Oxidative Stabilities of Soybean Oils with Elevated Palmitate and Reduced Linolenate Contents

Nuo Shen[a], Walter Fehr[b], Lawrence Johnson[a], and Pamela White[a,*]

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

ABSTRACT: Oils from soybean lines, developed to contain different amounts of palmitate (16:0) and linolenate (18:3), were evaluated for oxidative stability. Oils were extracted in the laboratory from the soybean seeds and refined, bleached, and deodorized. Two replications, separated at the point of conditioning, were evaluated for each genotype, including Hardin 91 (normal beans), P9322 (10.6% 16:0 and <2.6% 18:3), A91-282036 (26.3% 16:0 and 9.8% 18:3), and HPLL (23.2% 16:0 and 3.5 % 18:3). Elevating 16:0 and/or lowering 18:3 increased the oxidative stability of soybean oils as measured by peroxide values. Soybean oils with elevated 16:0 had higher solidification temperatures than did oils with normal 16:0 content, and soybean oils with low 18:3 content had higher solidification temperatures than did oils with normal 18:3 contents. *JAOCS 74,* 299–302 (1997).

KEY WORDS: Hydrogenation, oxidative stability, peroxide values, soybean oils, *trans* fatty acids.

Soybean oil is less stable at high temperatures than oils with greater amounts of saturated fatty acids. The oxidative stability of soybean oil can be improved by changing its fatty acid composition. Neff *et al.* (1) demonstrated, in soybean oils of various fatty acid and triacylglycerol compositions, that the rate of peroxide formation increased with an increase in the number of double bonds. They were able to generate similar conclusions by using canola oils with different fatty acid compositions and interesterified blends of soybean oil and palm olein (2,3).

Because of its relatively high content of polyunsaturated fatty acids, soybean oil is often partially hydrogenated to enhance the oxidative stability for high-temperature use. During hydrogenation, *trans* fatty acids (*t*FA) are formed. Because of concerns over *t*FA, the U.S. Food and Drug Administration is reviewing whether to require labels of processed foods to include information on dietary *t*FA contents (4).

Without hydrogenation, oxidative stability of soybean oil can be enhanced by increasing content of saturated fatty acids and decreasing polyunsaturated fatty acid contents by breeding. Researchers at Iowa State University have developed soybean lines that contain elevated palmitate (16:0, 23–25% vs. 11.0% as normal) (5) with different linolenic acid (18:3) contents (2.7–9.3%) (6). Naturally saturated oils also should have fewer processing costs and should result in more profit for the farmers and/or less cost for the consumers. Miller and White (7,8) reported that oil from the soybean line A6, containing 20% stearate (18:0), was more stable than oils from commercial cultivars, and several other studies have shown superior oxidative stability of soybean oils with low linolenic acid content (1,9–11). Soybean oils with high palmitic acid and reduced linolenic acid contents also may have greater oxidative stabilities than traditional soybean oils. The objectives of this study were to investigate the oxidative stability of soybean oils with combinations of high 16:0 and either normal (~8%) or reduced (~3%) 18:3 contents.

MATERIALS AND METHODS

Materials. The commercial soybean cultivar (Hardin 91), a low-linolenic acid (18:3) and normal-palmitic acid (16:0) soybean cultivar (P9322), an elevated-16:0 and normal-18:3 soybean line (A91-282036, abbreviated as A91), and an experimental soybean line with high 16:0 and low 18:3 (HPLL) were grown near Ames, IA, in 1993 (Table 1).

Despite attempts to match the 16:0 contents of A91-282036 and HPLL and the 18:3 contents of P9322 and HPLL, these values were not the same because of genetic differences among the genotypes. The individual and combined influences of 16:0 and 18:3, however, could still be studied in the oils extracted from these beans.

Oil extraction. The moisture content of the soybeans was measured by using a Gac2000 Grain Analysis Computer (Dickey-John Corporation, Auburn, IL). To equalize moisture contents among all soybean lines, moisture was adjusted to 11% by spraying the appropriate amount of water over the beans, thoroughly mixing them, and storing them overnight at 5°C. Random moisture evaluations of the beans after storage indicated homogeneity in moisture distribution. Samples from each genotype were divided into two lots after moisture conditioning to give two replications for each of the four soybean genotypes.

