After Storage at 28 C

Oil type	Fatty acid composition by GLC, %					
	16:0	18:0	18:1	18:2	18:3	20:0
Low linolenic (A5)						
Beginning values	10.5	4.1	37.7	44.2	3.7	0
Ending values	10.5	4.6	36.3	44.2	4.4	0
High stearic (A6)						
Beginning values	8.0	24.0	20.2	40.0	6.3	1.5
Ending values	8.5	24.1	20.0	39.3	6.7	1.5
Pella						
Beginning values	10.5	4.2	23.8	54.1	7.2	0
Ending values	10.9	4.7	23.8	54.4	6.3	0
BSR 101						
Beginning values	9.8	4.4	22.8	53.8	9.1	0
Ending values	10.3	4.5	23.0	54.3	7.8	0

[a]Relative area %.

TABLE 2

Peroxide Values[a-d] of Oils
During Storage at 28 C

Oil type	Peroxide values (meq/kg sample) at day						
	0	6	10	18	27	46	67
Low linolenic (A5)	1.0[b]	1.1[c]	2.4[c]	9.5[c]	13.9[c]	19.9[c]	28.7[c]
High stearic (A6)	1.3[a]	1.6[b]	2.1[c]	6.6[d]	12.1[d]	24.1[c]	28.3[c]
Pella	0.5[d]	0.8[d]	2.8[b]	13.2[b]	22.6[b]	37.6[b]	64.3[b]
BSR 101	0.7[c]	2.0[a]	5.1[a]	19.2[a]	48.1[a]	70.1[a]	100.6[a]

[a-d]Values in the same column with different superscript letters are significantly different (P<0.05).

slight but likely unimportant increase in the percentage of 18:3 after oxidation. However, if the total percentages of unsaturated FA (calculated by adding the percentages of the unsaturated FA for each oil) are compared, all four oils showed a similar drop in the total percentage of unsaturation.

When the oils were stored, PV (Table 2) for A5 and A6 were significantly lower (P<0.05) than for Pella and BSR 101 by day 10 and continued to be so for the remainder of storage. By day 67, PV for A5 and A6 were not significantly different from each other, even though A6 contained almost two times more 18:3 than did A5. For A5, Pella and BSR 101, PV increased in the same order as their 18:3 contents, but for A6, PV was less than expected when the amount of 18:3 is considered. However, A6 contained much less 18:2 and 18:1 than did the other oils.

The CD values are shown in Table 3. By day 18, CD values for A5 and A6 were significantly less (P<0.05) than the value for BSR 101. By day 46, values were significantly different (P<0.05) among the oils (A5 <A6 <Pella <BSR 101); they remained so on day 67. The differences in CD values between oils closely followed the differences in their 18:3 amounts.

Few significant differences in flavor were noted among the oils (Table 4). Only on day 0 was A5 significantly better (P<0.05) than the oils from the commercial vari-

TABLE 3

Percentages of Conjugated Dienoic Acid[a-d] in Oils
During Storage at 28 C

Oil type	Conjugated dienoic acid (%) at day						
	0	6	10	18	27	46	67
Low linolenic (A5)	0.17[b]	0.14[b]	0.15[a,b]	0.25[c]	0.31[c]	0.36[d]	0.49[d]
High stearic (A6)	0.24[a]	0.20[a]	0.18[a]	0.28[b]	0.33[b,c]	0.46[c]	0.57[c]
Pella	0.14[b]	0.12[c]	0.13[b]	0.27[b,c]	0.36[b]	0.55[b]	0.80[b]
BSR 101	0.16[b]	0.12[c]	0.13[b]	0.34[a]	0.48[a]	0.76[a]	1.04[a]

[a-d]Values in the same column with different superscript letters are significantly different (P<0.05).