*To whom correspondence should be addressed at Department of Food Science and Human Nutrition, 2312 Food Sciences Building, Iowa State University, Ames, IA 50011.

TABLE 1
Calculated Oxidizability of Soybean Oils and Fatty Acid Composition (relative area %)[a]

Soybean oil	Days	Fatty acid composition by GLC (%)[b]					Calculated oxidizability[c]
		16:0	18:0	18:1	18:2	18:3	
Hardin 91	0	10.3	5.2	25.2	52.0	7.4	7.2
	32	10.8	5.0	25.3	51.8	7.2	
P9322	0	10.6	4.7	26.4	55.5	2.6	6.5
	32	11.0	5.0	26.9	54.7	2.5	
A91-282036	0	26.3	4.5	15.0	44.4	9.8	6.8
	32	27.1	4.4	15.2	43.8	9.4	
HPLL	0	23.2	4.8	21.6	47.3	3.5	5.8
	32	23.4	4.8	21.4	47.1	3.4	

[a]Of soybean oils extracted from laboratory-scale extraction before and after storage at 60°C in the dark for 32 d.
[b]Values are the average of duplicate analysis of two replications.
[c]Oxidizability = [oleate% + 10.3 (linoleate%) + 21.6 (linolenate%)]/100 (Ref. 23); GLC, gas–liquid chromatography.

The extraction method (Fig. 1) was developed from a method described by Hassanen (12) and Reuber (13) and is fully reported here. The replicates of flakes (0.70 kg) were put into two vessels and extracted simultaneously at 60°C and at a ratio of 2:1, hexane/flakes, in the laboratory extraction simulator. During extraction, miscella was pumped to the vessels that contained the flakes through which it percolated. The pumping rate was controlled to maintain 1.0 cm of miscella over the flakes. Flakes from each replicate were extracted three consecutive times (stages) with fresh hexane for 6 min of recirculation, plus 3 min for draining. The miscella collections from the three stages were pooled. The time interval from cracking to extracting was kept between 45 to 50 min for each extraction.

The miscella was desolventized in a rotary evaporator (Wheaton, Heidolph, Germany) at 60°C at 80 ppm. For each replicate, 2 h was needed for desolventization. After desolventizing, the crude oils were stored at −14°C under nitrogen until needed. The crude oil yields (in percentage of flake weight) were 23.3% for Hardin 91; 23.5% for P9322; 19.2% for A91; and 19.4% for HPLL.

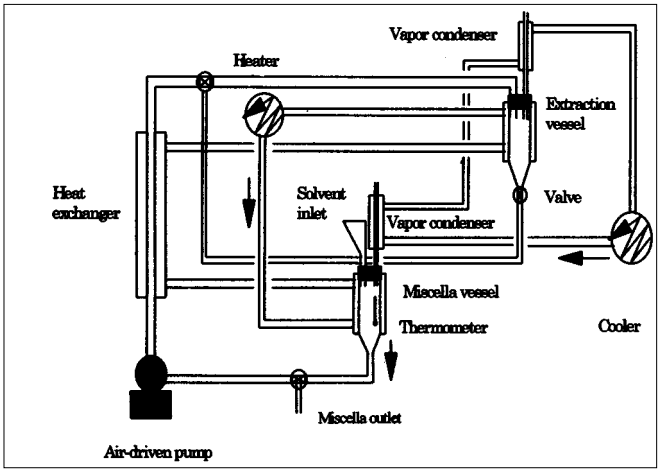

FIG. 1. Diagram of laboratory-scale extraction apparatus for extracting oil from soybean flakes with hexane.

Refining, bleaching, and deodorization. Crude oils from each replicate were refined, bleached, and deodorized as described by Shen *et al.* (14). Crude oils were refined according to AOCS Official Method Ca-9d-52 (15), bleached based on the AOCS Official Method Cc-8b-52 (15), and deodorized by high vacuum (<0.5 Torr) and high temperature (230 to 240°C for 2 h) as described by Stone and Hammond (16) and modified according to Moulton (17). Duplicate sets of each oil were refined, bleached, and deodorized separately. The oil was stored under nitrogen at −14°C until needed. The yields of refined, bleached, and deodorized (RBD) soybean oils were 78.7% of crude oil for Hardin 91; 75.1% for A91; 74.2% for P9322; and 78.4% for HPLL.