TABLE 4

Flavor Evaluation[a-b] of Oils
During Storage at 28 C

Oil type	Flavor scores[a] at day						
	0	6	10	18	27	46	67
Low linolenic (A5)	8.6[a]	7.4[a]	7.0[a,b]	7.2[a]	5.3[a,b]	4.3[a]	3.9[a]
High stearic (A6)	6.7[b]	6.6[a]	6.0[b]	6.2[a]	4.6[b]	3.3[a]	3.2[a]
Pella	7.8[b]	7.3[a]	7.8[a]	7.2[a]	6.7[a]	5.3[a]	4.0[a]
BSR 101	7.2[b]	7.6[a]	6.3[a,b]	6.7[a]	6.4[a]	5.0[a]	3.9[a]

[a]Flavor intensity scale ranged from 1 (strong) to 10 (bland). Eight trained panelists evaluated the oils.
[a-b]Values in the same column with different superscript letters are significantly different (P<0.05).

TABLE 5

Fatty Acid Composition[a] of Oils
Before Storage at 60 C

Oil type	Fatty acid composition by GLC, %						
	16:0	18:0	18:1	18:2	18:3	20:0	22:0
Low linolenic (A5)	10.0	4.6	32.7	48.0	4.2	0	0.4
High stearic (A6)	8.8	17.2	22.2	42.2	7.2	1.5	0.7
Hardin	11.2	4.6	24.3	51.4	7.3	0	0.3
BSR 101	10.2	4.6	20.5	55.1	9.2	0	0.3

[a]Relative area %.

TABLE 6

Peroxide Values[a-c] of Oils
During Storage at 60 C

Oil type	PV (meq/kg sample) at day				
	0	2	4	6	8
Low linolenic (A5)	0.3[a]	3.4[b]	16.8[c]	29.1[b]	37.7[b]
High stearic (A6)	0.5[a]	10.7[a]	19.0[b,c]	29.8[b]	40.2[b]
Hardin[b]	0.5[a]	9.8[a]	23.8[a]	NA[a]	45.8[a,b]
BSR 101	0.4[a]	8.7[a]	23.0[a,b]	36.0[a]	50.0[a]

[a-c]Values in the same column with different superscript letters are significantly different (P<0.05).
[a]Not analyzed.
[b]Values from one replication.

eties. This contrasts with the significant differences found between the oils in the PV and CD tests. Researchers generally agree that PV and CD are not fully capable of predicting flavor scores (22). Several reasons for this discrepancy are usually cited (22-24). One is that human testers are more sensitive than instruments or chemical tests. Humans can integrate all quality aspects, such as flavor, odor and consistency of an oil, into one score. The A6 oil was partially solid at room temperature, which made it difficult to incorporate into a stable emulsion. Although all the emulsions looked alike, perhaps the A6 sample was not as finely emulsified as the other oils and the panelists were influenced by the different texture.

Flavors not related to oxidation also can affect flavor and may have contributed to flavor intensity scores. Moulton et al. (25) noted that flavors contributed by phospholipids, tocopherols, chlorophyll, carotenoids, etc., are not measured by PV or CD, but could be detected by a sensory panel.

Oxidized flavor itself can have so many descriptors that it is difficult to train a panel to agree on the flavor intensity. Oils undergo complex interactions when stored in air; therefore, simple and straightforward correlations may not be expected between sensory analyses and chemical tests such as PV and CD (26).

Test 2, 60 C storage. The composition of the oils is presented in Table 5. There were only minor differences between the beginning and ending values, so only the beginning values are reported. The 18:2 and 18:3 contents of the A5 oil from the 1985 crop used in test 2 were slightly greater than those in test 1, and the 18:0 con-

content of A6 oil was slightly larger in test 1 than in test 2. Hardin was chosen as the commercial variety having an intermediate content of 18:3 because Pella was unavailable.