Accelerated stability tests at 60°C in the dark. Accelerated stability tests were conducted at 60°C. Eight replicate oil samples (0.040 kg) were stored in 100-mL beakers without covers at 60°C in the dark until sufficiently oxidized. Every other day, an aliquot of soybean oil was removed for analysis. Analyses on oil replicates were measured twice and averaged.

Peroxide values (PV) were measured according to the Stamm method as modified by Hamm *et al.* (18). Triacylglycerides were converted into fatty acid methyl esters (FAME) according to a method described by Hammond (19). The FAME were injected onto a Hewlett-Packard 5890 Series II gas chromatograph (Kennett Square, PA), which was equipped with a flame-ionization detector and split/splitless injector. A DB-23 fused-silica capillary column was used with dimensions of 0.25 mm × 15 m × 0.25 μm film thickness (J&W Scientific Inc., Rancho Cordiva, CA). Chromatographic parameters were set as follows: injector temperature 250°C, detector temperature 250°C, column temperature 200°C, and carrier gas (helium) flow rate 100 mL/min.

Cloud point test. A cloud point test was performed according to modifications of AOCS Official Method Cc 6-25 (15).

An aliquot (15 g) of each of the eight soybean oil replicates was placed in a 30-mL beaker. Each sample was heated to 130°C, then cooled to 50°C before being placed in an ice-water bath. During cooling in the water bath, the oil was stirred with a thermometer to prevent uneven crystallization of the oil. The end point was determined when the thermometer could not be seen at the back of the beaker when viewed horizontally.

Tocopherol contents. Tocopherol contents for each replicate of the RBD soybean oil were determined in duplicate according to Dove and Ewan (20). Although tocopherol contents are influenced by processing, amounts in the finished oils were analyzed because of potential effects on oxidation during storage. Differences in tocopherol levels from crude to deodorized oil were assumed to be relative from one oil type to another because of controlled processing conditions.

Statistical analysis. A randomized 2 × 2 factorial design was used for this experiment. Data from all treatments were analyzed by standard analysis of variance procedures (21). Differences in mean values among treatments were determined by the least significant difference test at $P = 0.05$ (21).

RESULTS AND DISCUSSION

Fatty acid compositions of the soybean oils, initially and after storage, are listed in Table 1. No significant differences were noted.

After day 10, soybean oils with reduced 18:3 and/or elevated 16:0 contents (P9322, A91, and HPLL) tended to have lower PV than did the oil with normal 18:3 and 16:0 contents (Hardin 91) (Table 2). After 8 d of storage, Hardin 91 had significantly greater PV than did HPLL oil. After day 14, soybean oil with reduced 18:3 content (P9322) had PV similar to those of soybean oil with high 16:0 content (A91). Both White and Miller (22) and Liu and White (9) reported that soybean oil with elevated stearate content had a significantly greater oxidative stability than did normal soybean oil, and soybean oils with low 18:3 contents oxidized at significantly lower rates than did oils with normal 18:3 contents.

In evaluating canola oils with different fatty acid compositions, Neff *et al.* (2) noted that the oxidative instability of canola oil was positively correlated with the amount of 18:3 content. Neff *et al*. (3) studied the oxidative stabilities of blends and interesterified blends of soybean oil and palm olein and concluded that oxidative stability was improved by lowering the 18:3 content.

The calculated oxidizability (23) of the soybean oils (Table 1) suggested the order of oxidation to be Hardin 91 > A91 > P9322 > HPLL. In this study, the PV of the oils during storage came close to these predicted values. A91 tended to have slightly lower PV than did P9322; however, these differences were significant only on day 10. Because the oxidizability of soybean oil is based on the percentage contents of unsaturated fatty acids, the influence of different 16:0 contents of soybean oils was not directly reflected in the calculated oxidizability. Because of the high 18:3 content (9.8%), A91 had the second greatest calculated oxidizability; however, A91 tended to have a slightly lower PV than P9322. P9322 had the greatest 18:2 content, whereas A91 had the lowest 18:2 content.

The soybean oils were evaluated by a modified cloud point test to characterize their potential use as refrigerated salad oils (Table 2). For a given fatty acid composition, the cloud point temperature is usually constant and depends mainly on the amount of saturated fat present (24). The four soybean oils were liquid at 25°C. The commercial soybean oil, with normal 16:0 and 18:3, had a significantly lower cloud point than did the other oils. The other three soybean oils, with either greater 16:0 contents or lower 18:3 contents, or both, had cloud points that were close to each other. This information was in agreement with the PV measurements, except for HPLL.

The tocopherol contents of soybean oils are shown in Table 3. There were significant differences among the oils in tocopherol contents for all homologues except γ-tocopherol. A91 had the greatest amounts of all other tocopherol homologues, whereas Hardin 91 had the lowest amounts of α- and β-tocopherol and tended to have the lowest amount of δ-tocopherol. The greater contents of these three tocopherol homologues in A91 may have helped its stability, considering that the calculated oxidizability of A91 was slightly greater than that of P9322, yet the two oils had similar PV throughout storage. HPLL was second in the amounts of these three tocopherol homologues, and had a much lower calculated oxidizability, both of which likely contributed to its significantly lower PV.

TABLE 2
Peroxide Values (meq/kg) and Cloud Points of Soybean Oils During Storage at 60°C in the Dark[a]

	Soybean oil genotypes			
Day	Hardin 91	P9322	A91	HPLL
Peroxide value				
0	0.2[a]	0.2[a]	0.1[a]	0.2[a]
2	0.3[a]	0.3[a]	0.3[a]	0.3[a]
4	0.5[a]	0.4[a]	0.4[a]	0.4[a]
6	0.7[a]	0.6[a]	0.6[a]	0.7[a]
8	1.7[a]	1.1[a]	0.9[a]	0.8[a]
10	7.8[b]	5.1[b]	1.4[a]	1.2[a]
12	16.2[b]	10.5[b]	4.1[a]	3.1[a]
14	24.9[b]	16.2[a,b]	11.5[a]	7.0[a]
16	35.0[b]	24.3[a,b]	23.3[a,b]	13.9[a]
18	45.5[c]	36.1[b,c]	34.8[b]	18.5[a]
20	60.8[b]	45.7[a,b]	43.1a[b]	24.7[a]
22	71.7[b]	54.7[a,b]	55.4[a,b]	35.2[a]
24	79.2[b]	62.9[a,b]	69.2[a,b]	43.7[a]
26	94.1b[b]	89.2[b]	83.1[b]	47.5[a]
28	110.0[b]	99.3[a,b]	98.5[a,b]	59.9[a]
30	139.2[b]	114.2[a,b]	111.5[a,b]	73.8[a]
Cloud point (°C)	2.5[a]	3.9[b]	4.0[b]	4.1[b]

[a]Values in the same row with different superscripts (a–c) were significantly different ($P \leq 0.05$).

TABLE 3
Tocopherol Contents (mg/kg) of Refined, Bleached, and Deodorized Soybean Oils[a]

Tocopherol homologue	Soybean oil genotypes			
	Hardin 91	P9322	A91	HPLL
α	43.9[a]	78.9[b]	105.1[c]	79.5[b]
β	1.9[a]	8.3[b]	18.0[d]	12.1[c]
γ	317.5[a]	317.6[a]	423.0[a]	332.4[a]
δ	80.2[a]	108.8[a]	238.1[c]	144.2[b]
Total	443.5	513.6	784.2	568.2

[a]Values in the same row with different superscripts (a–d) were significantly different ($P \leq 0.05$).

ACKNOWLEDGMENTS

The research was partly funded by the Iowa Soybean Promotion Board and by a grant from the United States Department of Agriculture for research programs of the Center for Designing Foods to Improve Nutrition. Soybean oil processing and extraction took place in the Center for Crops Utilization Research at Iowa State University. This is Journal Paper No. J-16773 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Project No. 3128).