The FA composition of the beans is affected, not only by the genetic makeup of the plant, but also by the climate. In particular, soybeans grown in southern climates yield oils with a lower 18:3 content than those grown in northern climates (27). Even within a given location, the FA contents will vary from year to year. After storage, PV (Table 6) for A5 and A6 were generally lower than PV for Hardin and BSR 101, but only a few differences were significant (P<0.05). The PV for A5 was significantly lower (P<0.05) than PV for Hardin and BSR 101 on days 2 and 4, and PV for A5 and A6 were significantly lower (P<0.05) than PV for BSR 101 on days 6 and 8. The CD values (Table 7) for A5 and A6 also tended to be lower than values for oils from the commercial varieties, but only on day 2 were the dif-

TABLE 7

Percentages of Conjugated Dienoic Acid[a-b] in Oils
During Storage at 60 C

Oil type	Conjugated dienoic acid (%) at day				
	0	2	4	6	8
Low linolenic (A5)	0.19[a]	0.23[b]	0.31[a]	0.49[a]	0.69[a]
High stearic (A6)	0.24[a]	0.31[a]	0.43[a]	0.53[a]	0.66[a]
Hardin[b]	0.20[a]	0.31[a]	0.39[a]	NA[a]	0.74[a]
BSR 101	0.22[a]	0.33[a]	0.43[a]	0.61[a]	0.90[a]

[a-b]Values in the same column with different superscript letters
are significantly different (P<0.05).
[a]Not analyzed.
[b]Values from one replication.

ferences significant (P<0.05) and only for A5. The flavor
evaluations of the oils again showed few significant
differences.

In both storage tests, A5 and A6 were more stable
than oils from the commercial varieties according to
the chemical tests. However, there were fewer signif-
icant differences between the oils in test 2. The flavors
of A5 and A6 tended to be more bland than the flavors
of the commercial varieties in test 2, but the differ-
ences were not generally significant. Possibly, the pane-
lists had difficulty in distinguishing between oxidized
and unoxidized flavors.

The 60 C storage test is often done as an accelerated
test to determine the oxidative stability of oil. Data
from the present study indicated that the differences in
oxidative stability as measured by PV and CD were
not as distinct in the 60 C accelerated test as in the 28
C test. White and Hammond (28) also found that the
differences in measured oxidative stability were smaller
in a 60 C storage test than in a room-temperature
oxidation test. However, in the current study, there
also were not as many differences in the total percentages
of unsaturated FA among the oils in test 2 as there
were in test 1. This could be a factor in the differences
in spread of the PV and CD between tests 1 and 2.
More research needs to be done to determine whether
the 60 C accelerated test provides an accurate repre-
sentation of oxidation at room temperature.

The oils from Hardin and A6 in test 2 had similar
18:3 contents. If the amount of 18:3 is the major cause
of rapid oxidation in SBO, then the rates of oxidation
should have been similar in these oils. This was not the
case, thus suggesting that the amount of 18:3 in SBO
is not the only factor controlling oxidative stability in
SBO. The total percentage of unsaturated FA in the oils
also did not predict the amount of oxidation.

Smouse (29) showed that key compounds identified
in oxidized SBO had 18:2 as well as 18:3 as their pre-
cursors. He concluded that, although the oxidation of
18:3 is still considered a cause of the off-flavors in
SBO, sufficient data had been published to show that it
may not be the only cause. Raghuveer and Hammond
(30) found that FA within a triglyceride structure in
SBO oxidized at different rates than pure methyl esters
of the same FA. The rates of some FA increased while
others decreased. They concluded that the presence of
one FA influenced the oxidation of another FA and/or

that the glyceride structure affected the relative rate of
oxidation.

Frankel (31) suggested that low levels of 18:3 hydro-
peroxides can catalyze the oxidation of 18:2, the pre-
dominant FA in SBO, and cause off-flavor development,
but that the minor constituents such as phospholipids,
sterols, hydrocarbons and pigments also can have det-
rimental effects, depending upon their relative concentra-
tions.

In both storage tests, the A5 and A6 oils generally
were more stable than the commercial varieties as mea-
sured by chemical tests; however, the sensory data
were inconclusive. Perhaps a more highly trained panel
would detect greater differences between the oils, or
perhaps there are other factors affecting the flavor
stability that are not reflected in the chemical tests
that were done in the study.

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