REFERENCES

1. Neff, W.E., E. Selke, T.L. Mounts, W. Rinsch, E.N. Frankel, and M.A.M. Zeitoun, Effect of Triacylglycerol Composition and Structures on Oxidative Stability of Oils from Selected Soybean Germplasm, *J. Am. Oil Chem. Soc. 69*:111–118 (1992).
2. Neff, W.E., T.L. Mounts, W.M. Rinsch, H. Konishi, and M.A. El-Agaimy, Oxidative Stability of Purified Canola Oil Triacylglycerols with Altered Fatty Acid Compositions as Affected by Triacylglycerol Composition and Structure, *Ibid. 71*:1101–1109 (1994).
3. Neff, W.E., M.A. El-Agaimy, and T.L. Mounts, Oxidative Stability of Blends and Interesterified Blends of Soybean Oil and Palm Olein, *Ibid. 71*:1111–1116 (1994).
4. Anderson, B.B., FDA May Announce *trans* Decision Soon, *INFORM 6*:103–106 (1995).
5. Fehr, W.R., G.A. Welke, E.G. Hammond, D.N. Duvick, and S.R. Cianzio, Inheritance of Elevated Palmitic Acid Content in Soybean Seed Oil, *Crop Sci. 31*:1522–1524 (1991).
6. Graef, G.L., W.R. Fehr, L.A. Miller, E.G. Hammond, and S.R. Cianzio, Inheritance of Fatty Acid Composition in a Soybean Mutant with Low Linolenic Acid, *Ibid. 28*:55–58 (1988).
7. Miller, L.A., and P.J. White, Quantification of Carbonyl Compounds in Oxidized Low-Linolenate, High-Stearate and Common Soybean Oils, *J. Am. Oil Chem. Soc. 65*:1328–1333 (1988).
8. Miller, L.A., and P.J. White, High-Temperature Stabilities of Low-Linolenate, High-Stearate and Common Soybean Oils, *Ibid. 65*:1324–1327 (1988).
9. Liu, H.R., and P.J. White, Oxidative Stability of Soybean Oils with Altered Fatty Acid Compositions, *Ibid. 69*:528–532 (1992).
10. Mounts, T.L., K. Warner, G.R. List, R. Kleiman, J. Wilcox, W. Fehr, and E.G. Hammond, Effect of Altered Fatty Acid Composition on Soybean Oil Stability, *Ibid. 65*:624–628 (1988).
11. Neff, W.E., T.L. Mounts, W.M. Rinsch, and H. Konishi, Photooxidation of Soybean Oils as Affected by Triacylglycerol Composition and Structure, *Ibid. 70*:163–168 (1993).
12. Hassanen, N.Z.S., Extraction of Oilseed Model Systems with Alcohol, Ph.D. Dissertation, Texas A&M University, College Station, Texas, 1985.
13. Reuber, M.A., New Technologies for Processing *Crambe abyssinica,* M.S. Thesis, Iowa State University, Ames, Iowa, 1992, pp. 52–84.
14. Shen, N., W. Fehr, L. Johnson, and P. White, Oxidative Stabilities of Soybean Oils That Lack Lipoxygenases, *J. Am. Oil Chem. Soc. 73*:1327–1336.
15. AOCS, *Official and Tentative Methods 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. Hammond, E.G., Organization of Rapid Analysis of Lipids in Many Individual Plants, in *Modern Methods of Plant Analysis. New Series, Volume 12. Essential Oils and Waxes,* edited by H.F. Linskens and J.F. Jackson, Springer-Verlag, Berlin Heidelberg, New York, 1991, pp. 321–330.
20. 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).
21. SAS Institute Inc, *SAS® User's Guide: Basic,* version 5 edn., SAS Institute Inc., Cary, NC, 1985.
22. White, P.J., and L.A. Miller, Oxidative Stabilities of Low-Linolenate, High-Stearate and Common Soybean Oils, *J. Am. Oil Chem. Soc. 65*:1334–1338 (1988).
23. Fatemi, S.H., and E.G. Hammond, Analysis of Oleate, Linoleate and Linolenate Hydroperoxides in Oxidized Ester Mixtures, *Lipids 15*:379–385 (1980).
24. Mehlenbacher, V.C., *The Analysis of Fats and Oils,* The Garrard Press, Champaign, IL., 1960, pp. 365–374.

[Received March 12, 1996; accepted November 11, 1996]